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Determination of total antioxidant content in various drinks by amperometry

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Abstract

In the present work the total content of phenolic antioxidants in juice of some fruit and vegetables, in wines, water extracts of tea and herb were measured by amperometry. Efficiency of the method allowed determining the total antioxidant content in their binary and multimixes, including processes of frosting-defrosting and juice diluting as well. The deviation of experimentally received values of the total antioxidant content in some drink mixes from the values calculated proceeding from the additivity principle of the antioxidant content in separate drinks has been revealed.

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1. Introduction

Now it is scientifically proved that a salutary effect of a lot of food-stuff and drinks, including juice of fruit and vegetables, wines, water extracts of tea and herb, on human health is related mainly to antioxidant activity of natural phenol combinations. They are contained in significant amounts in this food-stuff and are capable to break chain free radical oxidation reactions proceeding in a human body^{1,2}. In the food-processing industry various kinds of drinks, including mixtures of them are produced. Thus, the content of phenol type antioxidants can change both towards increase and decrease. Therefore, determination of the total antioxidant content not only in separate drinks,

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but also in mixes of drinks and extracts, represents a practical interest. From the scientific point of view, comparison of the antioxidant content in separate drinks and their mixes will allow studying possible interference of different drink components into each other.

The aim of the present work is to determine the total phenol type antioxidant (AO) content in various alcoholic and non-alcoholic drinks by amperometry.

2. Experimental

The essence of the use of amperometry consists in measurement of the electric current arising at oxidation of investigated substance on a surface of a working electrode at a certain potential. An oxidation of only OH - groups of natural phenol type AO (R-OH) there is at this potential. The electrochemical oxidation proceeding by the scheme $R-OH \rightarrow R-O' + e^- + H^+$ can be used as a model for measurement of free radical absorption activity which is carried out according to the equation $R-OH \rightarrow R-O' + H^*$. Both reactions include the rupture of the same bond O-H. In this case, the ability of the same phenol type AO to capture free radicals can be measured by the value of oxidizability of these compounds on a process electrode of the amperometric detector³. An amperometric device "TsvetJauza-01-AA", in which this method is used, represents an electrochemical cell with a glassy carbon anode and a stainless steel cathode to which a potential of 1, 3 V is applied⁴. An analyte is introduced into eluent by a special valve. As the analyte passes through the cell the electrochemical AO oxidation current is recorded and displayed on the computer monitor. The integral signal is compared to the signal received in the same conditions for comparing a sample with known concentration. Quercetin (Q) gallic acid (GA) is used as a comparison sample. The root-mean-square deviation for several identical instrument readings makes no more than 5% ⁴. A measurement error of the total phenol type antioxidant content taking into account reproducibility of the results is 10%. The method involves no model chemical reaction and measurement time makes 10–15 min.

3. Results and discussion

3.1. Berry, fruit and vegetable juice and their mixes

Objects of the research were squeezed juice of berries (a sea-buckthorn berry, a bilberry, a raspberry, a grape and a black currant), fruit (an orange, a grapefruit, a pomegranate, an apple, a peach and a plum) and vegetables (a carrot, a beet, cabbage, a tomato, red Bulgarian pepper (paprika) and an eggplant). Juices were prepared in a juice extractor, then filtered through the paper filter «a dark blue tape» and if necessary diluted with the distilled water. For preparation of binary mixes separate juices were mixed for 5 minutes in the ratio 1:1, and for mixes of 5 different juices – in the ratio 1:1:1:1:1. A quercetin (Q) was used as a comparison sample in those measurements.

In the diagram (Fig.1) the total AO content in squeezed juice of berries is resulted. It has appeared that their highest content - in juice of black currant and bilberries, apparently, is related to a considerable quantity of very active phenolic acids and anthocyanins in them^{5,6}. Further there are sea-buckthorn berries, rich in carotin, vitamins C, B1, B2, B6, and flavonoids. Much less phenols have appeared in juice of red grapes. As to binary mixes of berry juice (Fig. 2), some deviation of experimentally measured values of the total AO content from the values calculated proceeding from an additive principle of binary components (from Fig. 1) is observed. For a raspberry-black currant mix, for example, this deviation is in favour of the measured values and has made approximately 15% that exceeds an error of measurements. Apparently, in juice mixture of these berries there are complex chemical transformations leading to the occurrence of other, more active phenolic compounds or complexes increasing the total oxidizability of phenols on the anode of the amperometric detector, i.e. raising "the effective" content of phenols. Probably the increase of mix efficiency is reached also at the expense of regeneration of active phenol radicals by less active phenols or other components of the mix (for example, ascorbic acid). Such processes are known as varieties of chemical synergism⁷. In the majority of berry mixes decrease of the total AO content in comparison with calculated values, especially in the mixes containing sea-buckthorn berries and grapes (20-30%) has been observed. It is probably related to the fact that juice of these berries contains substances which decay in juice mixes. Less effective phenols formation in mixes is not excluded either.







Fig.2. Total AO content (C, mg/l Q) in berry juice mixes: 1 – sea-buckthorn berry+bilberry, 2 – sea-buckthorn berry+raspberry,
3 – sea-buckthorn berry+grape, 4 – sea-buckthorn berry+black currant, 5 – bilberry+raspberry, 6 – bilberry+grape, 7 – bilberry+black currant,
8 – raspberry+grape, 9 – raspberry+black currant, 10 – grape+black currant, 11 – mix of 5 berry juices.
Left columns (a) – measured values, right (b) –values calculated by additivity.

Similar diagrams have been received for fruit juice and their mixes (Fig.3, Fig.4). From all investigated fruit juices a pomegranate juice has the greatest total AO content that, apparently, is related to a considerable quantity of folic acid, ascorbic acid, tannin and many vitamins contained in it. For grapefruit-pomegranate and apple-pomegranate juice mixes the content deviation from additive values on 10-12% in favour of the measured values is observed. Probably here, as well as in berry juice mixes, we have chemical synergism at the expense of a considerable quantity of ascorbic acid in a pomegranate, which is a synergist of many antioxidants. For mixes with orange juice the measured total phenol content is 10-20% less than additive values. Orange juice contains numerous vitamins, flavonoids, 11 amino acids, niacin and other components. Probably, as a result of complex chemical interactions of these substances with components of other juice less effective phenol antioxidants are formed, i.e. the chemical antagonism is observed⁷. In a multimix of 5 fruit juices the measured value of the total AO content dominates (10%).



Fig.3. Total AO content (C, mg/l Q) in juice of fruits: 1 - orange, 2 - grapefruit, 3 - pomegranate, 4 - apple, 5 - peach, 6 - plum.



Fig.4. Total AO content (C, mg/l Q) in fruit juice mixes: 1 – orange+grapefruit, 2 – orange+pomegranate, 3 – orange+apple, 4 – orange+peach, 5 – orange+plum, 6 – grapefruit+pomegranate, 7 – grapefruit+apple, 8 – grapefruit+apple, 9 – pomegranate+apple, 10 – pomegranate+peach, 11 - apple+plum, 12 – mix of 5 fruit juices: orange+grapefruit+pomegranate+apple+a peach. Left columns (a) – measured values, right (b) –values calculated by additivity.

In vegetable juices (Fig.5, Fig.6) the greatest quantity of antioxidants is contained in red pepper juice and beet juice due to the presence in them of considerable quantity of folic acid, ascorbic acid, vitamins of B-group (in pepper). In all tested mixes the additivity of separate pairs of juice or reduction of measured values of the phenol content in comparison with the additive values reach 25-30%, for example, for all mixes containing pepper, including multimix. Probably, this fact denotes antagonism mentioned above. It is not excluded that during juice mixing there is a decay of unstable phenol compounds, containing in squeezed juice, and vitamin C destruction.



Fig.5. Total AO content (C, mg/l Q) in juice of vegetables: 1 - carrot, 2 - beet, 3 - cabbage, 4 - tomato, 5 - red pepper, 6 - eggplant.



Fig.6. Total AO content (C, mg/l Q) in vegetable juice mixes: 1 – carrot+beet, 2 – carrot+cabbage, 3 – carrot+tomato, 4 – carrot+red papper, 5 – beet+cabbage, 6 – beet+tomato, 7 – beet+red papper, 8 – cabbage+tomato, 9 – cabbage+red papper, 10 – tomato+ red papper, 11 – mix of 5 vegetable juices: carrot+beet+ cabbage+ a tomato+red papper.

Left columns (a) - measured values, right (b) -values calculated by additivity.

The research of influence of frosting-defrosting processes of some berry and fruit juice on the AO content in them has been done. Initial juice was at a room temperature during measurements (5-6 hours), then was frozen at the temperature of -12° C for 7 days and then was unfrozen to room temperature. In the diagram (Fig.7) the total AO content results before and after frosting process are presented. Results show that in all juices, except black currant one, the total AO content after frosting-defrosting processes has not practically changed. The AO content has increased by 40% in black currant juice. Probably, during the process of freezing it there are chemical processes leading to increase the total AO content by means of restoration of vitamin C destroyed on air.



Fig.7. Total AO content in juice of berries and fruit: 1 – apple, 2 – grape, 3 – pomegranate, 4 – grapefruit, 5 – black currant. Left columns (a) – before frosting process, right (b) – after frosting-defrosting process.

Influence of unfrozen juice, diluted in 10 times, on efficiency of the received binary mixes was studied either. In the diagram (Fig.8) results of influence of frosting-defrosting and diluting processes on the AO content in juice mixes are presented. Results of the research of binary mixes of specified juices after the frosting-defrosting processes, and mixes of the diluted juice show that both in diluted juice and not diluted one at their mixing the excess of measured values of the total AO content over additive values is not observed. In the majority of the diluted juice mixes reduction of measured values in comparison with additive ones, reaching 25% for apple-black currant and grape-black currant mixes is observed. Besides, additivity (absence of interaction between mix components) or antagonism is observed in the investigated mixes and antagonism is amplified by juice dilution.



Fig.8. Total AO content in juice mixes: 1 – apple+grape, 2 – apple+pomegranate, 3 – apple+grapefruit, 4 – apple+black currant, 5 – grape+pomegranate, 6 – grape+grapefruit, 7 – grape+black currant, 8 – pomegranate+grapefruit, 9 – pomegranate+black currant, 10 – grapefruit+black currant. Left columns (a) – not diluted juice mixes after frosting-defrosting processes, middle (b) – 10 time-diluted juice mixes, right (c) –values calculated under additivity condition

Thus, researches of the total phenol AO content in mixes of berry, fruit and vegetable juice have shown that at juice mixing there are complex chemical transformations in them resulting both in increase and decrease of the total AO content in mixes in comparison with the sum of additive contributions of the AO content in a separate juice. To explain this it is necessary to track time firmness of these products from the point of view of their AO properties.

3.2. Teas and their mixes

The aim of this study was to determine the total AO content in water extracts of some kinds of tea and herbal additives and to compare these values with the AO content of their mixes. Thus, possible influence of mixes components on each other was investigated. A rapid amperometric method also helped to trace the dynamics of change in the AO content of samples immediately after extraction.

Objects of the research were water extracts of three kinds of tea (1 - Chinese green, 2 - grey with bergamot and 3 - Ceylon black), a mint pepper (*Mentha piperita L.*) and dried lemon peels. Extracts of 10 binary mixes of those samples with different ratios of components were also investigated. Dry teas and herbs were ground in a mortar till particles of the size of 1-2 mm. Then herb and tea mixes (0.5 g) were immersed into 50 ml of distilled water with T = 318 K and held for 10 min without thermo stating. Then extracts were carefully filtered through a paper filter "a dark blue tape" and if necessary diluted before measurements.

The dynamics of the total phenol AO content (C, mg GA/g) depending on extract storage time for 5 samples is shown in Figure 9. The most significant decrease of C was observed in extracts of tea (20-25%), especially green, which is apparently due to the decay of unstable phenol compounds (catechins, theaflavins, tearubigins, etc.). For the extract of lemon peels the phenol AO content is much smaller and almost unchanged in the first few minutes after extraction.



Fig. 9. Dynamics of the total AO content C (in a gallic equivalent) depending on extract storage time: 1 - chinese green tea, 2 - grey tea with bergamot, 3 - mint, 4 - ceylon black tea, 5 - lemon peels (increased 5-fold).

The results of measurements of the total phenol AO content (C, mg/g) for tea extracts and additives (Fig. 10) and extracts of their mixtures with different ratios of components (Fig. 11) are shown. The total AO content in the mixes is calculated by the additive contribution of the AO content in components of mixes, taken from Figure 10 in accordance with their rations. The results of C values, shown in Figures 10 and 11, were obtained by 5-6 values on average (Fig.9). The highest AO content is found in a Chinese green tea extract and the least one - an extract of citrus peels. With regard to tea mixes and additives (Fig. 11), measured values of the AO content in the extracts of studied mixes are decreased compared with the additive contribution of mix components. Thus, there is their strong antagonism. It is very significant for the extracts of tea-lemon peel mixes.



Fig.10. Total AO content (C, mg GA/g) in extracts of: 1 - Chinese green tea, 2 - grey tea with bergamot, 3 - Ceylon black tea, 4 - mint, 5 - lemon peels.



Fig. 11. Total AO content (C, mg GA/g)) in extracts of tea and additives mixes: 1 - tea 1 + tea 2 (1:1), 2 - tea 1 + tea 3 (1:1), 3 - tea 1 + mint (4:1), 4 - tea 1 + lemon peels (3:2), 5 - tea 2 + tea 3 (1:1), 6 - tea 2 + mint (4:1), 7 - tea 2 + lemon peels (3:2), 8 - tea 3 + mint (4:1), 9 - tea 3 + lemon peels (3:2), 10 - mint + lemon peels (2:3). Left columns (a) - measured values, right (b) -values calculated by additivity.

Teas contain a large number of different compounds⁸. The main ones are as follows: catechins are powerful AO (in green tea - 15-30% of the dry leaf, in black tea - 9%); theaflavins are responsible for intensity of red colour of tea; tearubigins are compounds giving more intense staining of a brewing tea than theaflavins. Theaflavins are contained in black tea in amount of 3-6%, in green tea these compounds are less, because they are formed from catechins in the fermentation process. Tearubigins are formed in the fermentation process (in black tea - 12-18%) as well. Teas contain caffeine and various amino acids (glutamine, alanine, asparagine, methionine, etc.), the content of which is the criterion of its quality. Tea also contains glucose, fructose, vitamins (C, K, P and B group), phenol acids (primary - gallic acid) and metal complexes. As for mint, it contains an essential oil (2-3%), the main component of which is menthol, tannin, carotene, rutin and other compounds⁹. Lemon peels contain lemon oil (α -limonene, terpenes, citral), citric and ascorbic acid, thiamine, riboflavin and etc⁹. During the extraction of tea and additives mixes, complex biochemical reactions of interactions of mixes components occur in extracts, leading to the formation of less effective compounds. Their antioxidant properties are recorded by amperometry. This is the chemical antagonism⁷. Perhaps, at the extraction of mixes there is a competition of different substances, which leads to less extraction of some of them. Some extracts, for example mix of tea with lemon peels, are always more decolourized in comparison with pure tea extracts. It speaks about possible destruction of tearubigins and theaflavins by some compounds of lemon peels. Probably, these factors are lead to decrease of the phenol AO content in extracts of binary mixes in comparison with additive values of the AO content in the components of mixes.

Thus, for extracts of tea and herbal additive mixes an essential deviation of experimental values of the AO content in binary mixes from values calculated by the additive principle has been found.

3.3. Various alcohol drinks

In this part of the present work comparative research of the total AO content in various alcoholic drinks is described. Objects of the research were more than 100 drinks: dry, semidry, dessert, fortified red and white wines, cognacs, liquors and infusions received from manufactures or their distributors at an exhibition in Moscow in 2009. Gallic acid (GA) was used as a sample for calibration.

Diagrams (Fig. 12 and 13) represent comparative measuring data of the total AO content in some typical samples of dry, semi-dry, semi-sweet wines and champagnes (Fig. 12), and fortified wines and cognacs (Fig. 13). Information on these drinks is provided in Table1.



Fig.12. Total phenol AO content (C, mg/l GA) in samples of dry, semi-dry and semi-sweet wines.



Fig.13. Total phenol AO content (C, mg/l GA) in samples of fortified wines, infusions, liquors and cognacs.

№ of	Company, country	Name, type of wine, wine age	C, mg/l GA
sample			
1	Navarra, Spain	Palacio de OTAZU, red semi-dry, 2001	328.1
10	Batitu Reserv, Chile	Cabernet Sauvignon, dry red, 2007	245.5
14	Batitu Classic, Chile	Merlot, dry red, 2008	206.2
16	Legenda Kryma	Aligote, dry white, 2006	127.3
17	Koktybel, Crimea	Aligote, dry white, 2006	83.8
18	Inkerman, Crimea	Cabernet Kachinske, dry red, 2006	199.9
19	Inkerman, Crimea	Cabernet Coptobe, dry red, 2009	312.5
20	Legenda Kryma	Cabernet, dry red wine, 2009	254.8
21	Legenda Kryma	Muscat Sater, white semi-sweet, 2009	115.1
22	Legenda Kryma	Muscat Sater, semi-sweet red, 2009	153.3
23	Koktybel, the Crimea	Monte Rouge, semi-sweet red, 2008	206.5
25	Koktybel, the Crimea	Cahors, red special, 2008	174.3
27	Parteniyskaya Dolina, the Crimea	Tauris, red port, 2009	99.2
28	Magarach, the Crimea	Chernovoy Magarach, red port, 2005	180.1
29	Krymskoe Shampanskoe, Sevastopol Winery,	Semi-sweet champagne, 2009	155.9
	the Crimea		
30	Sevastopol Winery, the Crimea	Sparkling white muscat, 2009	84.4
31	Artemovskoe Shampanskoe, the Crimea	Brut champagne white, 2009	92.1
32	Novy Svet, the Crimea	Brut champagne, 2005	132.9
34	J. Bouchon, Chile	Sauvignon Blanc, dry white, 2009	60.9

Table 1. Total content of antioxidants in some typical samples of dry, semi-dry, semi-sweet wines, champagnes, fortified wines and cognacs.

37	Krevansky combine Ararat, Armenia	Noah, cognac, 2002	29.2
38	Hardy, France	V.S.O.P., Cognac, 2002	59.6
43	Ganuzh, Azerbaijan	semi-sweet red (coupage)	238.1
44	Baku, Azerbaijan	Cognac, 2001	18.3
45	Moscva, Azerbaijan	Cognac, 1998	26.3
46	Shirvan, Azerbaijan	Cognac, 1995	45.3
47	Apsheron, Azerbaijan	Ordinary cognac, 2005	5.5
48	Port wine Adam, Azerbaijan	Port wine, 2008	63.6
49	Martini Vermouth, Azerbaijan	Vermouth white, 2009	28.7
50	Dar Bogov, Amtel	Ginseng infusion, 2009	5.3
53	Baron Du Rua, France	Blanc Mouellux, white semi-sweet,2009	57.3
54	Vilage Saint Deni, France	Rouge Mouellux, semi-sweet red, 2009	185.8
57	Severnaya Zvezda, St. Petersb.	Cognac, 2006	23.1
58	Severnaya Zvezda, St. Petersb.	Cognac, 2004	33.2
59	Generalissimus, St. Petersburg	Cognac, 2006	19.3
62	Russkaya Loza, Krasnodar	Chardonnay, dry white, 2007	103.3
66	Carmen, Chile	Merlot, dry red, 2007	347.0
70	Saint-Anac, France	Moellux Dor De Alix, white semi-sweet, 2007	86.5
71	Cocoa	Liquor, 2009	7.6
72	Strawberries	Liquor, 2009	75.0
73	Mint	Liquor, 2009	6.2
75	El Toril, Mexico	Tequila, 2009	4.8
77	Vismos, Moldova	White Agate, cognac, 2004	69.7
78	Barton & Guestier, France	Sauvignon Blanc, dry white, 2009	136.0
87	Lambouri, Cyprus	Lambouri, dry white, 2007	102.7
94	Tokaji, Hungary	Furmint, dry white, 2005	137.5
96	Tokaji, Hungary	Furmint, white semi-sweet 2002	185.9
103	Dalvina, Macedonia	Cabernet Sauvignon, dry red, 2008	399.5
106	Skovin, Macedonia	Makedonsko Belo, dry white, 2008	210.1
108	Skovin, Macedonia	Syrah Cabernet, dry red, 2007	427.1
113	Woop Woop, Australia	Shiraz, dry red wine, 2008	321.7
115	Farrese, Italy	Trebyano a'Arbutstso Farnese, dry white, 2008	128.9
116	Isla Negra, Chile	Cabernet Sauvignon Merlot, red semi-dry, 2009	410.4

4. Conclusion

The total content of phenolic antioxidants in juice of berries, fruit and vegetables, in water extracts of tea and herb and in various alcohol drinks were determined by amperometry. Besides, efficiency of the method allowed determining the total antioxidant content in their binary and multimixes. The deviation of experimentally received values of the total antioxidant content in some mixes from the values calculated proceeding from the additivity principle of the antioxidant content in separate juices has been revealed. This method can be applied for quality control of various alcoholic and non-alcoholic drinks.

Dry and semi-dry red wines have the highest values of the total phenol AO content (from 200 to 430 mg/l GA), that is essentially more than dry and semi-dry white wines do (from 50 to 200 mg/l GA). The phenol content of red semi-sweet wines also dominates in comparison with semi-sweet white wines and champagnes. In the work¹⁰ the total content of phenols has been measured in dry red wines by the Folin-Ciocalteau method with gallic acid as a standard. Values of the phenol content occurred in 2-5 times more than in the present work. Apparently, it is possible to explain that besides phenols, other substances presenting in wines, such as restoring sugars, proteins and amino acids can react with a reagent of Folin-Ciocalteau¹¹. The amperometric method excludes it. Smaller values of the total phenol content in red wines, received experimentally, can be explained by total oxidation of these phenols passing through an amperometric cell⁴. Red port wines and Cahors have leading positions among fortified wines (Fig. 13). The level of phenol compounds in cognacs is significantly lower (20 - 70 mg/l GA). At this level the cognac sample Ne47 is sharply behind (5.5 mg/l GA). Similar results for infusion of ginseng (Ne 50), for tequila (Ne 75), cocoa and peppermint liqueurs (Ne 71, Ne 73) were observed. The same minor values of C were recorded for 40% ethanol. Relatively low values of the phenol AO content in these samples may indicate that they are probably falsified.

It is known from numerous studies⁸ of red grape wines, that more than 100 polyphenolic compounds, including flavonoids and polyphenols of non-flavonoid nature were identified in them¹². The main contribution to their AO activity is made by compounds of the following groups: anthocyans, catechins, oligomeric procyanidins, flavonols,

stilbenes, glucosides and etc.⁸. Anthocyans are main pigments of grape and cause a red wine color¹². Catechins and procyaniding whose content is more than 90% of total polyphenols of grape and wine, are the most active AO. Phenolic acids, particularly gallic and caffeic and flavonols: quercetin, rutin, myricetin, reducing blood cholesterol level, have high AO activity as well. The above and other phenol compounds⁸ contribute mainly to their part in high values of the total content of antioxidants and their activity for red wines. Comparative analysis of white and red wines^{14,15} shows a smaller (5-10 times) total polyphenol content in white wines than in red wines. It is associated with the fact, that in the fermentation of grape juice skins and seeds of grapes anthocyans, catechins and other polyphenol compounds are included. During the fermentation of white wine, grape juice is used only. Therefore, much less active AO such as epicatechins, gallic acid, quercetin, rutin, miricitin and proanthocyanidins are found in white wines¹⁴. Inclusion of skins and grape seeds in production of white wines significantly increases their antioxidant activity¹⁵. Quality cognacs contain antioxidants, which are tannin, lignin, oils and enzymes getting to the final product due to the extraction of them by alcohol from wood of oak butts. Cognac alcohol becomes golden color and is filled with woody-vanilla aromas due to the extraction of timber oxi- aromatic acids, the main of which are lilac, ellagic, vanillic, as well as vanillin and lilac aldehyde⁸. Thanks to the presence of tannins in cognacs, they help from gastroenteric disturbunce. Cognac also expands vessels of the brain and raises an organism tone, in case, if it is used only in small doses (30-50 ml) according to its medical properties.

Thus, amperometry allows determining the total AO content in various grades of wines and cognacs quickly and relatively cheaply and identifying low-quality production.

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