

ANTIOXIDANT ACTIVITY OF JUICE OF SMALL-FRUIT CROPS

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A comparative study of antioxidant activity of juices of black and red currant, raspberry and blackberry, cherry and sweet cherry, bilberry and blueberry, black chokeberry, and strawberry is carried out. The dependences of fluorescence intensity of fluorescein on a logarithm of concentration of juice from which IC_{50} indicators are graphically defined are received.

Keywords: antioxidant activity; juice of black and red currant, raspberry, blackberry, cherry, sweet cherry, bilberry, blueberry, black chokeberry, strawberry; fluorescein.

The excess concentration of free radicals in an organism is the central risk factor of cardiovascular and oncology diseases, and other pathologies. Flavonoids have strong antioxidant properties and can be used as preventive treatment for various diseases. Such flavonoids as Quercetinum and Rutinum as well as antotsiana, and other phenolic glycosides acting as inhibitors of free radicals [1–3] are the part of many berries.

A comparative study of antioxidant activity (AOA) of these flavonoids in juice of black and red currant, raspberry and blackberry, cherry and sweet cherry, bilberry and blueberry, black chokeberry, and strawberry is conducted.

The method of AOA determination in relation to activated oxygen forms (AOF) is based on the measurement of fluorescence intensity of the oxidized compounds and its reduction under the influence of AOF. In the present study fluorescein, which possesses high extinction coefficient and close to 1 fluorescence quantum yield, is used for detecting free radicals. The generation of free radicals is carried out using Fenton's system, in which hydroxyl radicals are formed through the interaction of iron compound (Fe^{2+}), etilendiamintetrauksusny acid (EDTA) and hydrogen peroxide [4; 5].

The dependences of fluorescence intensity of fluorescein on a logarithm of concentration of juice are received for all the samples. The study is conducted on 0,01–10 % juice concentration. The juice samples begin to show AOA at the concentration of 0,01–0,02 %. With the subsequent increase in juice concentration the increase in suppression of free radicals action and the increase of fluorescein fluorescence are observed. The studied samples restore fluorescein fluorescence to 51–78 % (A_{max}) at their concentration of 0,2–1 % (table 1). IC_{50} indicators, which are the concentrations of juices at which 50 % inhibition of free radicals is reached, are graphically defined. The IC_{50} indicators of black currant, sweet cherry, raspberry, blueberry, black chokeberry, strawberry, red currant, bilberry, blackberry, cherry respectively are $2,95 \cdot 10^{-2}$ – $13,2 \cdot 10^{-2}$ % (table 1) that demonstrates high antioxidant abilities of the juices studied. The comparison of the couples of juices of the berries having similar structure is carried out. The highest antioxidant activity is defined in blackcurrant juice. The fluorescence intensity of fluorescein is restored up to 78 % at the juice concentration of 0,2 %.

Table 1

Indicators of antioxidant activity of juices

Juices	A_{max} , %	C, %	$IC_{50} \cdot 10^{-2}$, %
black currant	78	0,2	2,95
sweet cherry	76	1	3,47
raspberry	64	0,2	6,17
blueberry	64	1	6,3
black chokeberry	61	0,2	7,5
strawberry	66	1	8,77
red currant	56	0,2	9,1
bilberry	55	0,2	12,3
blackberry	59	0,2	13
cherry	51	0,2	13,2

Due to the high content of flavonoids, juices of small-fruit crops can be considered as highly effective inhibitors of free radicals.

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ANALYSIS OF HUMAN CHORIONIC GONADOTROPIN USING BOTTOM-UP PROTEOMIC APPROACH

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Human chorionic gonadotropin (hCG) is mainly the product of placental syncytiotrophoblast cells. It can also be secreted by several normal non-placental tissues and trophoblastic or non-trophoblastic neoplasms. Human chorionic gonadotropin is included in the lists of illegal drugs in some sports. In this study the methodological approach to human chorionic gonadotropin detection by HPLC-mass spectrometry is developed.

Keywords: Human chorionic gonadotropin, human chorionic gonadotropin structure, high performance liquid chromatography, mass-spectrometry.

Human chorionic gonadotropin has a molecular weight of 38 000 Da with 237 amino acids organized in two subunits, alpha and beta, each consisting of a single polypeptide chain. Seventy percent of its structure is represented by the protein chains and 30 % by carbohydrate chains. The carbohydrate chains covalently bound to the peptide chains are of two types: O-linked and N-linked oligosaccharides. Regarding endogenous forms of hCG, there are various ways to categorize and measure them, including total hCG, free β -subunit hCG, β -core fragment hCG, hyperglycosylated hCG, nicked hCG, alpha hCG, and pituitary hCG.

In this study the methodological approach to human chorionic gonadotropin detection by high resolution mass spectrometry based on their prior tryptic hydrolysis (“bottom-up method”) is developed. The peptides obtained from tryptic hydrolysis are separated by HPLC method on reversed-phase column and are analyzed using a high resolution mass spectrometer Agilent 6550 iFunnel Q-TOF. The designed approach allows detecting 7 hCG peptides (figure 1).

The 3 peptides of the alpha subunit and 4 peptides of the beta subunit at various degrees of protonation are detected. Analyzing the peptides obtained after the tryptic hydrolysis of hCG, it is found that two peptides derived from the cleavage of the β -subunit correspond to peptides that come out during hydrolysis of the beta-core fragment.