DETERMINATION OF SOME BIOACTIVE COMPOUNDS WITH ANTIOXIDANT ACTIVITY FROM TOMATOES GROWN TO I.N.C.D.B.H. STEFANESTI, ROMANIA

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Abstract
Tomato is after potato the second most consumed vegetable in the world and approximately 30 % of tomato production is consumed as processed products. Tomatoes are recommended for people suffering from diabetes, who have respiratory infections, kidney problems, constipation or gastric hyperacidity. This vegetable is usually consumed at the maximum organoleptic quality of the fruits, the quality obtained to the maturity stage. Tomatoes have a high nutritional value, due to its content of different types of nutrients: sugars, organic acids, phenolic compounds, vitamins, minerals. This paper presents the results of some quality parameters for four varieties of tomatoes (Arges 11, Arges 16, Arges 123 and Arges 20) grown in 2014 at I.N.C.D.B.H., Stefanesti, Arges, Romania. The content of some bioactive compounds with antioxidant activity (polyphenols, flavonoids, anthocyanins, and carotenoids) and the colour parameters (colour intensity, colour tint, lightness, redness, yellowness, chroma and hue angle) from these varieties of tomatoes were determined by UV-Vis absorption spectroscopy. Results obtained established the correlation between the content of carotenoids (lycopene or β-carotene) and the chromatic parameters (redness and yellowness). Since the tomato species were grown in the same conditions for agriculture, geography and climate, the results showed variability in the bioactive compounds content from the analysed tomatoes due to the influence of the variety.

Keywords: anthocyanins, carotenoids, colour, flavonoids, polyphenols, tomatoes

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1. INTRODUCTION

Tomato is after potato the second most consumed vegetable in the world and approximately 30 % of tomato production is consumed as processed products. Both fresh and processed tomatoes have a high nutritional value, due to its content of different types of nutrients: sugars, organic acids, phenolic compounds, vitamins, and minerals. In the latest years, tomatoes became interest subjects for researchers due to their high biological value, antioxidant activity and functional characteristics (Fraser et al., 1994; Arias et al., 2000; Owusu et al., 2014; Abiso et al., 2015; Rubio-Diaz et al., 2011; Helyes et al., 2012; Jones et al., 2003; Urbonaviciene et al., 2012; Adenike, 2012; Popescu and Iordan, 2012), the antioxidant activity being determined by the presence of some bioactive compounds, like polyphenols, flavonoids, anthocyanins, carotenoids, and vitamins.

Tomatoes are usually consumed at the maximum organoleptic quality of the fruits (Arias et al., 2000; Abiso et al., 2015), the quality obtained to the maturity stage (the red colour stage but before excessive softening). Therefore, the tomatoes colour is the most important external characteristic to evaluate the ripeness and the postharvest life. The red colour of tomatoes is conditioned by the presence of lycopene (Urbonaviciene et al., 2012), besides which ones the anthocyanins coloured in orange and yellow are also found (carotene, xanthophyll and xanthophyll esters). The green colour of the unripe tomatoes is due to the presence of chlorophyll (Fraser et al., 1994). In tomatoes, the amount of xanthophyll does not exceed 0.1 mg per 100 g fruit (Fraser et al., 1994; Rubio-Diaz et al., 2011); sometimes xanthophyll missing.

Vegetables are rich sources of phenolic compounds, molecules that can act as antioxidants to prevent heart disease and reduce the inflammation, the incidence of

cancers and diabetes (Dai and Mumper, 2010; Mohanlal et al., 2013; Sawadogo et al., 2012).
Phenolic compounds from plants comprise simple phenols, coumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids and flavonoids. Phenolic acids in plants occur in the form of esters, glycosides or amides, but rarely in free form. They have two parent structures: hydroxycinnamic and hydroxybenzoic acids. Hydroxycinnamic acid derivatives include ferulic, caffeic, p-coumaric and sinapic acids while hydroxybenzoic acid derivatives consist from gallic, vanillic, syringic and protocatechinic acids (Khoddami et al., 2013; Fernández-Panchon et al., 2008).
Flavonoids (figure 1) are a group of polyphenolic compounds which have attracted attention as free radical scavengers with antioxidant activity (Heim et al., 2002). This family includes flavones, flavanones, flavonols, isoflavonols, catechins, anthocyanins, anthocyanidins, and proanthocyanidins (Khoddami et al., 2013; Heim et al., 2002; Dabija et al., 2011). They have a fused ring system consisting of an aromatic ring and a benzopyran ring with a phenyl substituent.

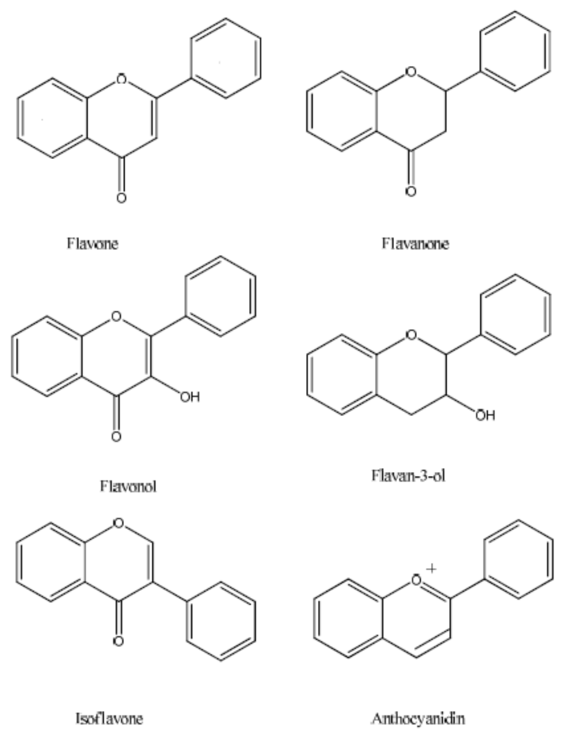

Figure 1. Chemical structures of flavonoids

Flavonoids are often responsible alongside the carotenoids and chlorophylls for their colours (blue, purple, yellow, orange and red). They function as both UV-protection for the plant and pollination aids by providing specific colours or patterns to flowers.

Anthocyanins (figure 2) are a group of natural phenolic compounds in vegetables, mainly detected in flowers and fruits, responsible for bright colours such as orange, red and blue (Tanaka et al., 2008). The characteristic red colour of tomatoes is mainly due to the presence of cyaniding-3-glucoside.

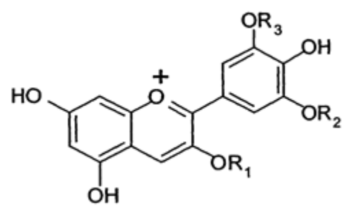

Figure 2. Chemical structure of some anthocyanins (pH<5). R_1: different sugars, R_2: H in pelargonidin, OH in cyaniding and delphinidin, OCH_3 in malvidin, petunidin and peonidin, R_3: H in pelargonidin, cyaniding and peonidin, OH in delphinidin and petunidin, OCH_3 in malvidin.

In the glycosylated forms, anthocyanins are soluble pigments stored in the acidic vacuole of cells, in flowers and fruits, where they are synthesized to attract pollinators and animals for seed dispersal (Mohanlal et al., 2013). The presence of anthocyanins in plant-derived food is also important because their intake in the human diet is associated with protection against coronary heart disease and an improvement in sight. They might prevent cholesterol-induced atherosclerosis, could have anti-inflammatory and anticarcinogenic activities and could aid in the prevention of obesity and diabetes (Lila, 2004).
Carotenoids, such as lutein, β-carotene, and lycopene, are important components of antioxidant defence against lipid peroxidation in living cells (Ramadan et al., 2012). Structurally, carotenoids are isoprenoid compounds with polyene chains that may contain up to 15 conjugated double bonds (figure 3). The most common carotenoids

found in tomatoes are lycopene, red pigment and β-carotene, yellow pigment.

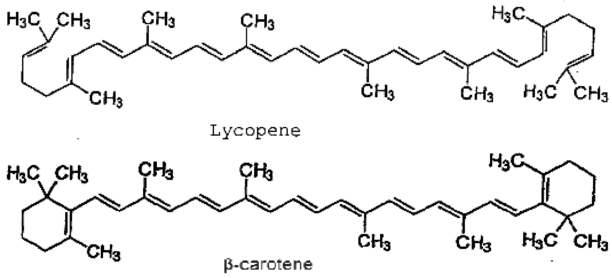

Figure 3. Chemical structure of some carotenoids

Carotenoids consumption has been associated with a lower risk in several types of cancer and a lower incidence of coronary heart disease (Costache et al., 2012). These compounds are responsible for the red, yellow and orange colour of fruits and vegetables.

Carotenoids act as a precursor of vitamin A. Only α-carotene and β-carotene are converted to significant amounts of vitamin A in the body. β-carotene is the most plentiful carotenoid found in fruits and vegetables and it cleaves to form two molecules of vitamin A when it is ingested. β-carotene is also a powerful antioxidant and it has been shown that helps to fight against cancer and heart disease (Martinez-Valverde et al., 2002; Hadley et al., 2002; Shi and Le Maguer, 2000).

Lycopene, an aliphatic hydrocarbon shows strong antioxidant activity, this being the main reason that the development of tomato varieties with increased lycopene content is required. According to Hadley et al., 2002 and Shi and Le Maguer, 2000, lycopene has anticancer properties, protects against certain cancer types (prostate, breast and skin), vascular diseases (atherosclerosis) and diminishes the level of cholesterol in blood.

Phenolic compounds, flavonoids, anthocyanins, and carotenoids have been characterized in tomatoes grown in different countries (Arias et al., 2000; Abiso et al., 2015; Rubio-Diaz et al., 2011; Helyes et al., 2012; Jones et al., 2003; Urbonaviciene et al., 2012; Martinez-Valverde et al., 2002; Hadley et al., 2002; Shi and Le Maguer, 2000); it was found the variation of tomatoes composition with the tomatoes variety, the cultivation conditions, and the handling and storage methods.

In this paper, we proposed to determine the content of some bioactive compounds (polyphenols, flavonoids, anthocyanins, and carotenoids) from four local varieties of tomatoes cultivated in 2014, at I.N.C.D.B.H. Stefanesti, Arges, Romania, using UV-Vis absorption spectroscopy.

2. MATERIAL AND METHODS

Plant material

Four local varieties of tomatoes with determined growing (*Arges 11*, *Arges 16*, *Arges 123*, and *Arges 20*), cultivated in 2014, at I.N.C.D.B.H. Stefanesti, Arges, Romania, were harvested at mature.

Tomatoes were selected for their absence of defects, firmness, uniformity of size and red colour. After collection, the tomatoes were washed, wiped with a paper towel, cut into pieces and converted into a homogeneous puree with a vertical mixer. The samples were stored in a refrigerator.

Chemical substances

Standards of phenolic acids (gallic acid) and flavonoids (catechin) and Folin-Ciocalteu's reagent were purchased from Redox Bucharest - Sigma Aldrich, Romania. Methanol, ethanol, acetone, hexane, sodium hydroxide, sodium carbonate, sodium nitrite, hydrochloric acid and aluminium chloride were purchased from Merck Romania SRL.

Methods

The phenolic compounds were quantified by reaction with the Folin-Ciocalteu reagent (Helyes et al., 2012; Dabija et al., 2011; Ramadan et al., 2012; Martinez-Valverde et al., 2002; Serea and Barna, 2011a,b; Tudor-Radu et al., 2014; Lekbir et al., 2015), the flavonoids content was determined by the colorimetric assay with aluminium chloride (Helyes et al., 2012; Dabija et al., 2011; Martinez-Valverde et al., 2002; Tudor-Radu et al., 2014; Lekbir et al., 2015), the anthocyanins content was determined by extraction with the dilute

solution of hydrochloric acid 1% (Jones et al., 2003; Ramadan et al., 2012; Tudor-Radu et al., 2014), and the carotenoids content was determined by extraction with mixture of hexane, ethanol and acetone (Rubio-Diaz et al., 2011; Urbonaviciene et al., 2012; Adenike, 2012; Popescu and Iordan, 2012; Ramadan et al., 2012), using UV-Vis PerkinElmer Lambda25 spectrophotometer. The content of carotenoids (lycopene and β-carotene) was calculated using its extinction molar coefficients of 184900 $M^{-1}cm^{-1}$ at 470 nm and 172000 $M^{-1}cm^{-1}$ at 503 nm for lycopene (Rubio-Diaz et al., 2011) and 108427 $M^{-1}cm^{-1}$ at 470 nm and 24686 $M^{-1}cm^{-1}$ at 503 nm for β-carotene (Zechmeister and Polgar, 1943), in hexane.
Colour of tomatoes was measured with UV-Vis PerkinElmer Lambda25 spectrophotometer, in accordance to the parameters described in the method Glories (Glories, 1984) and by using the CIE L*a*b* system (Perez-Alvarez et al., 1999; Noui et al., 2014; Giosanu et al., 2013). The samples were prepared in triplicate and the results were expressed as average of three analyses ± standard deviation.

3. RESULTS AND DISCUSSION

Tomatoes have antioxidant characteristics due to the presence of some bioactive compounds such as polyphenols, flavonoids, anthocyanins, and carotenoids.

Table 1. Chemical composition of tomatoes, reported to 100 g fresh tomatoes

Nutrient \ Variety	*Arges 11*	*Arges 16*	*Arges 123*	*Arges 20*
Polyphenols, mg	**2178**	**2061**	**1992**	1939
Flavonoids, mg	**222**	**196**	**194**	193
Anthocyanins, mg	**22.77**	**13.66**	**22.85**	12.52
Lycopene, mg	**13.92**	**17.29**	**8.84**	10.45
β-carotene, mg	1.45	1.18	1.63	1.74

Note: Polyphenols, expressed as mg gallic acid;
Flavonoids, expressed as mg catechin;
Anthocyanins, expressed as mg cyaniding-3-glucoside.

The level of the bioactive compounds (polyphenols, flavonoids, anthocyanins, and carotenoids) in the body is an indicator of health; they function as biomarkers for food quality.
Table 1 shows the chemical composition of tomatoes investigated, specifying the content of polyphenols, flavonoids, anthocyanins, and carotenoids (lycopene and β-carotene).
In examined tomato varieties, the polyphenolic content ranged from 1939 mg (*Arges 20*) to 2178 mg (*Arges 11*) per 100 g fresh tomatoes, expressed as gallic acid equivalents. The high polyphenols content from the examined tomatoes indicated that these compounds contribute to the antioxidant activity and that the tomatoes can be regarded as promising candidates for natural plant sources of antioxidants with high value. Similar results were obtained from Helyes et al., 2012; Martinez-Valverde et al., 2002; Tudor-Radu et al., 2014.
The anthocyanins content was found between 12.52 mg (*Arges 20*) and 22.85 mg (*Arges 123*) per 100 g fresh tomatoes, expressed as cyanidin-3-glucoside equivalents, results being comparable to those presented by Jones et al., 2003; Tudor-Radu et al., 2014 and Mes et al., 2008, which reported the anthocyanins contents between 7.79 and 41.5 mg per 100 g fruit for the various genotypes of tomatoes.

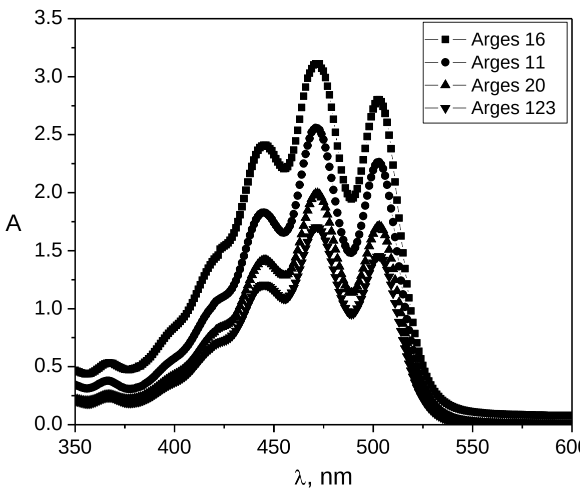

Figure 4. Visible spectra of supernatants of examined tomatoes

The visible spectrum of the tomatoes supernatants (figure 4) shows three absorption maxima located at 443, 471 and 502 nm for lycopene and at 425, 450 and 478 nm for

β-carotene. It is noted that the absorption maximum situated at 360 nm indicates the presence of *cis*-isomers of lycopene (Jones et al., 2003; Urbonaviciene et al., 2012; Zechmeister and Polgar, 1943; Mes et al., 2008; Yang et al., 2005).
In the examined tomato varieties, the lycopene content ranged from 8.84 mg (*Arges 123*) to 17.29 mg (*Arges 16*) and the β-carotene content varied from 1.18 mg (*Arges 16*) to 1.74 mg (*Arges 20*) per 100 g fresh tomatoes. Similar results were obtained from Helyes et al., 2012 and Martinez-Valverde et al., 2002.
Colour is a visual property, perceivable by humans, which derives from the spectrum of light (distribution of light energy versus wavelength). Colour is a rather subjective quality of fruits and therefore it is not easy to quantify (Glories, 1984, Perez-Alvarez et al., 1999). Degree of ripening of vegetables is usually estimated by colour charts (Perez-Alvarez et al., 1999; Noui et al., 2014).
In 1976 was developed the CIE L*a*b* colour system, which defines colour in accordance with to human perception and where all conceivable colours can be located within the colour sphere defined by three perpendicular axes, L* (from white to black), a* (from green to red) and b* (from blue to yellow). The value L* indicates the ratio of white and black colour. Negative values of a* indicates green colour, whereas positive ones indicates a red colour. Also, negative values of b* indicates blue colour and positive ones indicates a yellow colour (Noui et al., 2014; Giosanu et al., 2013).
Evaluation of the tomatoes colour by the Glories method (Glories, 1984) is based on determining the following chromatic parameters: the colour intensity (I), the colour tint (T) and the contribution of each colour (red, yellow and blue) at the tomatoes colour, using the formulas:

$$I = A_{420} + A_{520} + A_{620} \quad (1)$$

$$T = \frac{A_{420}}{A_{520}} \quad (2)$$

$$420\% = \frac{A_{420}}{I} \cdot 100 \quad (3)$$

$$520\% = \frac{A_{520}}{I} \cdot 100 \quad (4)$$

$$620\% = \frac{A_{620}}{I} \cdot 100 \quad (5)$$

where: 420 % is the yellow colour contribution, 520 % is the red colour contribution and 620 % is the blue colour contribution.

In all examined tomato varieties (Table 2), the red and yellow pigments have the major contribution (31-48 %) to the tomatoes colour while the blue pigments contribute in a lesser extent (<27 %) to the tomatoes colour.

Table 2. Chromatic parameters of the tomatoes determined by the Glories method

Nutrient \ Variety	*Arges 11*	*Arges 16*	*Arges 123*	*Arges 20*
I	**0.8343**	**2.3959**	**2.9033**	1.1067
T	**1.52**	**1.37**	**1.33**	1.32
420%	**47.18**	**44.05**	**43.01**	43.14
520%	**30.96**	**32.17**	**32.29**	32.55
620%	21.86	23.78	24.71	24.31

The colour of tomatoes was measured by the determination of L*, a* and b* chromaticity coordinates of the CIE L*a*b* scale (Perez-Alvarez et al., 1999, Noui et al., 2014; Giosanu et al., 2013). The measurements were taken in juice of tomatoes and the spectrophotometers (illuminant D65, 10° observer) was calibrated with a perfect speaker corresponding to the light considered white (Xn= 81.3, Yn= 86.2, Zn= 92.7).
Evaluation of the tomatoes colour in the CIE L*a*b* system is based on determining the following chromatic parameters: lightness (L*), redness (a*) and yellowness (b*), using the formulas:

$$L^* = 116 \cdot \left(\frac{Y}{Y_n}\right)^{1/3} - 16 \quad (6)$$

$$a^* = 500 \cdot \left[\left(\frac{X}{X_n}\right)^{1/3} - \left(\frac{Y}{Y_n}\right)^{1/3}\right] \quad (7)$$

$$b^* = 200 \cdot [(\frac{Y}{Y_n})^{1/3} - (\frac{Z}{Z_n})^{1/3}] \quad (8)$$

where:

$$X = 0.42 \cdot T_{625} + 0.35 \cdot T_{550} + 0.21 \cdot T_{445},$$
$$Y = 0.2 \cdot T_{625} + 0.63 \cdot T_{550} + 0.17 \cdot T_{445},$$
$$Z = 0.24 \cdot T_{495} + 0.94 \cdot T_{445},$$

assuming: $\frac{X}{X_n} \geq 0.01, \frac{Y}{Y_n} \geq 0.01, \frac{Z}{Z_n} \geq 0.01.$

The a* and b* variables are related with hue and saturation. Also, chroma (C*) were then calculated, according to the formula:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (9)$$

and hue angle (H°) according to the formula:

$$H° = \tan^{-1}(\frac{b^*}{a^*}). \quad (10)$$

The chroma value indicates the degree of saturation of colour and is proportional to the strength of the colour. The parameters L*, a* and b* are measured in NBS units, hue angle H° is measured in degrees from 0 to 360°. The NBS unit is a unit of the USA National Standard Bureau and corresponds to one threshold of colour distinction, which the trained human eye maybe notice. The average values of the colour parameters from the CIE L*a*b* system for the examined tomatoes are presented in Table 3.

Table 3. Chromatic parameters of the tomatoes in the CIE L*a*b* system

Variety / Nutrient	*Arges 11*	*Arges 16*	*Arges 123*	*Arges 20*
X	**61.92**	**17.72**	**9.69**	29.66
Y	**62.21**	**16.92**	**9.02**	29.04
Z	**60.96**	**11.45**	**5.13**	26.38
L*	**87.94**	**51.39**	**38.75**	64.74
a*	**8.5**	**11.5**	**10.5**	9.5
b*	**5**	**16.4**	**18.4**	7.6
C*	**9.86**	**20.03**	**21.19**	12.17
$\frac{b^*}{a^*}$	**0.58**	**1.43**	**1.75**	0.8
H°	30.11	54.96	60.26	38.66

For the varieties of tomatoes grown in 2014, at I.N.C.D.B.H. Stefanesti, L* values ranged from 38.75 to 87.94, a* values ranged from 8.5 to 11.5, b* values ranged from 5 to 18.4, C* values ranged from 9.86 to 21.19 and H° values ranged from 30.11 to 60.26. In the varieties of analysed tomatoes, L*, b*, C* and H° presented significant variation while a* presented small variation.

Similar results were obtained from Radzevičius et al., 2013; 2014, which reported that L* ranged from 42.3 till 50.7, a* varied from 12.9 till 26.1, b* varied from 28.8 till 36.5, C* varied from 32.5 till 44.1 and H° varied from 49.3 up to 66.6 for the various genotypes of tomatoes grown in Lithuania.

4. CONCLUSIONS

The colour is one of the most important attributes which affects the consumer perception, being also an indicator of the vegetable pigment concentration. The method of the chromatic characteristics assessment is the most complete model used for the description of colours in the visible spectra because it provides the possibility of assessing the difference between two close colours, by all parameters determined (shade, saturation and luminosity).

Results obtained for the varieties of tomatoes grown in 2014, at I.N.C.D.B.H. Stefanesti, Arges, Romania, established the correlation between the content of lycopene and the colour parameter a* and the correlation between the content of β-carotene and the colour parameter b*. The CIE L*a*b* method makes it easier to understand the relation between the visual appearance of the colour and the numeric value of the chromatic parameters from coloured vegetable materials.

Significant differences were detected between varieties of cultivated tomato in 2014, at I.N.C.D.B.H. Stefanesti, concerning the antioxidants compounds. Since tomatoes varieties were grown under the same agricultural, geographical and climatic conditions, the obtained results showed variability in the content of some bioactive compounds from tomatoes, like polyphenols, flavonoids, anthocyanins, and carotenoids, due to the influence of the variety.

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