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DETERMINATION OF CONTENT AND ANTIOXIDANT CAPACITY OF NATURAL FOOD COLORS E160A AND E160D IN KETCHUP

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Abstract. *After potato, tomato (*Solanum lycopersicum*) is the world's second-largest vegetable crop. More than 80% of tomato consumption comes from processed products such as ketchup, tomato juice, pickled tomatoes, sauces, paste, purée. Samples of mild ketchup from two different manufacturers (A and B) were selected for the analysis of the content of natural food colors E160a and E160d. Using a UV-Vis spectrophotometric method and Lambert-Beer law, a system of two linear equations with two unknowns was set up, which was used to determine the concentrations of colors E160a and E160d. The antioxidant capacity of the two selected samples was determined using the DPPH assay. The obtained results indicated that the content of colors E160a and E160d was higher in the sample of ketchup B. Also, the DPPH assay showed that the sample of ketchup B had a higher antioxidant capacity.*

Key words: *E160a (β-carotene), E160d (lycopene), ketchup, antioxidant capacity, DPPH assay*

1. INTRODUCTION

Ketchup is a sweet and sour spice typical for American nutrition. The technological process of obtaining ketchup consists of mixing tomato paste (65% solids, 30-32 °Bx) with sugar, wine vinegar, salt, aroma and trace preservatives. The mixture is pasteurized at 96 °C for 4-6 minutes and packed. The temperature must be carefully regulated to ensure the absorption of ingredients without overcooking. The whole process of ketchup production usually takes 2-3 hours (Galicia-Cabrera, 2007). Ketchup, as one of the basic

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products from tomatoes, is considered to be healthy food because it is low in fat and calories and is cholesterol-free, and rich in vitamins A and C, β-carotene (E160a), lycopene (E160d), and octadecanoic acid (Kim at al., 2011). Worldwide consumption of tomatoes has increased significantly in the last two decades, mainly due to the growing demand for tomato-based products such as ketchup, gazpacho, and tomato juice (Bugianesi et al., 2004, Simonetti et al., 2005).

E160a (carotenes: a mixture of α-, β- and γ-carotene) are natural orange food colors. The name carotene refers to a natural mixture of α-, β- and γ-carotene (see Fig 1).

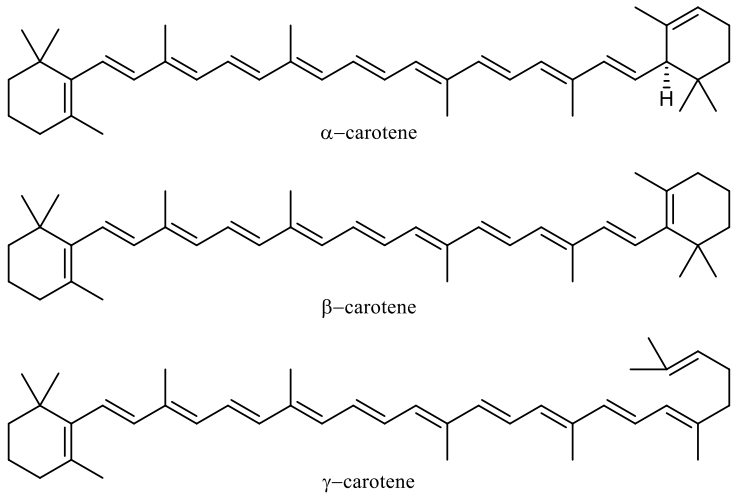
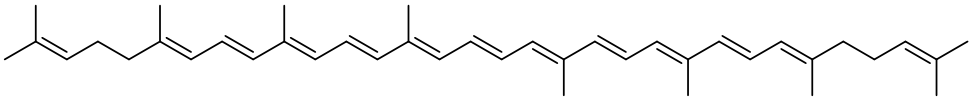


Fig. 1 Structural formulas of natural color E160a (α-, β- and γ-carotene)

The carotene mixture is obtained by chemical extraction from plants and contains about 85% β-carotene, 15% α-carotene, and 0.1% γ-carotene. Pure β-carotene can be produced synthetically or under the action of microorganisms. They can also be produced under the influence of genetically modified organisms, but the evaluation of the effect of the carotene obtained in this way has not yet been completed. They are synthesized by all photosynthetic organisms, some bacteria, and fungi. Animals cannot biosynthesize carotene and must ingest these nutritionally important components through foods of plant origin. In plants, carotene biosynthesis is important for growth and development; they play a role in photosynthesis, in preventing photo-oxidative damage to plants, and serve as precursors of phytohormones. Carotenes in the body are converted into vitamin A. In addition to provitamin activity, carotenes, and especially β-carotene, have other properties that can significantly contribute to human health. Thus, β-carotene reduces the risk of cataracts, coronary artery disease, and lung and breast cancer and boosts immunity (especially in the elderly). Studies show that α-carotene significantly reduces the incidence of tumors and has a protective effect on the skin, eyes, liver, and lungs. With increased intake, the skin (especially the palms and hands) can take on a yellowish tone, which is a completely normal and harmless phenomenon. They can be added to food by the rule of *quantum satis*, and are considered harmless. The reference daily intake (RDI) in mg per kg body weight for E160a is 5.0. Permitted use for E160a is in sausages and pâtés up to 20 mg/kg.

They may be added to other foods without restriction (by the rule of *quantum satis*). E160a food dyes are used for 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. They are also used in bakery products, biscuits, cakes, candy products, pudding powder, creams, desserts, non-alcoholic refreshing drinks, salad dressings, etc. (Vinković Vrček and Lerotić, 2010).

E160d (lycopene) is a natural red-orange dye from the carotenoid group. It is most common in tomatoes, pomegranates, watermelons, and red grapefruit. Lycopene can inactivate free radicals that cause oxidative damage of lipids, proteins, and DNA in living cells. Studies also show that lycopene reduces the risk of lung, stomach, and prostate cancer, breast and colon, ovarian, endometrial, and pancreatic and bladder cancers, as well as cardiovascular disease. The risk of prostate cancer is reduced by almost 10-20% by consuming about 6 mg of lycopene per day. The absorption of lycopene from processed tomato products is incomparably better because, in raw tomatoes, lycopene is bound to other compounds and is more difficult to separate from them. Cooking destroys these compounds, which speeds up lycopene metabolism, and absorption is even better if there are unsaturated fatty acids in the meal (for example, from oil). Figure 2 shows the structural formula of the natural color E160d (lycopene).



lycopene

Fig. 2 Structural formula of natural color E160d (lycopene)

Lycopene (E160d) is obtained by chemical extraction from tomatoes (synonym *Lycopersicon esculentum* L.), but synthetic lycopene of identical chemical structure and the same properties is also produced. As a food dye, it can only be added to certain foods (Table 1).

Table 1 Permitted use for E160

Food products/groceries	Permitted use (mg / kg)
products intended for weight control, products used under medical supervision	50
jam, marmalades, homemade jams and similar fruit products, flavored melted cheese, smoked fish	100
non-alcoholic flavored beverages, liquid food supplements	100
desserts, including flavored milk products	150
bakery products (biscuits, cakes, waffles), snacks: extruded, spicy snack products, canned red fruit	200
some alcoholic beverages, aromatized wine-based drinks, aromatized wine cocktail products	200
mustard, dietary supplements in solid form	300
sauces (except tomato-based sauces), spices	500

2. MATERIALS AND METHODS

Ketchup (of the same type, mild) from two different manufacturers was procured in a nearby market. A sample of ketchup from one manufacturer was marked A, and a sample from another manufacturer B. 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).

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

Conventional solvent extraction methods (Perkins-Veazie et al., 2001; Sadler et al., 1990) 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 of 172 000 L mol^{-1} cm^{-1} at 503 nm was used to estimate E160d (lycopene) concentration, using the Beer-Lambert law (Ravelo-Perez et al., 2008; Zechmeister and Polgar; 1943).

2.2. 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 1.86×10^{-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 (A or B).

2.3. 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.

3. Results and Discussion

Figure 3 represents typical overlapped visible spectra of the hexane extract of E160a/E160d β-carotene-lycopene mixture in ketchup A (B) with absorption maxima at 450 nm, 472 nm, and 503 nm. Most carotenoids exhibit absorbance maxima at three wavelengths in a three-peak spectrum. As the number of conjugated double bonds increases, the λ_{max} shifts to longer wavelengths. Thus, the most unsaturated acyclic carotenoid, lycopene (E160d), with 11 conjugated double bonds is red and absorbs at the longest wavelengths (λ_{max} at 443, 471, 503 nm) (Rodriquez-Amaya and Kimura, 2004). Cyclization results in steric hindrance 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 respect to those of the chain, causing a hypsochromic effect (displacement of λ_{max} to shorter wavelength), a hypochromic effect (decrease in absorbance), and loss of fine structure (spectrum with less defined peaks). Thus, the molecule of β-carotene is yellow-orange and 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 choice of 503 nm to quantify lycopene is alright even though this wavelength value is not equal to λ_{max} (Ravelo-Perez et al., 2008).

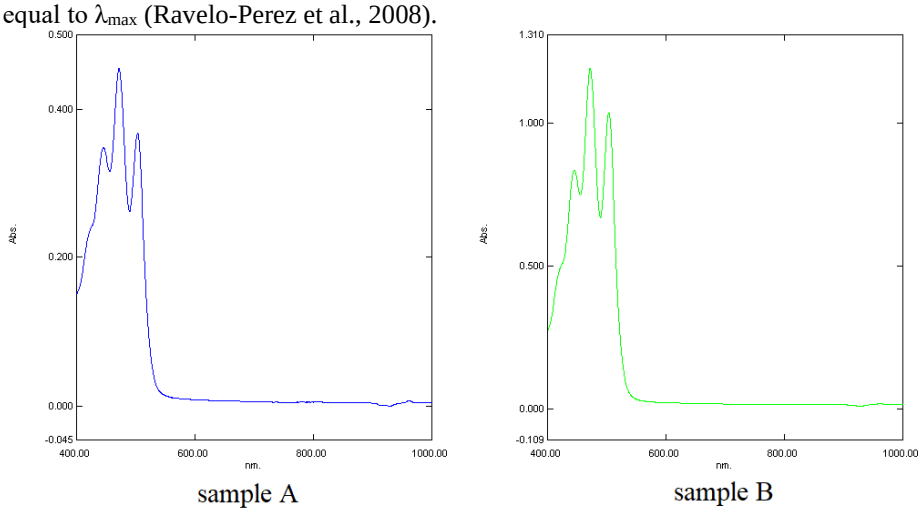


Fig. 3 Visible spectra of the hexane extract of E160a/E160d β-carotene-lycopene mixture

According to the law of Lambert 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; $\varepsilon^{450}_{\beta-car}$ and $\varepsilon^{450}_{lycopene}$ are molar absorptivities for β-carotene and lycopene at 450 nm while $\varepsilon^{503}_{\beta-car}$ and $\varepsilon^{503}_{lycopene}$ represent corresponding molar absorptivities for β-carotene and lycopene at 503 nm.

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

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

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

Equations (3) and (4) were used to calculate the concentrations of E160a and E160d, respectively, and the results are shown in Table 2 (see Fig 4, left).

Table 2 Calculated concentration of E160a and E160d in the extracts of samples of ketchup A and B in n-hexane

[a]Sample	Absorbances (nm)		[b]E160a conc. ± SD (μM)	[c]E160d conc. ± SD (μM)	E160a conc. (μg/g)	E160d conc. (μg/g)
	450	503				
A	0.33	0.33	0.855±0.036	1.796±0.019	22.950±0.965	48.209±0.510
B	0.74	0.90	1.101±0.044	5.072±0.027	29.554±1.181	136.145±0.725

[a] Sample weight is 10.00 g and the volume of hexane extract is 500 ml
[b] calculated using equation (3)
[c] calculated using equation (4)
The results are the means of triplicate analysis with reported standard deviation.

The antioxidant capacity of the tested samples was determined using a spectrophotometric method – DPPH assay (see Fig 4, right). The results are given as the mean value of the three samples (C_{sr} ± SD, n = 3) and are shown in Table 3.

Table 3 Comparison of antioxidant activity values of the ketchup samples from two different manufacturers (A and B) using DPPH assay

Sample	DPPH C_{sr} ± SD (μM TE/g)	RSD (%)
A	0.4173±0.0039	0.9451
B	0.6342±0.0124	1.9585

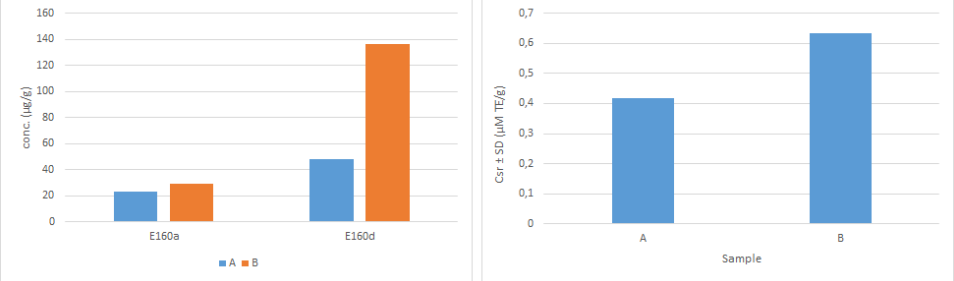


Fig. 4 E160a and E160 content in ketchup samples A and B (left); Antioxidant capacity of ketchup samples A and B (right)

4. CONCLUSION

Given that there is a large number of different producers of mild ketchup on the market, it was interesting to compare products in terms of the content of natural colors E160a and E160d, as well as their antioxidant capacity. The results showed that the sample of mild ketchup marked with B is better in terms of the content of natural colors E160a and E160d, as well as in terms of antioxidant capacity, so the ketchup from that manufacturer is a better choice for the customer. The reasons for the difference in the examined parameters should be sought in the quality of the initial raw material-tomato, as well as the geographical origin-land from which the tomato originates. Also, the possibility of differences in the quality of the tested ketchup parameters may lay in some technological parameters of ketchup production from the two selected manufacturers.

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ODREĐIVANJE SADRŽAJA I ANTIOKSIDATIVNOG KAPACITETA PRIRODNIH PREHRAMBENIH BOJA E160A I E160D U KEČAPU

*Posle krompira, paradajz (*Solanum lycopersicum*) je druga najveća povrtarska kultura na svetu. Više od 80% potrošnje paradajza potiče od prerađevina kao što su kečap, sok od paradajza, kiseli paradajz, sosevi, paste, pire. Za kvalitativnu i kvantitativnu analizu sadržaja prirodnih prehrambenih boja E160a i E160d izabrani su uzorci blagog kečapa dva različita proizvođača (A i B). Primenom UV-Vis spektrofotometrijske metode i korišćenjem* Lambert-Beer-*ovog zakona postavljen je sistem dve linearne jednačine sa dve nepoznate koji je poslužio za određivanje koncentracija boja E160a i E160d. Antioksidativni kapacitet dva izabrana uzorka određen je primenom DPPH testa. Dobiveni rezultati ukazuju da je sadržaj boja E160a i E160d veći u uzorku kečapa B. Takođe i DPPH test je pokazao da uzorak kečapa B ima veći antioksidativni kapacitet.*

Ključne reči: *E160a (β-karoten), E160d (likopen), kečap, antioksidativni kapacitet, DPPH test*