POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2004, Vol. 13/54, No 3, pp. 261–268

JUICES AND BEVERAGES WITH A CONTROLLED PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY

Lubomiła Owczarek, Urszula Jasińska, Marzanna Osińska, Sylwia Skąpska

Department of Fruit and Vegetable Product Technology, Institute of Agricultural and Food Biotechnology, Warsaw

Key words: concentrated fruit juices, herbal extracts, sensory acceptability of beverages, fruit-herbal beverages, total phenolics, trolox equivalent antioxidant capacity

The aim of the study was to design sensory-attractive beverages containing a significant but naturally occurring level of total phenolics and with a potentially high antioxidant effect. The concentrated fruit juices (cranberry, raspberry, black currant and others as references) and herbs (tea and dried elderberry fruits) were selected as raw materials. The study was realised in three steps: (1) development of the sensory-accepted herbal extracts and fruit beverages – characterised as phenolic sources, (2) development of the fruit-herbal beverages based on the pattern formulae of the selected fruit products, (3) determination of the total phenolics, antioxidant capacity and sensory properties of newly developed fresh compositions, and after six months of storage.

It was found out that the total phenolics of fruit-tea beverages exceeded significantly those of their fruit counterparts and, except for one, those of the reference red grape juice. The fruit-tea drinks and red grape juice were recognised among all products tested as revealing comparable and the highest antioxidant capacities. The antioxidant capacity of the fruit and fruit-herbal beverages analysed correlated with their total phenolic content ($r=0.85$; $p\leq0.02$), which could be accepted as the basic characteristic of the antioxidant properties of the products declared as functional. Fruit and mixed beverages were categorised in accordance with both of these characteristics. The total phenolics, antioxidant and sensory properties of developed fruit-herbal beverages stored did not undergo substantial changes during six months. So, the products could be proposed as good sources of antioxidants supporting the organism defence system against oxidative stress.

INTRODUCTION

Phenols represent substances which had earlier been perceived as non digestible rest of food but not energy sources, or else as compounds which have a negative impact on the sensory attributes of the processed and stored products of plant origin [Łoś *et al.*, 1996]. Currently phenols are perceived as factors performing a regulatory and protective function in a human body. Dietary flavonoid intake for example, mostly from tea, was found to significantly reduce death from coronary heart disease [Hertog *et al.*, 1993].

Natural phenolic compounds, including tocopherols, flavonoids and phenolic acids, are capable of performing the repair and adaptation functions in a human organism due to the antioxidant activity. This property of phenolics enables the neutralisation of reactive oxygen species (ROS) preventing their emergence in the organism by inhibiting the initiation or propagation phases of the oxidation reaction, which ultimately leads to oxidation changes in lipids, proteins and nucleic acids [Bartnikowska, 1995].

To an increased interest in natural antioxidants nowadays has contributed the fact that the use of synthetic antioxidants with a butylated phenol structure such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) for technological purposes is restricted by law, due to the reported carcinogenicity of the compounds [Branen, 1975].

Fruits and vegetables rich in flavonoids and phenolic acids are the basic source of dietary phenols. The basic flavonoid subgroups of fruits are: (1) anthocyanins (cyanidin and other colourings of the skin, *e.g.* red grape delfinidin, peonidin, malvidin, pelargonidin, petunidine); (2) flavanols (proanthocyanidins and catechins); and (3) flavonols (derivatives of rutin, quercetin, myricetin and kaempferol) [Prior *et al.*, 1998; Kalt *et al.*, 1999; Wawer, 2001; Zielińska *et al.*, 2001]. The phenolic acids in fruits are represented by hydroxylated derivatives of benzoic acid (phenylcarboxylic acids), as well as cinnamic acids (phenylpropene acids) and chlorogenic acid [Nartowska, 2001]. Chlorogenic acid, *p*-coumaroylquinic acid and phloridzine with phloretin xyloglucoside were recognised as the major identifiable antioxidants of apple juice, contributing in 32.0, 6.8 and 11.2 per cent, respectively, to its total antioxidant activity (TAA) measured using the TEAC (Trolox Equivalent Antioxidant Capacity) method. The ascorbic acid share in the antioxidant compound pool (TAA) of juice was stated in these assays as only 1 per cent [Miller *et al.*, 1995]. The phenolic content and antioxidant properties vary depending on

Author's address for correspondence: Lubomiła Owczarek, Department of Fruit and Vegetable Product Technology, Institute of Agricultural and Food Biotechnology, ul. Rakowiecka 36, 02-532 Warszawa, Poland; tel.: (48 22) 606 36 15; fax: (48 22) 849 04 26; e-mail: owczarek@ibprs.pl

the kind of fruit and plant species [Wang & Stretch, 2001]. Generally, small intensely coloured berries, like these of some cultivars of *Vaccinium* species, are rich in anthocyanins highly influencing the total antioxidant capacity of fruits [Prior *et al.*, 1998; Kalt *et al.*, 1999].

Tea (*Camelia sinensis* L.) is also a rich source of phenols that contribute to more than 35 per cent of leaf tea dry weight. Tea leaves similarly to the stems and flowers provide flavonoids and other phenol compounds such as stilbenes, tannins, lignans, lignin, and others [Larson, 1988]. On the average, green tea has a large flavanol content, including mainly epigallocatechingallate; with smaller amounts of epicatechingallate, epigallocatechin and epicatechin. Only green tea includes flavandiols. Black tea, produced by fermentation of green tea, contains significantly less flavanols than green tea. However, both of these products have a comparable content of total flavonols: kaempferol, quercetin and myricetin. Only black tea, having a higher content of gallic acid and theogallin depside than green tea, includes theaflavins and thearubigens that are formed during the fermentation process of leaves as the result of the oxidation and enzymatic polymerisation of catechins [Vinson *et al.*, 1995; Liebert *et al.*, 1999; Nartowska, 2001].

The subject of this study was to design sensory-attractive beverages, with a high, though observable in nature, phenolic content and potentially high antioxidant capacity, using concentrated juices of such fruits as black currant, cranberry and raspberry, as well as green and black tea infusions and elderberry decoction. The study also aimed at the beverage classification according to their antioxidant properties, as well as the control of the retention level of phenolics and antioxidant capacity of products on storage. These data could be used as a basis for a producer's competence to declare antioxidative potential – the functional property of a product.

MATERIAL AND METHODS

Extracts of tea (*Camelia sinensis* L.) were prepared in series: from whole or ground leaves of green tea (Lucky Bird Sunrise®) and whole leaves of black tea (Assam MTF®), using two proportions of tea to boiling water: 1.0 g/100 mL or 1.3 g/100 mL, and dynamic (stirring with a magnetic stirrer) or static (without stirring) conditions of brewing for 15 min. The different combinations of conditions applied to tea extraction have been described in details in Table 1 (I–V).

The decoction of dried elderberry fruits (*Sambucus nigra* L.) Kawon® was prepared by adding 1.0 g of whole fruits into 100 mL of cold water, heating everything to boiling point and simmering for 15 min (VI in Table 1). The decoction and infusions were analysed immediately after being prepared.

Fruit juices and drinks for comparative purposes. The series of sensory-accepted fruit beverages were developed on the basis of concentrated juices of unspecified varieties of fruits: black currant (*Ribes nigrum* L.), cranberry (*Oxycoccus quadripetalus* Gilib.), raspberry (*Rubus idaeus* L.), red grapes (*Vitis vinifera* L.), and apple (*Malus domestica*). For research purposes, the juice concentrates were obtained from Agros-Łowicz and Powiśle production plants. Juices and beverages were composed in accordance with good technological practices. Product preparations, as acidic food items [Codex Alimentarius, 1995], were pasteurised under laboratory conditions in 200-mL glass jars at 94°C for 25 min, as these parameters have been proved [Buckenhüskes, 1988] to ensure the microbial stabilisation and safety of products on storage. Beverages were controlled after pasteurisation. Series of products (Table 1) were admitted as comparative (VII, VIII, X, XI, XIV) or reference materials regarding the levels (the lowest and highest in the beverages currently drunk) of the phenolic content (XVII, XVIII). Commercial apple juices (XIX and XX) were used as the parallel samples of the apple juice obtained (XVII).

Fruit beverages containing extracts of plant materials were prepared (Table 1) on the basis of the pattern formulae of products selected for comparative purposes (VII, X, XI, XIV) by mixing the appropriate concentrated fruit juice(s) with sensory-acceptable tea infusions or elderberry decoction instead of water ingredient. These fruit-herbal compositions (IX, XII, XIII, XV and XVI) were pasteurised and controlled as above.

The total phenolic content in beverages and plant water extracts was determined in accordance with the procedure of Singleton *et al.* [1965] using Folin-Ciocalteu reagent (Sigma, 1:10 diluted), photometrically. The phenolic content in products was expressed as gallic acid equivalents in mg/L, whereas gallic acid monohydrate (Sigma) was used as standard (concentration range 5–50 mg/100 mL).

The antioxidant capacity of the beverages was determined as the product ability to inhibit the process of oxidation of crocin chromogen by the hydroxyl radical whose donor was H_2O_2 in the presence of Cu^{2+} and Fe^{3+} [Hałasińska *et al.*, 2001]. The effect of this phenomenon was quantified using the spectrophotometric method to measure the colour retention of the reaction mixtures, compared with the mixtures of standard concentrations of synthetic antioxidant 6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid (Trolox-Aldrich). Crocin [8,8'-diapocarotenedioic acid bis(6-*O*-D-glucopyranosyl-D-glucopyranosyl) ester] was obtained by the methanol extraction of saffron (Sigma) which was initially purified using diethyl ether. The concentration of chromogen in the methanol extract was calculated from the absorption coefficient of crocin: $\varepsilon = 1.33 \times 10^5$ mol^{-1} cm^{-1} at $\lambda = 443$ nm wavelength [Nicoli *et al.*, 1997; Bressa *et al.*, 1996; Tubaro *et al.*, 1996]. Analyses of the antioxidant capacity of the test samples in the medium of 0.1 mol phosphate buffer (pH 7.0) were carried out at 40°C in 3 mL reaction mixtures composed of: 6.7×10^{-2} mmol Cu^{2+}, 4.5×10^{-2} mmol Fe^{3+}, 0.1% H_2O_2, and 6.67 μmol crocin (added after 15 min of pre-incubation from buffered 10 μmol working crocin solution). The absorbance of the mixtures was measured after 10 min of incubation. The antioxidant capacity of the beverages studied was expressed in Trolox Equivalents (TE), where 1 TE corresponds to the activity of 1 mmol Trolox.

The sensory analysis of products was performed by a panel with controlled sensitivity thresholds and after assessor training in recognising new product attributes. The analyses were first performed by identification and selection of sensory descriptors for products in development and by establishing the non-acceptability standards (examples of products whose sensory profile was perceived as objectionable). Hedonic assessment recommended in the research on food property optimisation was then applied to evaluate the appearance, odour and taste of the beverages developed and the method of unstructured graphic scales with end points [Pokorny, 1991] was used for this purpose. The assessment results were then adequately quantified in points from 1 to 10.

The properties of three lots of each product were analysed in at least three replications. The means and standard deviations, as well as regression equations and correlation values were estimated using Microsoft Excel 1997.

RESULTS AND DISCUSSION

Development of herbal component of beverages

Herbal extract series, differently prepared, have been globally presented in Table 1 (I-VI), as refers to the details of their extraction conditions.

Total phenolic contents of freshly prepared tea infusions and elderberry decoction, presented as the relevant class of bars (I–VI) in Figure 1, differed significantly from each other. Total phenolics of tea series (I–V) testified to the strong dependence of phenolic concentration on the conditions of the extraction process.

While comparing the total phenolics of green tea extracts with black tea extracts, obtained dynamically from whole leaves in the proportion to water of 1 g/100 mL (II and V), it was found out that green Lucky Bird yielded in the higher phenolic content of infusion (1314 mg/L) than the black Assam (939 mg/L). These results were apparently different from those of Liebert *et al.* [1999] who found out the same phenolic content (*ca.* 900 mg/L) in infusions from the whole-leaf green China Gunpowder and black English blend of Assam and India (tea/water proportion of 1.3 g/100 mL) in the final 10th minute of dynamic extraction. Apart from the differences in both studies (tea brands, tea/water proportion), their results would be probably more convergent after extraction prolongation by the cited authors up to 15 min, as they forecast a raising trend of their

TABLE 1. Preparation of experimental beverages

Basic materials	Extraction or preparation method	Symbol
HERBAL EXTRACTS		
Extraction conditions: static or dynamic brewing or simmering – disintegration degree of raw material - raw material/water proportion.		
Green leaf tea Lucky Bird Sunrise®	Static – whole leaves – 1.0 g/100 mL	I
	Dynamic – whole leaves – 1.0 g/100 mL	II
	Dynamic – whole leaves – 1.3 g/100 mL	III
	Static – ground leaves – 1.3 g/100 mL	IV
Black leaf tea Assam MTF®	Dynamic – whole leaves – 1.0 g/100 mL	V
Dried elderberry fruits, Kawon®	Static simmering – whole fruits – 1.0 g/100 mL	VI
CRANBERRY BEVERAGES		
Concentrated cranberry juice	Sugar addition and dilution with water to 11.5% d.m.	VII (P)
Concentrated cranberry juice	Sugar addition and dilution with water to 10.0% d.m.	VIII
1. Concentrated cranberry juice 2. *Green tea infusion*	Fruit-herbal beverage – (VII) diluted with tea infusion (I)	IX
BLACK CURRANT AND BLACK CURRANT/CRANBERRY BEVERAGES		
Concentrated black currant juice	Sugar addition and dilution with water to 10.2% d.m.	X (P)
1. Concentrated black currant juice 2. Concentrated cranberry juice	Sugar addition to the mixed black currant and cranberry concentrated juices (d.m. proportion 2:1) and dilution with water to 11.5% d.m.	XI (P)
1. Concentrated black currant juice 2. Concentrated cranberry juice 3. *Elderberry decoction*	Fruit-herbal beverage (XI)– diluted with elderberry decoction (VI)	XII
1. Concentrated black currant juice 2. Concentrated cranberry juice 3. *Green tea infusion*	Fruit-herbal beverage (XI) – dilution with green tea infusion (II)	XIII
RASPBERRY BEVERAGES		
Concentrated raspberry juice	Sugar addition and dilution with water to 10.4% d.m.	XIV(P)
1. Concentrated raspberry juice 2. *Green tea infusion*	Fruit-herbal beverage (XIV) – dilution with green tea infusion (I)	XV
1. Concentrated raspberry juice 2. *Black tea infusion*	Fruit-herbal beverage (XIV) – dilution with black tea infusion (V)	XVI
REFERENCE FRUIT JUICES		
Concentrated apple juice	Dilution with water to 10.4% d.m.	XVII
Concentrated red grape juice	Citric acid addition and dilution with water to 11.5% d.m.	XVIII
COMMERCIAL APPLE JUICES		
Apple juice 1	Used *per se*	XIX
Apple juice 2	Used *per se*	XX

(P) – formula pattern (fruit beverage)

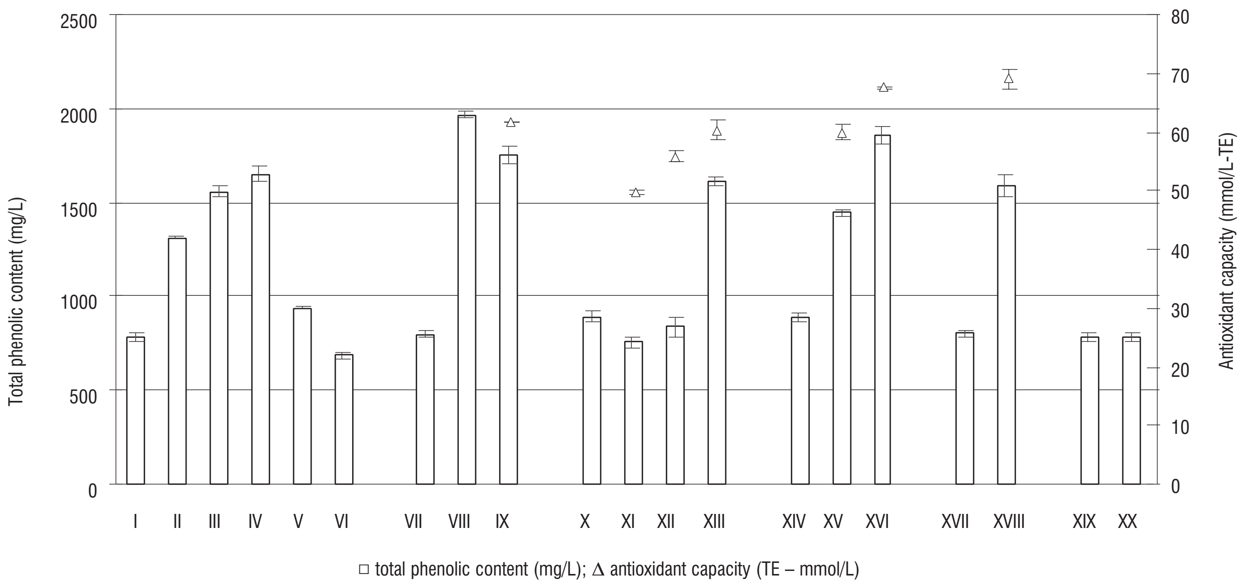

FIGURE 1. Total phenolic content and antioxidant capacity of the freshly-prepared products. I–XX – symbols of preparations (see Table 1).

phenolic extraction curve in the last minutes of green tea brewing.

The stated phenolic content in the infusions of the whole-leave green tea I and II, *prepared to compare the effects of the static extraction conditions with the dynamic one,* was: 783 mg/L (I) and 1314 mg/L (II), respectively. Taking into account the tea/water proportion for extraction providing both infusions, the values 783 mg/10 g (I) and 1314 mg/10 g (II) have consequently presented the yields of phenolics from tea material within the static and dynamic extraction conditions (Figure 2). Within the dynamic extraction *conditions two proportions of whole-leaf green tea to water (1.0 g/100 mL and 1.3 g/100 mL*) yielded in the following phenolic concentration of infusions: 1314 mg/L (II) and 1559 mg/L (III), depending on the proportion used. The yields of phenolics from tea material in these cases, 1314 mg/10 g (II) and 1199 mg/10 g (III), should be considered. To determine the effect of leaf disintegration on the phenolic concentration of tea extracts the conditions of the static extraction of ground green tea leaves in tea/water proportion of 1.3 g/100 mL (IV) were controlled. The total phenolic content of the infusion obtained was declared as 1651 mg/L but the yield of phenolics from the disintegrated tea leaves – as 1270 mg/10 g (Figure 2).

As it was demonstrated, the gradual increase of the total phenolic content in green tea infusions I–IV (Figure 1), following the changes in their production conditions (Table 1), was not reflected to the same extent in the succeeding raise of phenolic yields from the tea material (I–IV Figure 2). This fact enabled concluding that only extraction conditions leading to obtaining infusion II (dynamic extraction of whole leaves within the lower tea/water proportion) could be admitted as the cost effective method of tea extraction intensification, as compared with the static extraction providing infusion I (Table 1). Considering the inhibited ratio of phenolic extraction from tea when the higher proportion of tea to water was used (III, IV), an oncoming exhaustion of tea material could be assumed. It was also supposed by others [Liebert *et al.*, 1999] who studied the time-dependent effect of some factors on phenolic content in the whole-leaf extracts of tea. Further studies would be needed to elucidate conditions of exhaustion of phenolic extraction from tea.

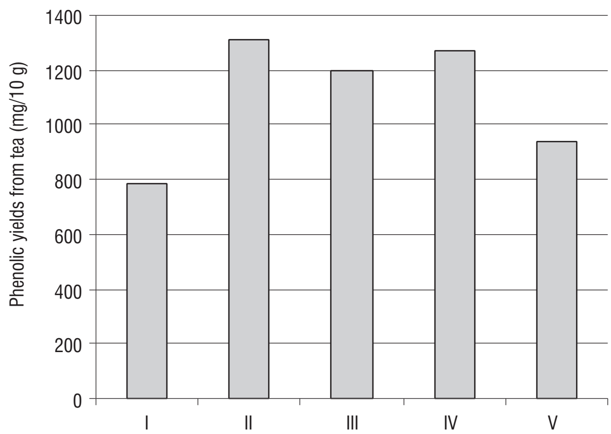

FIGURE 2. Comparison of the yields of total phenolics from tea materials depending on the extraction Conditions. I–V – symbols of preparations (see Table 1).

Since an achievement of conditions of extraction exhaustion was not the first goal of the study – the extraction experiments were rather focused on searching the process parameters and phenolic levels in extracts that could assure good sensory perception of products. Thus, the sensory profile of freshly prepared infusions and decoction (I–VI) was assessed using the descriptor method. The turbidity as an attribute of appearance and the distinct notes of bitterness or astringency of extracts were especially sought as having potentially the most profound negative impact on the desirability of the planned fruit-herbal products. The descriptors of attributes considered for infusions and decoction were agreed in the assessor discussion and established as a panel verdict (Table 2).

From the data presented in Table 2 it appears that green tea extracts I–II (phenolic contents up to 1314 mg/L, as presented in Figure 1) could be sensory accepted. The perception of infusion series III–IV, described as too turbid and strongly bitter, was deemed to be an effect of their too high total phenolic content (1559 and 1651 mg/L, respectively), potentially transgressing the phenolic concentration limit separating the sensory acceptability from unacceptability of

TABLE 2. Descriptive assessment of principal properties of the herbal extract sensory profile.

Symbol	Appearance: lucidity	Taste: bitterness
I	Clear	Medium
II	Opalescent	Medium
III	Turbid; sediment	Very strong
IV	Opaque	Very strong
V	Clear	None
VI	Opalescent	None

Symbols – see Table 1.

tea. Thus, after eliminating the infusions not meeting the sensory expectations, only extracts I and II of green tea, as well as the parameters of the extraction process providing these infusions were ultimately selected for further assays.

Fruit patterns of the planned products

Fruit beverages developed for comparative purposes (Table 1), accepted as the formula patterns for the planned fruit-herbal drinks (VII, X, XI, XIV) or reference products (XVII–XX), all meeting the standard and sensory requirements, differed as to their total phenolic contents (Figure 1). Those differences ranged from 757 mg/L (black currant--cranberry beverage XI) to 892 mg/L (raspberry beverage XIV) within the first series of the beverages. Total phenolic content in the apple juices (XVII, XIX-XX) ranged from 778 to 804 mg/L and in the reference red grape juice (XVII) it was established as 1587 mg/L. The results presented allowed us to determine the range of phenolic concentrations of the fruit beverages commonly consumed. This range has been restrained by the limit values representing the total phenolics of apple juice, on the one hand, and the average concentration of phenolics in red grape juice, on the other hand.

Since the impact of preparation conditions on the fruit beverage properties is highly complex, the discussion on the total phenolics of fruit beverages developed should be replaced by the following explanations. On an industrial scale, juices with properties defined in the standards are regenerated by an appropriate addition of water to semi-finished products, *e.g.* concentrated fruit juices selected as a component of the planned new beverages. Regeneration of juices from concentrated juices of fruits being reach or, on the contrary, poor in the total acids – such as currants or, on the contrary, grapes – requires sensory modifications in the form of addition of sugar or contrary – any of the acids permissible in food preparation. These additives are admitted within the limits of standardisation. The cranberry juice VIII, regenerated here especially for the demonstrative purposes from the highly acid cranberry concentrate (total acidity, as citric acid, 65.25 %), in spite of the permitted sugar addition, could not be assessed as drinkable beverage for it was too acidic. The preparation of the sensory-accepted cranberry beverage (VII) required the use of additional water apart from the higher sugar supplementation.

Irrespective of the study's first goal, defined as *designing beverages being a good source of natural antioxidants*, sensory acceptability – deemed to be the ultimate criterion where food is concerned – was adopted as the first criterion for assessing the usefulness of the fruit beverage formula patterns for assays. Therefore, versions of fruit juices or beverages developed but not fully harmonised organoleptically, were rejected from the assay irrespective of their total phenolic content. An example may be the cranberry juice VIII that was not balanced within the notes of sweetness and acidity being at the same time the best source of total phenolics (1964 mg/L) – better as its palatable counterpart VII (799 mg/L) – both shown in Figure 1. The sensory assessments of the two cranberry drinks should explain the role of all sensory evaluations in the course of food product designing or optimisation. Besides, the presented example could elucidate the possible technological reasons of low total phenolic contents of drinkable fruit products, even though they are to be based on good sources of phenolics like cranberry fruits [Kähkönen *et al.*, 1999; Prior *et al.*, 1999; Wang & Stretch, 2001].

The antioxidant activity of comparative beverages, established as 49.8 mmol/L (TE) for black currant-cranberry (XI) and 69.0 mmol/L (TE) for red grape juice (XVIII) were admitted in the study as the limits of the scale encompassing the average antioxidative potential of freshly-produced, common fruit drinks (Figure 1).

Fruit-herbal beverages

The criteria of the sensory acceptance for the developed fruit-herbal products included the best possible lucidity of beverages, good perceived odour and the taste not marked with the distinct notes of bitterness. Fruit-herbal drinks, selected as sensory accepted from the versions obtained on the basis of the formula patterns of fruit products (VII, X, XI, XIV) using extracts of green tea (I, II), black tea (V) and elderberry decoction (VI), have been restrained to five beverages (IX, XII–XIII, XV–XVI in Table 1). The results of quantified hedonic evaluation of appearance and flavour attributes of the freshly-prepared, selected fruit-herbal drinks have been presented in the range "F" of Table 3.

TABLE 3. Sensory characteristics of the stored beverages.

Symbol	Assessment	Appearance	Odour	Taste
IX	F	7.6 ± 1.01	6.9 ± 0.88	7.9 ± 1.00
	S	4.9 ±0.91	6.5 ± 0.71	5.5 ± 0.93
XI	F	8.8 ±1.04	8.3 ± 1.04	9.0 ± 0.74
	S	7.7 ±1.75	8.3 ± 0.82	7.3 ± 1.08
XII	F	8.2 ± 1.02	7.8 ± 0.73	8.8 ± 0.55
	S	6.8 ± 0.96	7.8 ± 1.01	7.8 ± 0.76
XIII	F	7.6 ± 0.66	6.3 ± 0.78	7.8 ± 0.89
	S	6.3 ± 0.54	6.7 ± 0.68	6.8 ± 0.71
XV	F	8.6 ± 0.85	7.4 ± 0.76	8.5 ± 0.65
	S	6.0 ± 0.45	7.5 ± 0.44	7.3 ± 0.51
XVI	F	6.5 ± 0.74	7.8 ± 1.05	7.2 ± 0.91
	S	4.5 ± 0.72	9.0 ± 0.59	8.8 ± 1.12
XVIII	F	8.0 ± 1.00	7.9 ± 0.95	8.5 ± 0.95
	S	6.1 ± 0.85	9.5 ± 0.91	9.1 ± 1.02

Symbols – see Table 1; F – as freshly prepared; S – after six months of storage.

Fruit-herbal beverages developed and two comparative fruit products (XI, XVIII) were stored for 6 months in a dark store at 18–20°C. Their pH value, total phenolics, antioxidant capacity and sensory properties were controlled at the beginning and at the very end of the storage period. All of the beverages studied have represented highly acidic pasteurised food items of the initial pH ranging from 2.84 to

3.46, *i.e.* preventing the growth of the most species of bacteria and filamentous fungi [Adams & Moss, 2001]. The final pH test showed that the beverages kept almost the initial pH value during six months, for at the end of the storage period an inessential and straight drop of pH, ranging from 0.10 to 0.20 (±0.028), was stated. The sufficiently-stable pH level of beverages proved their microbial stability on storage.

Products presented as having an antioxidative potential require the properties determining their functionality to be proved. To this end, at first the total phenolic content of freshly prepared fruit-herbal beverages was measured. The values presented in Figure 1 have confirmed the significant increase in phenolics of the fruit-tea beverages developed, as compared with their fruit counterparts. The observed increases were: 551 mg/L (raspberry beverages XIV and XV), 855 mg/L (black currant-cranberry beverages XI and XIII), 949 mg/L (cranberry beverages VII and IX), and 965 mg/L (raspberry beverages XIV and XVI). No increase in the total phenolic content was observed in the case of fruit-herbal beverage (XII) formulated on the basis of black currant – cranberry pattern (XI) using the elderberry decoction (VI) instead of water. This result, unexpected for us, should be admitted as a consequence of unknown interference within the composition of beverage XII as the phenolic content of its individual components was reliably measured using Folin-Ciocalteu procedure. In this study, gallic acid was chosen as the standard because in the Folin--Ciocalteu method this substance responded as highly as catechin [Singleton, 1974; cited after Heinonen *et al.*, 1998]; both occurring profusely in the total phenolics of fruits (*i.e.* grapes) and tea. Further studies using efficient techniques would be needed to research the antioxidant potential of such valuable fruit compositions like beverage XII containing mixed berry ingredients.

The series of our results enabled us to classify the developed fruit and fruit-herbal beverages in terms of the total phenolic content. These beverages can be divided into two groups: (1) fruit juices and beverages (VII, X–XI, XIV, XVII) and fruit-herbal beverage with elderberry decoction (XII) containing total phenolics below 1000 mg/L, (2) fruit--tea products (IX, XIII, XV–XVI) and red grape juice containing more phenolics than 1000 mg/L (1443–1857 mg/L).

The initial antioxidant capacity of fruit-herbal beverages, presented in Figure 1, varied from the lowest 55.8 mmol/L (TE) for the black currant-cranberry product with elderberry decoction (XII) to 67.5 mmol/L (TE) for the raspberry beverage with black tea (XVI). Those values belonged to the range of 49.8–69.0 mmol/L (TE) which was delimited by the antioxidant capacity of fruit products for comparative purposes – black currant-cranberry beverage (XI) and red grape juice (XVIII), respectively. The antioxidative potentials of freshly-prepared beverages correlated with their adequate total phenolic contents ($r=0.85$; $p\leq0.02$). The calculated regression equation in which antioxidant capacity (mmol/L-TE) was used as the dependent variable (Y) and the total phenolic content was used as the independent variable (X) was:

$Y = 0.013X+42.5$

[X = total phenolic content (mg/L)]; $n=5$.

From Figure 1 it can be seen that the allied pairs of results related to the antioxidant properties of the two products based exclusively on fruit materials (XI–XII) including raw material from black currant – known as one of the best sources of vitamin C – exerted the strongest negative influence on the correlation studied. It could be explained as the potential ascorbic acid – and not phenolics – share in the antioxidant capacity of these fruit beverages, as it was experienced by Miller *et al.* [1995] who studied vitamin C contribution to the total antioxidant capacity of apple drinks fortified with ascorbic acid.

In food production special attention should be paid to the product shelf life and quality retention by products, especially the functional ones, on storage. The results of comparison of the initial and final antioxidant properties of the developed beverages, in terms of per cent retention of the total phenolic content and antioxidant capacity, have been presented in Figure 3. The fruit-herbal (IX, XII–XIII, XV–XVI) and comparative (XI, XVII) beverages after six months' storage retained about 75–98% of their initial antioxidant capacity and 96–100% of the total phenolics. It was observed that the antioxidant potential of red grape juice (XVIII) decreased on storage at the slowest rate (97.5% retention at the storage end). The higher decrease of the antioxidant activity (75.3–77.8%) was stated in the case of two beverages containing no tea (XI–XII) and revealing initially, as it was mentioned above, the antioxidative potential not proportionally high to their total phenolic content. The hypothesis on vitamin C contribution to the initial antioxidant capacity of those beverages could help to explain its decrease on storage because of the possible ascorbic acid decay after six months' keeping of products at 18–20°C. Fruit-tea beverages developed revealed better retention of their initial antioxidant capacity (81.6–88.9%) on storage; the best retention values, 88.0% and 88.9%, were stated for product XVI being the composition of raspberry juice with black tea infusion (V) and for product XIII containing black currant, cranberry and green tea (II) ingredients. It could be suggested that vitamin C of product XIII, composed according to the same fruit formula pattern (XI) as product XII, was better protected against destruction by green tea phenols than that in product XII by elderberry phenolics. At the very end of product storage the calculated regression equation for antioxidant capacity (TE – mmol/L) (Y) and total phenolic content (X) was:

$Y = 0.018X+26.98$

[X = total phenolic content (mg/L)];

$r=0.80$; $p\leq0.02$; $n=5$.

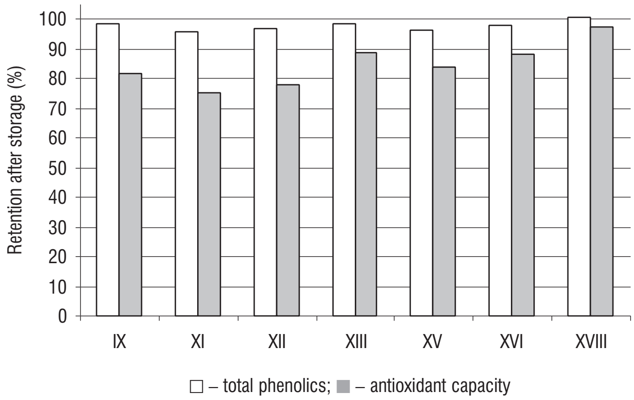

FIGURE 3. Per cent retention of the total phenolics and antioxidant capacity of fruit-herbal and comparative fruit beverages after six months of storage. Symbols of beverages – see Table 1.

The results of the study showed that the new beverages were developed on the basis of raw materials whose antioxidant properties were moderately powerful and stable. Contrary to Wang and Stretch [2001] who showed that the total phenolics and antioxidant activity of stored cranberry fruits were raising during three months in proportion to the increase of storage temperature (0°C-5-10-15°C), we found out that the total phenolics of our products stored at 18–20°C were stable and their antioxidant capacity decreased after six months to 77.8–88.9%. Generally, antioxidant properties of the concentrated fruit juices and herbs selected for assay could be well stabilised after obtaining the semi-finished product and the probable increase of their antioxidative potential during the first thermal processes applied [Gazzani *et al.*, 1998].

The beverages being under control because of their increased level of total phenolics deserved our special attention as to their sensory quality. After six months' observations of the developed products we found out that all of them maintained the acceptable sensory properties on storage. The quantified results of hedonic assessment of the main sensory attributes of beverages freshly prepared ("F") and after six months of storage ("S") have been presented in Table 3. The appearance of fresh beverages, predominantly characterised by red colour and clearness or slight opalescence, was evaluated within the range of 6.5 points (XVI – raspberry concentrated juice diluted with black tea infusion) to 8.8 points (XI – mixed black currant and cranberry juice diluted with elderberry decoction). After six month's storage the appearance of beverages changed and the lowest notes 4.5 and 4.9 were given to the products (XVI) and (IX), respectively, both revealing the highest concentration of phenolics. The odour of all beverages was evenly well perceived and evaluated at both test dates (6.3–9.5 points). The taste of beverages presented the most appreciated attribute of products – from the moment after preparation till the end of the storage experiment.

CONCLUSIONS

1. Developed, sensory-attractive fruit-herbal beverages revealed high, stable during six months' storage, total phenolic content and/or antioxidant capacity levels, exceeding those of their fruit counterparts and herbal components, as well as comparable with those of the reference red grape juice.

2. The factors strongly determining the highest phenolic contents and antioxidant properties of newly-developed fruit-herbal beverages were, apart from the total phenolic levels in semi-finished products: the appearance and palatability of plant extracts and their compositions with concentrated fruit juices.

3. The ranking of beverages obtained from plant extracts and/or concentrated fruit juices which are prepared taking into consideration their phenolic content, allows us to make suggestions as to the categorisation of the products, including fruit beverages on the market, as sources of phenols (up to 1000 mg/L) and good sources of phenols (1000–1600 mg/L). The fruit-tea drinks developed can be classified as good sources of total phenolics.

4. In respect of the antioxidant capacity – expressed as Trolox mmol/L (TE) – of juices and beverages containing concentrated fruit juices, red grape juice (69.0 mmol/L), and fruit-tea beverages developed (60.0–67.5 mmol/L), they can be deemed to have a high level of antioxidant capacity.

5. The determined correlation of the value of antioxidant capacity and the total phenolic content in the analysed beverages, enables simple measurements such as total phenolic content to be generally applied in order to prove the actual antioxidant properties of these products.

REFERENCES

1. Adams M.R., Moss M.O., Food Microbiology. 2000, Second Edition. The Royal Society of Chemistry, U.K., pp. 25–29.
2. Bartnikowska E., Health benefits of dietary antioxidants. Pol. J. Food Nutr. Sci., 1995, 4/45, 4, 3–22.
3. Branen A.L., Toxicology and biochemistry of butylated hydroxy anisole and butylated hydroxy toluene. J. Am. Oil Chem. Soc., 1988, 52, 59–63.
4. Bressa F., Tesson N., Dalla Rosa M., Sensidoni A., Tubaro F., Antioxidant effect of Maillard reaction products: application to a butter cookie of a competition kinetics analysis. J. Agric. Food Chem., 1996, 44, 692–695.
5. Buckenhüskes H., Gierschner K., Hammes W.P., Theorie und Praxis der Pasteurisation. Optimierung des Pasteurisationseffektes bei Sauergemüse. Ind. Obst. Gemüseverwert, 1988, 73, 315–322.
6. Codex Alimentarius, Volume I B. General requirements (Food Hygiene). Recommended International Code of Hygienic Practice. CAC/RCP 23 1979, Rev. 2 (1993), Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, Rome 1995.
7. Gazzani G., Papetti A., Massolini G., Daglia M., Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. J. Agric. Food Chem., 1998, 46, 4118–4122.
8. Hałasińska A., Trzcińska M., Sieliwanowicz B., Polyphenol oxidase from *Gliocladium virens*. Pol. J. Food Nutr. Sci., 2001, 10/51, 1, 25–30.
9. Heinonen I.M., Lehtonen P.J., Hopia A.I., Antioxidant activity of berry and fruit wines and liquors. J. Agric. Food Chem., 1998, 46, 25–31.
10. Hertog M.G.L., Feskens F.J.M., Hollman P.C.H., Katan M.B., Kromhout D., Dietary antioxidant flavonoids and the risk of coronary heart disease: the Zupthen elderly study. Lancet, 1993, 342, 1007–1011.
11. Kalt W., Forney C.F., Martin A., Prior R.L., Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. J. Agric. Food Chem., 1999, 47, 4638–4644.
12. Kähkönen M.P., Hopia A.I., Vuorella H.J., Raucha J.-P., Pihlaja K., Kujala T.S., Heinonen M., Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 1999, 47, 3954–3962.
13. Larson R.A., The antioxidants of higher plants. Phytochem., 1988, 27, 969–978.
14. Liebert M., Licht U., Böhm V., Bitsh R., Antioxidant properties and total phenolics content of green and black tea under different brewing conditions. Z. Lebensm. Unters. Forsch. A, 1999, 208, 217–220.
15. Łoś J., Wilska-Jeszke J., Pawlak M., Enzymatic oxidation of polyphenols in fruit products and model solutions. Pol. J. Food Nutr. Sci., 1996, 5/46, 1, 83–93.

16. Miller N.J., Diplock A.T., Rice-Evans C.A., Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. J. Agric. Food Chem., 1995, 43, 1794–1801.
17. Nartowska J., Natural compounds with antioxidant properties. Farm. Pol., 2001, 57, 741–745 (in Polish; English abstract).
18. Nicoli M.C., Anese M., Manzocco L., Lerici C.R., Properties of coffee brews in relation the roasting degree. Lebensm.-Wiss. Technol., 1997, 30, 292–297.
19. Pokorny J., Sensory problems related to product optimisation. 1991, *in*: Materials of the Conference on "Sensory Analysis of Food – Methods and Applications". Polish Society of Food Technologists and Centre for Agrotechnology and Veterinary Sciences PAS in Olsztyn, Warszawa, pp. 38–40.
20. Prior R., Cao G., Martin A., Sofic E., McEwen J., O'Brien C., Lischner N., Ehlenfeldt M., Kalt W., Krewer G., Mainland C.M., Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J. Agric. Food Chem. 1998, 46, 2686–2693.
21. Singleton V.L., Rossi J.A., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 1965, 16, 144–158.
22. Tubaro F., Micossi E., Ursini F., The antioxidant capacity of complex mixtures by kinetic analysis of crocin bleaching inhibition. J. Am. Oil Chem. Soc., 1996, 73, 2, 173–179.
23. Vinson J.A., Dabbagh Y.A., Mamdouch M.S., Jang J., Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. J. Agric. Food Chem., 1995, 43, 2800–2802.
24. Wang S.Y., Stretch A.W., Antioxidant capacity in cranberry is influenced by cultivar and storage temperature. J. Agric. Food Chem., 2001, 49, 969–974.
25. Wawer I., Anthocyanidins – structure and antioxidant activity. Farm. Pol., 2001, 57, 728–731 (in Polish; English abstract).
26. Zielińska A., Rodowski D., Wawer I., Cranberry – composition, antioxidant and therapeutic properties. Farm. Pol., 2001, 57, 731–733 (in Polish; English abstract).

Received January 2003. Revision received August 2003 and accepted April 2004.

SOKI I NAPOJE O KONTROLOWANEJ ZAWARTOŚCI FENOLI I POJEMNOŚCI PRZECIWUTLENIAJĄCEJ

Lubomiła Owczarek, Urszula Jasińska, Marzanna Osińska, Sylwia Skąpska

Zakład Technologii Przetworów Owocowych i Warzywnych,
Instytut Biotechnologii Przemysłu Rolno-Spożywczego, Warszawa

Cel badań stanowiło opracowanie atrakcyjnych sensorycznie napojów zawierających znaczące, choć występujące naturalnie poziomy fenoli ogółem, o potencjalnie wysokim działaniu przeciwutleniającym. Stosowane surowce obejmowały zagęszczone soki owocowe (żurawinowy, malinowy, z czarnych porzeczek oraz inne jako materiał odniesienia) i zioła (herbata, susz owoców bzu czarnego). Badania prowadzono etapami: (1) opracowanie metod otrzymywania akceptowanych sensorycznie wyciągów ziołowych i napojów owocowych, charakteryzowanych jako źródła fenoli, (2) opracowanie metod otrzymywania napojów owocowo-ziołowych opartych na recepturach wybranych produktów owocowych, (3) oznaczanie fenoli ogółem, pojemności przeciwutleniającej i właściwości sensorycznych nowoopracowanych kompozycji napojów świeżych i po sześciu miesiącach przechowywania.

Stwierdzono, że stężenie fenoli ogółem w napojach owocowo-ziołowych istotnie przewyższało poziomy tych związków w owocowych odpowiednikach produktów, i z wyjątkiem jednego napoju, również poziom fenoli w winogronowym soku odniesienia (rys. 1, tab. 1). Wśród ogółu badanych produktów napoje owocowo-ziołowe i sok winogronowy rozpoznane zostały jako przejawiające porównywalne i najwyższe wartości pojemności przeciwutleniającej (rys. 1, tab. 1). Pojemność przeciwutleniająca analizowanych napojów owocowych i owocowo-ziołowych była skorelowana z zawartością fenoli ogółem ($r=0.85$; $p\leq0.02$), którą można było uznać za podstawową właściwość tej kategorii produktów o deklarowanych cechach funkcjonalnych. Napoje owocowe i owocowo-ziołowe zostały sklasyfikowane według wartości obu tych cech. Zawartość fenoli ogółem oraz właściwości przeciwutleniające (rys. 3, tab. 1) i sensoryczne (tab. 3 i 1) przechowywanych napojów nie ulegały poważnym zmianom w okresie sześciu miesięcy. Wynika stąd, że opracowane produkty mogłyby być proponowane jako dobre źródła przeciwutleniaczy, wspomagające system obrony organizmu przed stresem tlenowym.