Antioxygenic properties of winter apples

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Abstract. This article shows the results of a comparative study on 9 varieties of winter apples grown on the territory of the Samara Region. The study contains an analysis of the phenol compound assay using the Folin-Ciocalteu method, an antioxidant ability using the DPPH(2,2- diphenyl-1picrylhydrazyl) free radical, the flavonoid assay, a restoring force, an antioxidant activity in the linolic acid system and physical and chemical features (the mass fraction of reducing sugar, soluble solids, titrated acids, the fruit pulp assay and the sugar-acid ratio). These data indicate the best and the worst apple varieties of the study group.
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Introduction

A great number of the latest clinical and experimental researches are dedicated to antioxidants and their influence on the human body. Antioxidants are like “hunters” for free radicals. They contribute to the regulation of the balance mentioned above. This balance helps the body grow, produce energy, resist infections and detoxificate chemicals and pollutants. Antioxidants work to reduce the level of a tissue injury and activate the regeneration process. The study proves that antioxidants are able to increase the human lifespan. The crucial interest of the antioxidant activity studies is fruits and vegetables. It was discovered they are 20% of the food [1] a person consumes.

Chemical content of apples is a subject of study of many world researchers. Thus, for example, Chinese researchers offer a new test method for apple sugar assay using the Fourier transform infrared spectroscopy. This method shows good results for a Fugi apple variety [2].

A great number of national studies are dedicated to recipe and technology development for the production of apple-based foods. Thus, a series of functional products based on apple puree was designed on the basis of the food combining principles [3]. The food includes fruit sauces, drinks and puree.

The production technology of the Apple BAA based on pomaces was designed [4] using the mecano-chemical activation method. The additive has a complex of medical and biological properties: antioxidative, antitoxic, hepatoprotective, radioprotective and immune modulating. A recipe of the whole series of a dessert jelly includes natural apple juice [5]. This natural product has a special smell, flavour, colour and fine texture.

There is an article [6] of a prominent interest dedicated to a study of apples as a valuable source of ascorbic acid, polyphenolic and pectin elements, acids and sugars. This chemical content may be offered to be included into a recipe of fermented beverages, soft drinks, kissels, bread and gingerbreads.

A number of scientists are carrying out researches to study other functional apple properties. There are also analysis researches dedicated to an antioxidant activity of different apple varieties. Thus, phenol compound assay of Limoncella apples was studied [7] using the HPLC method and an antioxidant activity of the same variety was analyzed on the basis of free radical bond and inhibition of formations of the elements capable of an interaction with 2-tiobarbituric acid.

American scientists determined the chemical structure of apple skin constituents responsible for an antiproliferative and antioxidative activity having taken Red Delicious apples as a sample. They managed to identify 29 compounds relating to triterpenoid, flavanoid, organic acid and plant sterol varieties [8].

The objective of our research is to study physical and chemical and organoleptic features of 9 winter apple varieties grown in the Povolzhskiy Region in 2011. The varieties are as follows: Lobo, Kutuzovetz, Berkutovka, Kuybyshevskoye, Renet Simirenko, Florina, Champion, Rossoshnskoye, Podarochnoye [9].

Materials and methods

Chemicals and reagents

Folin-Ciocalteu reagent in sodium carbonate, gallic acid, catechin, were purchased from Fluka (Germany). DPPH (2,2-diphenyl- 1-picrylhydrazyl), Tween 40, hydrogen peroxide,

sodium nitrite, aluminum chloride, thiobarbituric acid, trichloroacetic acid were purchased from Sigma-Aldrich Chem. mp. (USA).

Fruit collection

The apple fruits were gathered in the Samara Region of the Russian Federation near the Volga river in September-October of 2011 and stored at the temperature of 0°C and humidity of 98%. The objects of study were the apples (pulp + skin + juice, directly squeezed juice) of the following varieties: Lobo, Kutuzovetz, Berkutovka, Kuybyshevskoye, Renet Simirenko, from the fruit collection of the State Budgetary Institution of the Samara Region "Research Institute of Horticulture and Medicinal Plants "Zhigulevskie Sady", Florina, Champion, Rossoshnskoye, Podarochnoye from the collection of "Sad", ltd. of the Privolzhsky Region and "Sadovod", ltd. of the Syzran Region.

Determination of total phenols

Total phenolic content of methanolic fruit extracts was assessed using a modified version of the Folin–Ciocalteu assay (Singelton, Orthofer, & Lamuela-Raventos, 1999). Gallic acid was used as a standard and the aqueous gallic acid solution (200 mg l ^{1}) was di-luted with distilled water to give appropriate concentrations for a standard curve. For the analysis, 100 ll of methanolic fruit extract or gallic acid standard, 100 ll of methanol, 100 ll of Folin–Ciocal-teu reagent and 700 ll of Na_2CO_3 were added into 1.5 ml micro-centrifuge tube. The samples were vortexed immediately and the tubes were incubated in the dark for 20 min at room temperature. After incubation all samples were centrifuged at 13,000 rpm for 3 min. The absorbance of the supernatant was then measured at 735 nm in 1 ml plastic cuvettes using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Japan). The results were expressed in mg gallic acid equivalent/100 g dry weight (mg GAE 100 g ^{1} DW).

Determination of total flavonoids

The flavonoid content of the methanolic extracts were measured using a colorimetric assay (Zhishen, Mengcheng, & Jianming, 1999). A known volume (0.5 ml) of the extract or standard solution of quercetin was added to a 10 ml vol-umetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% w/v NaNO2 was added to the flask. After 5 min, 0.6 ml of 10% w/v AlCl3 was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. Absorbance was read at 350 nm against the blank (water) and flavonoid content was expressed as mg querce-tin equivalents (QE) in 100 g of fresh material.

DPPH radical scavenging activity

The scavenging activity of samples was measured in accordance with the method of Brand-Williams (Brand-Williams, Cuvelier, & Berset, 1995). The method was based on the reduction of methanolic DPPH in the presence of a hydrogen-donating antioxidant. DPPH solution was an intense violet colour and showed an absorption band at 515 nm. Adsorption and colour lowered when DPPH was reduced by an antioxidant compound. The remaining DPPH corresponded inversely to the radical-scavenging activity of the antioxidant. DPPH (2 mg) was dissolved in 54 ml of MeOH. Aliquots of investigated extract (50, 100, 200, 300, 500 and 1000 lg) were dissolved in 2 ml of MeOH. Then 1.0 ml of each solution was added to 1.0 ml of DPPH solution at room temperature. The absorbance at 515 nm was measured against a blank (2 ml MeOH in 2.0 ml of DPPH solution) using a UV-1601 Shimadzu spectrophotometer. The results were expressed as percent-age of reduction of the initial DPPH adsorption by test samples:

% of reduction of the initial DPPH adsorption =

ADPPH(t)_A sample (t)/ADPPH (t) _ 100,

ADPPH(t) is absorbance of DPPH at time t and A sample (t) is absorbance of sample at t the same time.

Determination of Antioxidant Activity in a Linoleic Acid System.

The total antioxidant activity of FEHP was carried out by use of a linoleic acid system (26). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 mL of phosphate buffer (0.2 M, pH 7.0), and then the mixture was homogenized. A 0.5-mL ethanol solution of different concentration of FEHP (50-500 íg/mL) was mixed with linoleic acid emulsion (2.5 mL, 0.2 M, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0). The reaction mixture was incubated at 37 °C in the dark to accelerate the peroxidation process. The levels of peroxidation were determined according to the thiocyanate method by sequentially adding ethanol (5 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), sample solution (0.1 mL), and ferrous chloride (0.1 mL, 20 mM in 3.5% HCl). After the mixture was left for 3 min, the peroxide value was determined by reading the absorbance at 500 nm on a spectrophotometer.

FRAP assay

The FRAP assay was carried out according to Stratil et al. (2006) with slight modifications. The FRAP solution was freshly prepared on the day of use, by mixing acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ solution (10 mM TPTZ in 40 mM HCl) in a proportion of 10:1:1, respectively. Following this the FRAP solution was

heated, while protected from light, until it had reached a temperature of 37 LC. Appropriate dilutions of methano-lic fruit extracts were prepared. One hundred microlitres of the di-luted sample extract (or for blank 100 ll methanol and for Trolox standard curves 100 ll Trolox of appropriate concentration) and 900 ll of FRAP solution were added into a micro-centrifuge tubes. The tubes were vortexed and left at 37 LC for exactly 40 min, and the absorbance was measured at 593 nm. The Trolox standard curves were used to calculate the antioxidant activity of the sam-ples in relation to Trolox and were expressed as mg Trolox equiv-alent/100 g dry weight sample (mg TE 100 g ^{1} DW).

Total sugar content

The Luff–Svhoorl technique was used as described in NP-1420 and samples were analyzed as quadruplicates. This method is based on the amount of cuprous oxide obtained after reduction of a cuprous sulphate solution (copper II) by sugars in an alkaline environment. An iodometric titration is used for the determination of cuprous oxide.

Acidity

A potentiometric titration using a combined glass membrane electrode was used according to NP-1421:1977.

Mass concentration of soluble solids

This is an important indicator nowadays. It is the content of soluble solids that determines the status of the drink as nectar, restored juice or 100% directly squeezed juice. To study this indicator, indirect determination of the refractive index of the soluble non-volatile substances is performed. The determination is performed using a refractometer in accordance with the State Standard Specification 28562-90.

The content of pulp

The determination of the content of pulp in apple juice was performed by separating the pulp from the liquid during centrifugation and subsequent gravimetric determination of the amount of pulp by the sediment in accordance with the State Standard Specification 51442-99 [10 and 11].

Results and discussion

Determination of phenolic compounds

According to the results of apple phenolic assay, there is a curious relation (see Fig. 1).

We may observe that the biggest number of phenolic elements corresponds to Kuybyshevskoye, Florina and Kutuzovetz apple varieties (354 mg, 354 mg and 352 mg of gallic acid per 100 g of a feedstock). Besides, their number exceeds two times the same ratio of Berkutovka apples (acid per 100 g of a feedstock).

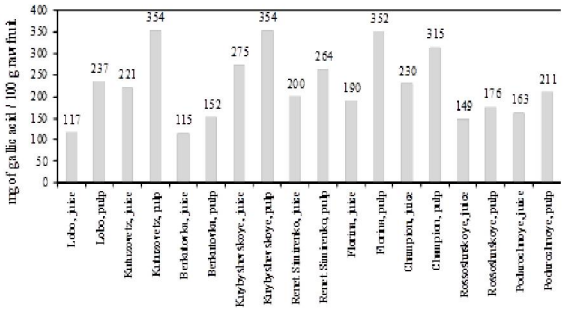

Fig. 1. Content of phenolic compounds in apple

Flavonoid assay

According to the data shown in the Fig.2, we may suggest the following: similar to the phenolic elements, the apple pomace has bigger number of flavonoids than the juice of the same apple varieties (Kuybyshevskoye juice/pulp – 284 mg of catechin/322 mg of catechin).

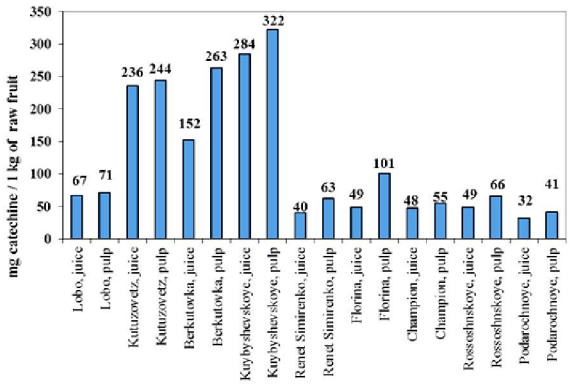

Fig. 2. Total content of flavonoids in apples

The undisputed leader is the winter apple variety Kuybyshevskoye. Its number of pulp flavonoids (322 mg of catechin) exceeds 8 times the number of pulp flavonoids of the winter apple variety Podarochnoye (41 mg of catechin).

Assessment of the level of antioxidant activity in apples

According to the experimental data shown in the Table 1, we may identify the apple varieties with the greatest potential to absorb DPPH radicals.

The highest level of antiradical activity calculated according to the method corresponds to Champion and Kuybyshevskoye apples. But the linolic acid system of antioxidant activity demonstrates the highest results of the following apple varieties: Kuybyshevskoye, Berkutovka and Kutuzovetz.

Table 1. The chemical composition of apples of different varieties of the 2011-2012 harvest

Studied object	E_{c50}, mg/cm^3	Antioxidant activity in a linoleic acid system, % of inhibition of linoleic acid oxidation
Lobo, juice	77	4,7
Lobo, pulp	69	5,0
Kutuzovetz, juice	89	15,4
Kutuzovetz, pulp	29	19,6
Berkutovka, juice	139	19,2
Berkutovka, pulp	93	20,7
Kuybyshevskoye, juice	28	19,7
Kuybyshevskoye, pulp	16	22,6
Renet Simirenko, juice	89	13,9
Renet Simirenko, pulp	85	15,3
Florina, juice	82	14,6
Florina, pulp	86	15,5
Champion, juice	26	Not found
Champion, juice	14	Not found
Rossoshnskoye, juice	82	7,1
Rossoshnskoye, pulp	49	7,8
Podarochnoye, juice	106	14,7
Podarochnoye, pulp	84	15,3

FRAP assay

According to the data shown in the Fig.3, there is an apple variety that demonstrates the highest restoring force.

These are Kuybyshevskoe apples (29,16 mM Fe^{2+}/1 kg of a feedstock). This variety exceeds almost two times another leader – Florina apples (14,04 mM Fe^{2+}/1 kg of a feedstock) and almost seven times a loser of the assay – Berkutovka (4,32 mM Fe^{2+}/1 kg of a feedstock).

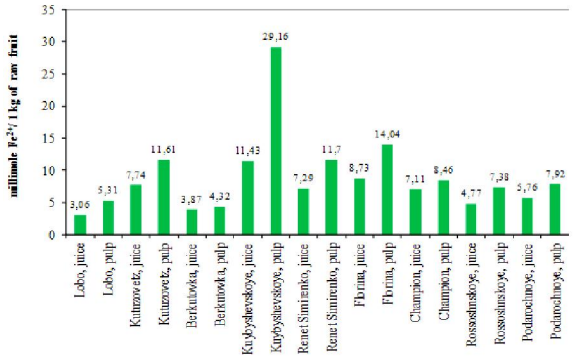

Fig. 3 Restoring force by FRAP method

Composition analysis

Nowadays apple juice is produced by all the juice manufacturers of different labels and is presented in every price segment – starting from the cheapest group up to the premium one. Many juice drinks are based on apple juice. High-quality drinks are vintage juices made from the fruits of the high-quality varieties released in the area of their production. The process of apple tree selection for juice manufacturing includes the following criteria: its production, taste and compliance with standards. The results of technological assessment show the properties of 9 apple varieties for not-concentrated juice production and allow us to single out the best of them (see Table 2).

Table 2. Results of the study of physical and chemical parameters of apple juice

Apple Varieties	Indicators					
	Mass concentration of soluble solids, %	Mass concentration of reducing sugars, %	Mass concentration of titratable acids (n malic acid equivalent), %	Content of the pulp	Juice secretion	Sugar-acid index
Lobo	9,9	9,7	0,3	12,7	1,78	32,33
Kutuzovetz	12,0	9,5	0,5	7,4	2,14	19,00
Berkutovka	10,0	7,8	0,5	3,1	1,51	15,60
Kuybyshevskoye	9,0	10,2	0,5	6,5	1,77	20,40
Renet Simirenko	15,1	10,5	0,6	3,3	1,75	17,50
Florina	15,3	13,7	0,5	4,2	1,50	27,40
Champion	12,5	11,1	0,6	4,2	1,89	18,55
Rossoshnskoye	13,5	9,6	0,7	10,1	1,89	13,71
Podarochnoye	13,2	11,0	0,7	4,2	1,41	15,71

According to GOST R 52184-2003, the technological criteria of juice comprise the following requirements: mass ratio of soluble solids of apple vintage juice shall be not less than 11%, mass ratio of titrated acid shall be not less than 0,3 % (see Table 3).

Table 3. Physicochemical indicators of nonclarified directly squeezed juice in accordance with the State Standard Specification R 5284-2003

Brand of juice	Mass concentration, %	
	PCB, no less than	Titratable acids, no less than
Apple (vintage) juice	11	0,3
Apple juice from summer ripening apples	9	0,2
Apple juice from autumn ripening apples	9,5	0,3
Apple juice from wild growing apples	8	1,1

According to the data of Table 2, the apple varieties that are not suitable for not-concentrated juice manufacturing are as follows: Lobo, Berkutovka and Kuybyshevskoye. The most appropriate in the category of the sugar content (more than 10%) are Podarochnoye, Champion, Florina, Renet Simirenko and Kuybyshevskoye apples. But the most valuable varieties for manufacturing and selection purposes are the varieties of more than 13% sugar content which is Florina.

According to the analysis and synthesis of the data of technological assessment of a considerable number of Samara fruit crop genotypes, there are apple tree varieties (Rossoshanskoye, Florina, Champion) which obtain an optimal combination of chemical and technological fruit indices meeting modern technological requirements and suitable for not-concentrated juice manufacturing.

Conclusions

We have studied the chemical assay of the following apple varieties: Lobo, Kutuzovetz, Berkutovka, Kuybyshevskoye, Renet Simirenko, Florina, Champion, Rossoshanskoye and Podarochnoye of 2011/2012. The data show that this apple fruit crop contain valuable natural components and physiologically functional ingredients which are not only an essential part of a healthy sustenance but also are able to correct and prevent a body system malfunction and alimentary (connected with nutrition) diseases [12 and 13].

That is why, being a raw material of natural content of active components, apples may be a potential base for healthy food manufacturing. But physiologically functional ingredients are digested marginally directly from a plant raw material. Juice processing i.e. a conversion of a product into a soluble state – juice fraction – facilitates digestion of nutrients considerably.

According to the analysis and synthesis of the data of technological assessment of a considerable number of Samara fruit crop genotypes, there are apple tree varieties (Rossoshanskoye, Florina, Champion) which obtain an optimal combination of chemical and technological fruit indices meeting modern technological requirements and suitable for not-concentrated juice manufacturing. The most significant factor to influence the chemical assay and an antioxidant ability is an apple variety. It has been proved that apple pulp contains a bigger number of phenolic elements and has a higher antioxidant activity.

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References

1. Saura-Calixto, F. and I. Goci, 2006. Antioxidant capacity of the Spanish Mediterranean diet. Food Chemistry, 94 (3): 442-447.
2. Yan-de, L., Y. Yi-bin, F. Xiaping and L. Huishan, 2007. Experiments on predicting sugar content in apples by FT-NIR Technique. Journal of Food Engineering, 80(3): 986-989.
3. Arutyunova, G.U. and L.Y. Rodionova, 2008. Functzionalniye pishcheviye izdeliya na osnove kostochkovikh plodov. Izvestiya vuzov. Pishchevaya Tekhnologiya (in Russian), 1: 39-41.
4. Martovshchuk, E.V., V.I. Martovshchuk, N.I. Alsheva, D.U. Kashkara and E.A. Verbitzkaya, 2009. Khimitcheskiy sostav I pishchevaya tzennost BAD “Yabloko. Izvestiya vuzov. Pishchevaya Tekhnologiya (in Russian), 4: 120-121.

5. Dzharulayev, D.S., M.-Z.V. Vagabov E.M. and Rasulov, 2008. Noviy sposob proizvodstva desertnogo zhele. Pishcevaya promyshlennost (in Russian), 7: 24-25.
6. Kiseleva, T.F., I.S. Zaiytzeva, D.B. Pekov and N.V. Babiy, 2009. Viyavleniye predposilok kompleksnoy pererabotki plodovo-yagodnogo syrya Sibirskogo rayona. Tekhnika I tekhnologiya pishchevykh proizvodstv (in Russian), 3: 7-11.
7. D'Abrosca, B., S. Pacifico, G. Cefarelli, C. Mastellone and A. Fiorentino, 2007. 'Limoncella'apple, an Italian apple cultivar: phenolic and flavonoid contents and antioxidant activity. Food Chemistry, 104: 1333-1337.
8. He, X.J. and R.H. Liu, 2008. Phytochemicals of apple peels: isolation, structure elucidation, and their antiproliferative and antioxidant activities. Journal of Agricultular and Food Chemistry, 56 (21): 9905-9910.
9. Aljadi, A.M. and M.Y. Kamaruddin, 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chemistry, 85 (4): 513–518.
10. Wu, L.C., H.W. Hsu, Y.C. Chen, C.C. Chiu, Y.I. Lin and J.A. Annie Ho, 2006. Antioxidant and antiproliferative activities of red pitaya. Food Chemistry. 95: 319-327.
11. Sun, T., J.R. Powers and J. Tang, 2007. Evaluation of the antioxidant activity of asparagus, broccoli and their juices. Food Chemistry, 105 (1): 101-106.
12. Mohammadzadeh, S., M. Sharriatpanahi, M. Hamedi, Y. Amanzadeh, S. E. Sadat Ebrahimi and S. N. Ostad, 2007. Antioxidant power of Iranian propolis extract. Food Chemistry. 103 (3): 729-733.
13. Liu, Q. and H. Yao, 2007. Antioxidant activities of barley seeds extracts. Food Chemistry. 102 (3): 732-737.

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