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INFLUENCE OF STORAGE ON THE QUALITY OF NATURAL ANTIOXIDANTS IN FRUIT BEVERAGES

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Key words: fruit beverages, polyphenols, antioxidant properties, storage

The study material consisted of the following fruit beverages: (1) apple, (2) apple-orange-peach, (3) apple-cherry, and (4) apple-chokeberry, produced by Fructo-Maj in Milejów near Lublin. Contents of phenolic acids, anthocyanins, and ability to neutralize free synthetic ABTS and DPPH radicals were determined just after producing and 3 months of storage.

Beverage storage had a negative influence on polyphenols content. A decrease of polyphenolic activities resulting from the storage process was reported. The highest antioxidant properties were recorded for apple-chokeberry beverage, the lowest – for apple-orange-peach juice.

INTRODUCTION

Fruit beverages are popular due to their fairly great ability to retain properties of raw materials they had been achieved from.

Consumers force the foodstuff industry to meet their taste therefore various types of fruit juice mixtures are produced.

An increase of interests in natural antioxidants has been observed in the 1990's, when numerous epidemiological studies confirmed the efficiency of a diet abundant in fruits and vegetables as well as their processed products against aging and many civilization diseases. Free-radical background of many health disturbances contributed to intensification of research on natural antioxidants and their pro-health influence on human's organism. Fruit beverages gained exceptional importance due to their availability and efficiency of substances they contain that are of natural antioxidant character and much exceeding the health results of their supplementation in a form of pharmaceuticals [Szczypka *et al.,* 1997; Łoś *et al.,* 1996; Horubała, 1999; Flueki *et al*., 1968].

According to qualitative norms, fruit beverages are products achieved from concentrated fruit juices. They are lucid liquids with no fruit tissue, thus without pectins, starch, and tannins. Besides beverages, there are juices on domestic market. In reference to their physicochemical properties, they differ with fruit or vegetable tissue content and refractometric extract content [PN-A-79031. 2000.05]. Clarification process, that is necessary in technology of concentrated juices, removes part of biologically-active antioxidants within fruit tissue, which is reflected in antioxidant capacity. Thus, fruit beverages, from the nutritional point of view, are very poor in antioxidants. Besides this disadvantage, fruit beverages have also virtues: they are much cheaper than juices, and sooth thirst, which is quite important for consumer's customs [Mitek *et al.,* 2003; Swain *et al.,* 1959; Brand-Williams *et al.,* 1995].

Consumer's acceptance of fruit beverages is apparent and directed not only by vitamin spectrum, low caloricity, and presence of easy available simple sugars or minerals, but also other biologically-active components, including natural antioxidants with polyphenols, flavonoids (anthocyanins, flavanols, flavonols, flavanons, and dihydrochalkones) as well as numerous group of phenylpropenoic acids (caffeic, chlorogenic, pherullic, coumaric) [Wilska- Jeszka, 2000; Re, 1999].

Due to the improving the sensoric traits of food, the most important polyphenol groups are: anthocyanins that are responsible for red colour, phenolic acids, catechins, and proanthocyanidins being tannin precursors and substrates of enzymatic browning that give the taste and aroma of many food products (tea, cocoa, wine, beer, fruit and vegetable juices).

Polyphenols are characterised by easy getting into redox reactions. These compounds are easily oxidized and may mediate in oxidation of substrates that do not react with gaseous oxygen, which is extremely important in human's health prophylaxis [Maniak *et al.*, 1996].

Polyphenols can be degraded during storage. It is the result of oxidative polymerization that decreases beverage quality. Inappropriate storage conditions (excessively low or high temperatures, light and oxygen access) are inhibitors of those processes [Dietrich, 2004; Swain *et al*., 1959].

The aim of the work was quantitative determination of antioxidants contents in juices.

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TABLE 1. Contents of phenolic acids and anthocyanins in fruit beverages.

Items	1a	1b	2a	2b	3a	3b	4a	4b
Phenolic acids (mg/L)	43	41	52	49	57	49	100	83
Percentage*	4.7	0	5.8	0	14.0	0	17.0	0
Anthocyanins (mg/L)	0	0	0	0	2.1	1.8	15.1	10.0
Percentage*	0	0	0	0	14.3	0	33.8	0

MATERIAL AND METHODS

Apples, cherries, and chokeberries are the most commonly cultivated in our climatic zone, thus beverages made of these fruits – namely apple as a basis – were the study material. The following beverages were tested: (1) apple, (2) apple-orange-peach, (3) apple-cherry, and (4) apple-chokeberry.

Apple juice is a base of tested beverages, while another fruit addictives contained in beverages are just forming only antioxidant level. The beverages are studied just after production and after three months of storage by antioxidant lose testing. The beverages were stored according to the standards. The study was made in three trials. The results are arithmetic mean from the trials.

The beverages analysed were determined for: (1) total content of phenolic acids by means of Folin-Ciocalteau's spectrometry described by Singelton *et al*. [1999] with modification of Groistein *et al*. [2000]. It consists in measurements of reductive properties of polyphenols and absorbance of a complex that is formed as a result of reaction between polyphenols and tungsten-molybdate agent; (2) anthocyanins by means of spectrometric method according to Flueka & Francis [1968]. Absorbance results are put into the empirical formula for calculating the anthocyanin amount in a sample; (3) ability to neutralize free DPPH radicals by antioxidants contained in beverages. Antioxidant properties of beverages were tested by determination of neutralization of stable synthetic DPPH radicals (1.1-diphenyl-2-pikrylhydrazyl); (4) ability to reduce 50% of DPPH radicals at the presence of polyphenols in accordance to the method described by Brand-Wiliams *et al.* [1995]. Juice samples were previously purified on a mini column Sep-Pak (Waters); antioxidant capacity was calculated as the per cent of inhibition in relation to the reference and expressed in EC_{50} as the juice volume needed to reduce 1 mol of DPPH radicals by 50% [Sanchez-Moreno *et al.,* 1999]; (5) total antioxidant activity (CAP) in the experimental system with ABTS radical and potassium persulfate ($K_2S_2O_8$) by means of Trolox Eqiuvalent Antioxidant Capacity method. Value of TEAC was determined spectrometrically by measuring the antioxidant activity based on the ability to inhibit the formation of cationic radical $ABTS^+$ (2.2-azino-bis(3-ethylbenzthiazolin-6-sulfonic acid)).

Antioxidant activity of TEAC was defined as equaled to the concentration (mmol/L) of Trolox in reference to 1 mL of juice. In order to determine the TEAC value, per cent of $ABTS^+$ absorbance inhibition was recorded after juice addition in reference to calibration curve.

Designations used in the experiment are as follows: (1a) apple juice, (1b) apple juice, after 3-month storage, (2a) apple-orange-peach, (2b) apple-orange-peach after 3-month storage, (3a) apple-cherry, (3b) apple-cherry after 3-month storage, (4a) apple-chokeberry, and (4b) apple-chokeberry after 3-month storage.

RESULTS AND DISCUSSION

Contents of phenolic acids and anthocyanins in tested beverages were directly proportional to antioxidant properties (Table 1). The storage process negatively affected the contents of anthocyanins and phenolic acids, which may prove the high lability of these compounds. Similar conclusions were drawn by Dietrich *et al.* [2004]. Similar antioxidant activity has been described for phenolic-rich beverages such as wines and teas [Gardner *et al*., 1997; Rice-Evans *et al*., 1996] and has lead to suggestions that some phenolic compounds may prevent oxidative damage *in vivo* and thus protect against the development of diseases such as heart disease and cancer [Wiseman, 1999]. Candidate phenolic antioxidants in foods include avonoids, anthocyanins, catechins, chalcones and hydroxybenzoic and hydro-xycinnamic acids many of which are present in fruit juices [Hertog *et al.,* 1993]. Selection of appropriate conditions for storing the foodstuff is a challenge for a producer.

The highest percentage of phenolic acids was recorded in apple-chokeberry beverage, the lowest – in apple-orange-peach ones. The dependence between phenolic acids and anthocyanins contents *vs*. the ability to neutralize free ABTS and DPPH radicals was observed (Figures 1 and 2). Apple-orange-peach beverages, that do not contain anthocyanins, are the least abundant in antioxidants, *e.g.* they have the weakest ability to neutralize free radicals. Addition of juices made of colorful fruits elevates the nutritional value of these beverages. It should be underlined that antioxidant properties of beverages depend on polyphenols concentration in fruits, of which they were produced.

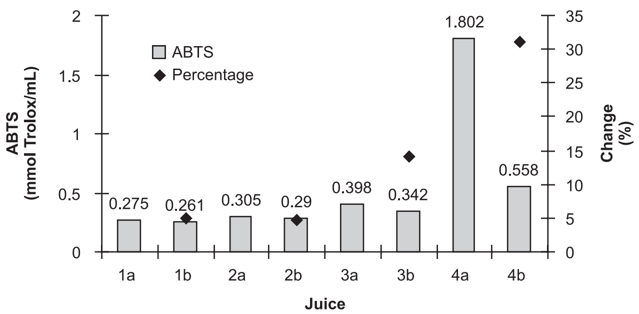

Figure 1. Antioxidant capacity determined with ABTS method in fruit beverages.

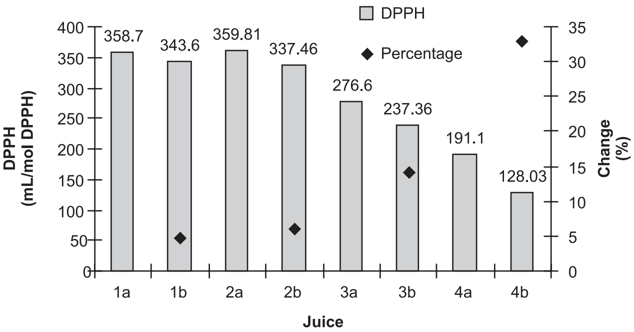

Figure 2. Antioxidant capacity determined with DPPH method in fruit beverages.

CONCLUSIONS

1. Apple-orange-peach, apple-cherry, and apple-chokeberry beverages revealed great differences in antioxidant contents 0.275-0.558 mmol Trolox/mL.

2. Beverages with chokeberry addition appeared to be the most abundant in phenolic acids and anthocyanins; the apple plus citrus beverages were the poorest in that context.

3. In every tested case, the storage process affected the decrease of polyphenols contents.

4. Beverages with chokeberry addition had the strongest ability to neutralize free DPPH and ABTS radicals in relation to the control; the apple with citrus addition ones – the weakest.

5. The highest loss of phenolic acids was observed in beverages contained chokeberry juice; the lowest losses occurred in apple beverages with citrus juice addition.

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WPŁYW CZASU PRZECHOWYWANIA NA JAKOŚĆ NATURALNYCH PRZECIWUTLENIACZY W NAPOJACH OWOCOWYCH

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Materiał badawczy stanowiły napoje owocowe: (1) jabłkowy, (2) jabłkowo-pomarańczowo-brzoskwiniowy, (3) jabłkowo-wiśniowy, (4) jabłkowo-aroniowy wyprodukowane przez Fructo-Maj w Milejowie/k Lublina. Badano zawartości: kwasów fenolowych, antocyjanów oraz zdolność „zmiatania" wolnych, syntetycznych rodników ABTS i DPPH po wyprodukowaniu i po 3 miesiącach przechowywania.

Przechowywanie napojów wpływało niekorzystnie na zawartość polifenoli. Określano obniżenie aktywności polifenoli wynikające z procesu przechowywania. Najwyższe właściwości antyoksydacyjne posiadały napoje jabłkowo-aroniowe, najniższe napoje jabłkowo-pomarańczowo-brzoskwiniowe.