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Original scientific paper

DETERMINATION OF NATURAL DYES (E160A AND E160D) CONTENT AND ANTIOXIDANT ACTIVITY IN DRY ROSEHIP

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ABSTRACT

The most famous of all forest fruits is pomegranate (*Rosa carina*), which is used to make extremely tasty jam and marmalade. Fruits that are collected for jam or marmalade and those that are collected for tea are in different stages of ripening. In addition to vitamin C, rosehip is rich in vitamins B2 and K as well as provitamin A (beta-carotene, E160a) and lycopene (E160d). A sample of dried rosehip was selected for qualitative and quantitative analysis of the content of E160a (β-carotene) and E160d (lycopene) dyes. Using UV-VIS spectrophotometric method and Beer-Lambert's law, a system of two linear equations with two unknowns was set up, which was used to determine the concentrations of E160a and E160d dyes. Antioxidant capacity of sample was determined by DPPH method. The E160a (β-carotene) and E160d (lycopene) content was 64.8326 ± 0.4128 μg/g and 20.9746 ± 0.459 μg/g in n-hexane extract of dry rosehip, respectively. Antioxidant activity of prepared fruit and rosehip leaves extracts was determined by DPPH method. Result of analysis expressed as EC_{50} (mg/mL) value was 1.87. Rosehip is mainly consumed as a tea for enjoyment and refreshment. It poses a beneficial effect on the heart and kidneys, and is ideal for preventing the formation of stones in the kidneys and urinary tract. There are no harmful effects for human health because it is a natural astringent, antibacterial agent, antioxidant, arteriosclerotic, antiscorbutic, antidiarrheal, diuretic, depurative, choleretic, so it can be taken in larger quantities.

Key words: beta-carotene, DPPH method, lycopene, *Rosa canina*.

INTRODUCTION

Rosehip (*Rosa canina* L.) is a species of *Rosaceae* family that grows in Europe, Western Asia and North Africa. Rosehip fruit is bright to deep red colored, and gets ripened in August-September (Turkben et al., 2005). It found usage in food industry for making marmalades, soups and wine or tea (Razungles et al., 1989).

It is known as a source of vitamin C and polyphenols (Chrubasik et al., 2001), but also contains: carotenoids, bioflavonoids, tannins, pectin, sugars, organic acids, amino acids and essential oils (Ercisli, 2007). It is worth noticing that rose hip fruit contains rosehip provitamin A (beta-carotene, E160a) and lycopene (E160d).

E160a is mixture of α-, β- and γ- carotene, a natural orange food color. It is obtained by chemical extraction from various plant species (tomato, rose hip, etc.) and consist of about 85% β-carotene, 15% α-carotene, and 0.1% γ-carotene. The photosynthetic organisms, some bacteria and fungi, are able to synthetize β-carotene. The synthesis of carotenes is important for growing of plants. They have a function in photosynthesis, in preventing of photo-oxidative damaging in plants, and are precursors of phytohormones (Vinković Vrček and Lerotić, 2010).

In human body carotenes are converted into vitamin A. Generally speaking, carotenes, and especially β-carotene, are contributing to improvement of human health by boosting immunity. Also, they reduces the risk of coronary artery disease and cataracts. The incidences of tumors are reduced by α-carotene significantly and it improves the function of eyes, skin, liver and lungs (Yesilada, 2002; Chrubasik et al. 2006; Zhang et al., 2008). Carotenoid extract have anti *H. pylori* activity comparable to metronidazole (Horvath et al., 2012).

The carotenes can be added to food as colorants in *quantum satis* and are considered harmless. For E160a, the reference daily intake (RDI) in mg per kg body weight is 5.0. In sausages and pâtés permitted amount of use for E160a is up to 20 mg/kg. Examples for usage of E160a as a food dyes are: coloring butter, low-fat margarine, and other fat emulsions; fermented ripe orange; yellow, white cheese in pieces and non-flavored melted cheese; vegetables in vinegar, saltwater or oil (except olive oil); marmalade, jam, and similar fruit products, including energy-reduced products. E160a also found usage in production of some bakery products such as: biscuits, cakes, candy products, pudding powder, creams, desserts, etc. (Vinković Vrček and Lerotić, 2010).

E160d (lycopene) is a natural red-orange dye that belongs to carotenoid group. It is most present in tomatoes, watermelons, pomegranates and red grapefruit. Besides that it can be obtained by extraction from plant material synthetic lycopene is also produced. Free radicals can cause oxidative damage of lipids, proteins and DNA in cells and lycopene can inactivate them. Studies also show that lycopene reduces the risk of different cancers, as well as cardiovascular disease. By consuming about 6 mg of lycopene per day the risk of prostate cancer is reduced by almost 10-20% (Giovannucci, 1999).

The aim of this work is to determinate the content of natural dyes E160a and E160d and antioxidant activity in dry rosehip.

MATERIALS AND METHODS

For this experimental research was used fresh cynosbati fructus bought from the local market in Niš, Serbia. The fruits were washed, and left drying in the shade. After that, dried fruits were sliced and homogenized in Brown® blender. Acetone was obtained from Fisher Scientific (Loughborough, United Kingdom). Hexane, butylated hydroxytoluene, sodium acetate, glacial acetic acid, 2,2-diphenyl-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Steinheim, Germany).

Determination of E160a (β-carotene) and E160d (lycopene) content

Conventional solvent extraction methods (Sadler et al., 1990; Perkins-Veazie et al., 2001) were employed for carotenoid extraction. 10 g of each sample (ketchup A or ketchup B) were added to a mixture consisting of 250 ml hexane, 125 mL of acetone, 125 mL of ethanol (2:1:1, *V*/*V*/*V*), and 0.05% (*w*/*V*) butylated hydroxytoluene (BHT). The mixture was stoppered and placed on an orbital shaker to mix at 180 rpm for 150 minutes (temperature of mixing was 5 °C). After shaking, 75 mL of cold deionized water was added and the mixture was agitated for another 5 min. The suspension was left at room temperature for 10 minutes to allow the separation of polar and non-polar layers. The extract was re-dissolved in hexane. The hexane extracts were scanned in the

visible light wavelength range of 400-750 nm using Jenway 6105 UV/Vis spectrophotometer (Jenway, United Kingdom) in 1 cm path length quartz cuvette blanked with n-hexane and the maximum absorbance were observed at 450, 472, and 503 nm, respectively for the lycopene/β-carotene hexane layer mixture. The molar extinction coefficient with value 172 000 L mol^{-1} cm^{-1} at 503 nm was used to estimate E160d (lycopene) concentration, using the Beer-Lambert law (Zechmeister and Polgar, 1943; Ravelo-Perez et al., 2008).

Determination of antioxidant capacity - DPPH assay

Using the modified procedure described by Kaneda et al. (1995) the total antioxidant capacity of samples A and B was determined by DPPH assay. For analysis of antioxidant capacity, approximately 10 g of samples A and B were dissolved in 30 mL of ethanol solution (70 %, *V*/*V*). The samples were mixed for 10 minutes at 5 °C and then centrifuged at 9000 rpm for 10 minutes. The supernatant was poured off and the pellet was re-extracted with 15 mL of ethanol solution by the same procedure. The obtained supernatants were combined and the total volume was adjusted to 50 mL with 70% (*V*/*V*) ethanol solution. The extracts of samples (0.2 mL) were added to the DPPH solution (2.8 mL) (mixture of 1.86 x 10^{-4} mol/L DPPH in ethanol and 0.1 M acetate buffer (pH 4.3) in ration 2:1) and mixed vigorously. After 60 minutes of incubation in a dark place, the absorbance was measured at 525 nm. The standard curve was constructed using 1 mM Trolox solution and the results were expressed as mM Trolox equivalents (TE) per kilogram of a sample (Kaneda et al., 1995).

Statistical analysis

All measurements were conducted in triplicate and data were expressed as mean ± standard deviation. The significance of differences among means was tested using a t-test for independent samples.

RESULTS AND DISCUSSION

The visible spectra of the hexane extract of E160a/E160d β-carotene-lycopene mixture from dry rose hip with absorption maxima at 450 nm, 472 nm and 503 nm is showed in Figure 1. It is know that most of the carotenoids shows absorption maxima at three wavelengths in a three-peak spectrum. The λ_{max} shifts to longer wavelengths as the number of conjugated double bonds raises.

The most unsaturated acyclic carotenoid which is of interest in this work, lycopene (E160d), has 11 conjugated double bonds and is red colored with absorption at the longest wavelengths (λ_{max} at 443, 471, 503 nm) (Rodriquez-Amaya and Kimura, 2004). The steric hindrance is a result of a cyclization that happens between the methyl group at C-5 of the ring and the hydrogen atom at C-8 of the polyene chain. This hindrance takes the electrons of the ring double bond out of the plane with no affinity to those of the chain, causing a hypsochromic effect (displacement of λ_{max} to shorter wavelength), a hypochromic effect (decreased absorbance), and loss of fine structure (lower resolution spectrum).

The yellow-orange molecule of β-carotene despite possessing the same number of conjugated double bonds as lycopene, exhibits absorption peaks at 450 and 472 nm and a mere shoulder at 425 nm (Rodriguez-Amaya and Kimura, 2004). This is so because both carotenoids absorb substantially in the overlapping wavelength ranges. The wavelength of 503 nm to quantify lycopene is acceptable even this wavelength value is not equal to λ_{max} (Ravelo-Perez et al., 2008).

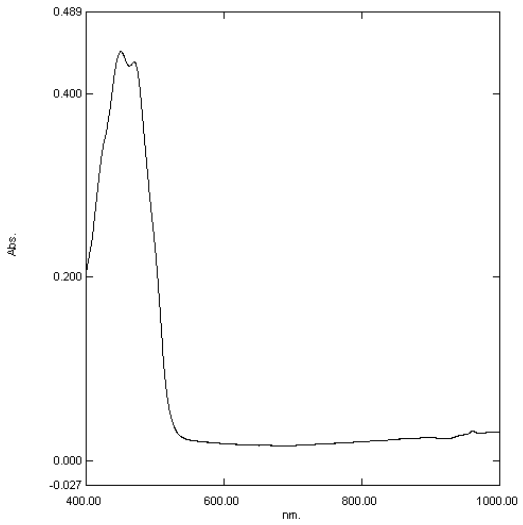

Figure 1. Visible spectra of the hexane extract of E160a/E160d β-carotene-lycopene

According to the law of Labert and Beer the absorbance at 450 and 503 nm (in a quartz cuvette 1 cm wide) of the carotenoid mixture of lycopene and β-carotene can be expressed through equations (1) and (2):

$$A_{450} = \varepsilon^{450}_{lycopene}[lycopene] + \varepsilon^{450}_{\beta-car}[\beta - carotene] \quad (1)$$

$$A_{503} = \varepsilon^{503}_{lycopene}[lycopene] + \varepsilon^{503}_{\beta-car}[\beta - carotene] \quad (2)$$

[lycopene] and [β-carotene] are the molar absorbances of lycopene and β-carotene, respectively; $\varepsilon^{450}_{lycopene}$ and $\varepsilon^{450}_{\beta-car}$ are molar absorptions for β-carotene and lycopene at 450 nm while $\varepsilon^{503}_{lycopene}$ and and $\varepsilon^{503}_{\beta-car}$ represent corresponding molar absorptions for β-carotene and lycopene at 503 nm.

The values for $\varepsilon^{450}_{\beta-car}$, $\varepsilon^{450}_{lycopene}$, $\varepsilon^{503}_{\beta-car}$ and $\varepsilon^{503}_{lycopene}$ are known: o $1.39x10^5$, $1.16x10^5$, $2.63x10^4$, $1.72x10^5$ L $mol^{-1}cm^{-1}$ (Zechmeister and Polgar, 1943; Krinsky et al., 1990; Clinton, 1998; Du et al., 1998), and therefore:

$$[E160a] = \frac{1.483 * A_{450} - A_{503}}{1.798 * 10^5} \quad (3)$$

$$[E160d] = \frac{A_{450} - 1.39 * 10^{15}[E160a]}{1.16 * 10^5} \approx \frac{A_{503}}{1.72 * 10^5} \quad (4)$$

To calculate the concentration of E160a and E160d in this work equations (3) and (4) were used, and results expressed as μM are 2.4153 ± 0.0154 and 0.7814 ± 0.0171 respectively. Finally, 64.8326 ± 0.4128 μg/g is concentration of E160a (β-carotene) and 20.9746 ± 0.459 μg/g for E160d (lycopene) in n-hexane extract of dry rosehip. The lycopene concentration in rose hip determined by Fan et al. is 6.8 mg per 100 g, and for α- and β- carotene 0.031 and 2.35 respectively (Fan et al., 2014). Allowed maximum amount (mg/kg) of lycopene in some foods is: 100 in jam, flavored melted cheese, smoked fish, non-alcoholic flavored beverages; 150 in desserts, flavored milk products; 200 in biscuits, cakes; 300 in mustard; 500 in sauces except tomato based sauces, spices. The antioxidant capacity of the tested samples was determined using a spectrophotometric method – DPPH assay. The result expressed as μM TE/g is 6.8421 ± 0.0288. Other researchers from Serbia reported that rosehip flavonoids from acetone extract shows concentration dependent antioxidant activity towards DPPH EC_{50} = 49 mg L^{-1} (Tumbas et al., 2012).

CONCLUSION

In order to describe chemical profile of dried Rose hip, which is used in everyday life for tea preparation, determination of content of natural dyes E160a and E160d and antioxidant activity was done. Results shows that this plant species has significant content of lycopene and carotenes which contribute to human health.. The difference in resultst between tea samples of rose hip are because of the geographical origin from which samples are and technological parameters during preparation by different manufacturers. Rose hip should be considered as a functional food and has a recommendation because of chemical composition and health benefits.

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