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THE USE OF FRUIT EXTRACTS FOR PRODUCTION OF BEVERAGES WITH HIGH ANTIOXIDATIVE ACTIVITY

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ABSTRACT

Free radicals and reactive oxygen species can cause many diseases of the circulatory and nervous system as well as tumors. There are many ways of preventing and treating these diseases including the consumption of products that contain significant amounts of antioxidant compounds, such as polyphenols and antioxidative vitamins. However, currently food stores offer mainly convenient food, ready-to-eat foodstuffs or highly processed products. During numerous technological treatments they have been deprived of many valuable compounds occurring in fresh products. Therefore, an important element of the food production technology is to ensure a proper composition of valuable human health-promoting compounds, mostly vitamins, minerals and polyphenols in final food product. Consumers often and willingly drink beverages. They are also a good starting base for supplementation. Drinks can be enriched with polyphenols, which may reduce the risk of lifestyle diseases, owing to their antiradical potential. The aim of this study was to use the fruit extracts for beverages enrichment in order to increase their antioxidative potential and polyphenol content. For the experiment the fruits of Cornelian cherry, lingonberry, elderberry, hawthorn and Japanese quince were used. Fruit was extracted with 80% ethanol, and then thickened by distillation under reduced pressure. Extracts were used to enrich the apple, orange and grapefruit beverages. Antioxidative activity and total polyphenols content in final beverages were determined. Also, sensory analysis was carried out. The fortification of tested beverages resulted in an increased antioxidative activity and total polyphenol content in case of all applied fruit extracts. Among the beverages composed, the best antioxidative properties were found in a beverage of red grapefruit, whereas the best organoleptically evaluated was the orange beverage. The scores of on the sensory evaluation revealed that the addition of extracts from Japanese quince fruit (in the case of apple and orange beverages) and lingonberry extract (grapefruit beverage) were preferred than the other samples.

Keywords: beverage; enrichment; antioxidative potential; polyphenol compound

INTRODUCTION

Current lifestyles and unhealthy eating habits contribute to the development of many diseases, known as lifestyle diseases, including heart disease, cardiovascular and nervous systems diseases and tumors. One of the main reason of mentioned diseases is the too high concentration of free radicals and reactive oxygen species. One of the method of treatment is introducing of properly balanced diet, which should be rich in compounds with antiradical potential, such as polyphenols **(Robards et al., 1999; Knekt et al., 2002)**. Exogenous antioxidants include, among others, vitamin E and C, carotenoids, and polyphenols. The antioxidant properties of polyphenolic compounds result, inter alia, from their ability to metal chelating, quenching of free radicals and preventing the Fenton and Haber-Weiss reactions, preventing for example a lipid peroxidation. They also react with intermediate products, generated in the course of peroxidation, leading to the termination of free radical reaction **(Manach et. al., 2004)**. Antioxidative properties have mainly phenolic acids and flavonoids. These abilities largely depend on the structure of the molecule, mainly on the amount and localization of hydroxyl groups **(Gawlik-Dziki, 2004)**. The antioxidative activity increases with increasing number of hydroxyl groups in the molecule. Moreover, antioxidative potential of molecules is higher when -OH groups are attached in position ortho- or para- than in meta-. It has been also demonstrated that glycosylation of flavonoids reduces their antioxidative activity comparing with the corresponding aglycones. Flavonoids exert also protective impact on vitamin C and E, and may decrease the activity of enzymes that are involved in peroxidation of membrane lipid. Antioxidative activity of phenolic acids could be increased by attaching additional methoxy groups to the ring **(Heimet et. al., 2002)**. Very good source of phenolic compounds in our diet are fruits and vegetables.

Fruit beverages, juices and nectars are one of the most commonly consumed groups of food products. They increasingly attract attention with new flavor compositions and interesting, tempting packaging. Physicians and nutritionists encourage people to make the consumption of vegetables and fruits more frequent, including tasty and readily available juices, which are an excellent source of vitamins and polyphenols in the human diet. However, not every fruit drink or juice has a positive effect on health. The final beverages often irretrievably had lost their health properties, mainly due to the technology used by the manufacturer or because of low quality of raw materials

(Oszmiański et al., 2007; Nosecka, 2008; Sokół-Łętkowska and Kucharska, 2008). The most valuable health benefits have cloudy fruit and vegetable juices. They contain significant amounts of polyphenolic compounds, vitamins, minerals and fiber. However, the majority of the commercially available juices are clarified juices. Unfortunately, as a result of the clarification many valuable components are lost, which is detrimental to consumers. When clarified juice is produced, the compounds with the highest antioxidative activity that originates from fruits are largely lost. Low molecular weight polyphenols are oxidized, while the high molecular polyphenols are removed during the clarification. However, the clarified juice can be enriched only in sugars and acids, and there is no possibility to add polyphenolic compounds in any form. On the other side, beverages are the best group of products to use with functional additives, which in other products could not be found **(Oszmiański and Wojdyło, 2006; Sokół-Łętkowska and Kucharska, 2008)**.

Food fortification has been defined as the addition of one or more essential nutrients to a food, whether or not it is normally present in that food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population group **(FAO/WHO, 1994)**. Food fortification is one of the possibilities of enrichment food products with ingredients that have been removed or inactivated during the production process. In addition, producers may provide new properties for foodstuff through addition of the ingredients, which are not naturally occurring in the product **(Gębczyński and Jaworska, 2009)**.

The aim of the study was to evaluate the possibility of applying the fruit extracts for enrichment fruit beverages in polyphenol compounds and hence increasing their antioxidative activity.

MATERIAL AND METHODOLOGY

Apple, orange and grapefruit juice concentrates (Tymbark, Poland) were used for preparing the beverages. Apple beverage (20% concentrated apple juice, 80% distilled water), orange drink (18% concentrated orange juice, 2% sugar syrup, 80% distilled water) and grapefruit drink (18% concentrated grapefruit juice, 14% sugar syrup, 68% distilled water) were prepared. Then the beverages were fortified with fruit extracts.

The extracts were obtained from fruits purchased from the pomological orchard of University of Agriculture in Krakow, located in Garlica Murowana near Krakow or from the organic farms in Malopolska and Podkarpacie areas. As the raw material for extracts elderberry fruits (*Sambucus nigra L.*), Japanese quince (*Chaenomeles japonica* (Thunb.), Cornelian cherry fruits (*Cornus mas L.*), lingonberry (*Vaccinium vitis–idea* L.), and hawthorn (*Crataegus oxyacantha* L.) were used.

Preparation of extracts

10 g of seedless fruit was soaked with 90 mL of ethyl alcohol (80% v/v) and extracted using a high speed homogenizer (19 000 rpm; 5 min; UltraTurrax T25 Basic, IKA). The extracts were filtered, adjusted to 100 mL by adding a solvent and stored at -20°C. Before introducing to the fruit beverages, extracts were concentrated four-fold by distillation under reduced pressure (60°C) under an inert atmosphere (N_2).

Evaluation of antioxidative activity (**Tarko et al., 2009**)

The antioxidative activity in final fruit beverages was determined by using the active radical cation ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), Sigma). ABTS radical was generated by chemical reaction between 7 mM aqueous solution of diammonium salt of 2,2'-azynobis (3-ethylbenzthiazoline-6-sulfonate) and 2.45 mM potassium persulfate solution ($K_2S_2O_8$). The solution was kept overnight in the dark at ambient temperature, to terminate the reaction and to stabilize ABTS cation. During analysis, the concentrated solution of ABTS was diluted with phosphate buffer saline (PBS) at pH 7.4, to obtain solution with absorbance value of A = 0.70 ±0.02 ($ABTS_{0,7}$) measured by a spectrophotometer (Beckman DU 650) at a wavelength of 734 nm. 100 µL of the appropriate diluted samples were added to 1 mL of $ABTS_{0,7}$ and the absorbance was measured at 6 minutes after mixing. The antioxidative capacity of the samples was calculated using a standard curve performed with solutions of synthetic vitamin E (Trolox; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma) and expressed in mg of Trolox/100 mL.

Determination of total polyphenol contents **(Tarko et al., 2009)**

Total polyphenol content was determined by the modified Folin-Ciocalteu method. 45 mL of redistilled water was added to 5 mL of diluted beverages. Then, 0.25 mL of Folin-Ciocalteu reagent (water dissolved at 1:1 v/v, Sigma) and 0.5 mL 7% Na_2CO_3 (POCh) were added. Samples were incubated for 30 minutes in the dark, before measuring the absorbance by a spectrophotometer at the 760 nm (against PBS as a blank). The results of total polyphenols content were obtained based on the calibration curve and were expressed as mg of catechin/100 mL of beverage.

Sensory evaluation **(PN-64/A-04022)**

Evaluation was carried out by the panel comprising 20 qualified and tested for their sensory sensitivity people. They assessed three basic quality factors. For the sensory evaluation the 5-point scale with following weights: flavor (0.5), odor (0.3) and color (0.2) was used.

Statistical analysis

There were a minimum of three repetitions of the analysis and the results are shown as the arithmetic mean with standard deviation (± SD). Statistical analysis was performed using InStat v. 3.01 (GraphPad Softwere Inc., USA). A single-factor analysis of variance (ANOVA) with post hoc Tukey's test was applied to determine the significance of differences between means. The Kolmogorov-Smirnov test was carried out to assess the normality of distribution.

RESULTS AND DISCUSSION

In the first stage of the study, beverages were prepared with addition of various extracts at doses from 1 to 10%. The sensory evaluation was performed and the obtained results showed that extracts of Japanese quince (a final concentration 2 and 5%), Cornelian cherry (2%) or lingonberry (2%) were suitable for the enrichment of apple beverages; Japanese quince extracts (2 and 5%) and lingonberry (2%) for orange beverages and Japanese quince extract (2%), Cornmelian cherry (2 and 10%) and lingonberry (2%) for grapefruit beverages. Extracts from the elderberry and hawthorn fruits were rated as not acceptable by the sensory panel.

Apple beverages were characterized by the lowest antioxidative activity and the total polyphenols content in comparison with other samples. For this reason their fortification raised antiradical capacity much more than in case of other beverages (Table 1). The increased antioxidative capacity of fortified beverages (by 10 to 32% in comparison with control beverage) has been shown. However, fortification with fruit extracts first of all affected the amount of polyphenolic compounds in apple beverages (from 48 to 138% more than in the beverage without additives). The highest differences were observed for the 5% addition of Japanese quince extract, which is a raw material rich in organic acids (malic and citric acids), catechin (140 mg/100 g) and leucoanthocyanins (270 mg/100 g) **(Domański et al., 1994; Fronc and Oszmiański, 1994)**. It could be also noted that only the addition of 2% of the Japanese quince extract had a positive effect on the score of sensory evaluation. Fortification of apple beverages with lingonberry and Cornelian cherry extracts was negatively evaluated by the sensory panel. A strong correlation between the content of total polyphenols and antioxidative activity of beverages (R2 = 0.94) was also shown.

In the case of enrichment of orange drink with fruit extracts a lower impact on the total polyphenol content was demonstrated than in the corresponding apple beverages. Enrichment of orange drink in Japanese quince extract and lingonberry extract contributed to an increase of their antioxidative capacity, as well as the total polyphenol content (Table 1). It has been shown that the additives in a similar way increased the quality parameters of analyzed orange drink. Lingonberry, like Japanese quince, is characterized by significant catechin and organic acids content **(Matuszkiewicz, 2006)**. The 5% addition of Japanese quince extract was associated with increased antioxidative activity and polyphenol content in orange beverage (by 24 and 56%, respectively). It is worth noting that the supplementation of orange beverages with extracts from Japanese quince, both at concentration of 2 and 5%, resulted in the higher scores of sensory evaluation. Japanese quince extracts are characterized by high acidity (3.5–4.5%) **(Lopez, 2006)**, which matched well with a relatively high content of organic acids in orange drink and for this reason, they could be attractive for evaluators. High sensory evaluation score is also related to the content of sugars in the fruit. Japanese quince contains improper sugar to acid ratio (three times more acids than sugars, while fruits intended for direct consumption should contain at least 10 times more sugar than acids) **(Lesińska, 1988)**. The proportions of acids and sugars in the fruits of Cornelian cherry disqualify these fruits for direct consumption, but the orange beverage has been much sweeter than apple beverage and therefore it better masked the sour taste sensation of added extracts.

Grapefruit drinks are a good raw material for enrichment due to high acidity, high sugar content and a dark color. These features well mask the undesirable sensory traits of introduced additives (eg. dark color of extracts). The enrichment of grapefruit beverages in fruit extracts slightly contributed to the change of their antioxidative capacity and total polyphenol content (Table 1). These values, especially the amount of polyphenols, did not differ significantly. Only 10% addition of Cornelian cherry extract caused an increase of the analyzed parameters values. However, such high content of the extract in the grapefruit beverage was negatively rated by the sensory panel, and scores were significantly lower than in the case of beverage without additives. Worth mentioning is the fact, that only in the case of this beverage, addition of Japanese quince extract did not increase the scores of sensory evaluation. It was also shown that fortification of grapefruit beverages with extracts of lingonberry increased

Table 1 Antioxidative activity. total polyphenols content and sensory evaluation of beverages enriched with plant extracts (mean ± SD).

Parameter	Beverages	Control	Japanese quince 2%	Japanese quince 5%	Lingonberry 2%	Cornelian cherry 2%	Cornelian cherry 10%
Antioxidantive activity [mg Trolox/100 mL]	Apple	177 ±1.6 a	206 ±2.2 b	234 ±3.4 c	194 ±1.1 d	207 ±1.3 b	–
	Orange	329 ±5.7 a	357 ±3.4 b	407 ±5.2 c	364 ±1.5 b	–	–
	Grapefruit	274 ±3.1 a	300 ±3.9 b	–	306 ±0.9 b.c	323 ±2.3 c	396 ±3.3 d
Total polyphenols content [mg catechin/100 mL]	Apple	34 ±1.7 a	57 ±2.3 b	81 ±0.8 c	51 ±1.1 d	51 ±1.0 d	–
	Orange	66 ±5.5 a	91 ±2.0 b	103 ±1.9 c	92 ±2.8 d	–	–
	Grapefruit	62 ±5.9 a	72 ±0.6 b	–	67 ±0.7 a	66 ±0.3 a	105 ±3.7 c
Sensory evaluation score [points]	Apple	3.89 ±0.3 a	4.01 ±0.4 b	3.88 ±0.4 a	3.79 ±0.5 c	3.70 ±0.2 c	–
	Orange	3.99 ±0.4 a	4.32 ±0.4 b	4.10 ±0.2 c	3.88 ±0.3 a	–	–
	Grapefruit	4.01 ±0.5 a	3.99 ±0.2 a	–	4.15 ±0.4 b	3.69 ±0.3 c	3.47 ±0.3 c

Note: means marked with different letter (within the analyzed parameter and beverage - lines) differ statistically at $p < 0.05$

sensory evaluation scores. In case of this beverage a very strong influence of the content of polyphenolic compounds on antioxidative activity was also shown ($R^2 = 0.96$).

CONCLUSION

This study has shown that addition of fruit extracts positively affected antioxidative activity and total polyphenolic content of beverages. Based on the sensory evaluation, the samples with addition of extract from Japanese quince fruit (in the case of apple and orange beverages) and lingonberry (grapefruit beverage) were preferred before the others. It can be assumed that beverages fortified with ingredients that may provide a health benefits will be very popular in the future, mainly due to increasing consumer awareness.

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