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Artigo

Evaluation of Lipophilic Antioxidant Capacity and Lycopene Content in Brazilian Tomatoes

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Avaliação da Capacidade Antioxidante Lipofílica e Conteúdo de Licopeno em Tomates Brasileiros

Resumo: O objetivo deste trabalho foi determinar a capacidade antioxidante lipofílica (CAL) e o conteúdo total de licopeno de várias amostras de tomate, sendo que uma delas (BRS-Zamir) é uma variedade especialmente desenvolvida pela EMBRAPA para possuir um alto teor de licopeno em sua composição. Nas cultivares BRS Zamir, Honey Grape e Holandês foram detectadas os maiores valores de CAL/licopeno enquanto que na variedade Sweet Cherry foram observadas as menores quantidades. O tomate BRS Zamir possui uma quantidade de licopeno que é superior em relação a diversos tipos de tomate, mas menor quando comparado a outros tomates desenvolvidos especificamente para obtenção de altos teores de licopeno.

Palavras-chave: FRAP; Tomato; Lycopene; DPPH; ORAC.

Abstract

This work aims at the determination of lipophilic antioxidant capacity (LAC) and total lycopene content of several Brazilian tomato cultivars, one of them (BRS Zamir) being a high-lycopene sample type developed by EMBRAPA. The BRS Zamir, Honey Grape and Holandês cultivars showed the highest LAC/lycopene values while the Sweet Cherry varietiy gave the lowest. The BRS cultivar possesses a lycopene amount which is superior regarding many tomato types, but lower in relation to other high-lycopene tomatoes.

Keywords: FRAP; Tomato; Lycopene; DPPH; ORAC.

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Evaluation of Lipophilic Antioxidant Capacity and Lycopene Content in Brazilian Tomatoes

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1. Introduction

Tomato (*Solanum lycopersicon L*) is a worldwide consumed fruit.[1] While being available in many forms for human consumption, it is an important dietary source of bioactive compounds with antioxidant activity, such as lycopene, polyphenols, ascorbic acid, b-carotene and lutein.[1] There is a great interest in the study of carotenoids from tomato samples, since a prominent deficiency of β-Carotene may lead to diseases such as xerophthalmia and blindness, while regular intakes of lutein can reduce the probability of occurrence of macular degeneration, which is related to natural aging, and cataracts.[2] The presence of all these bioactive compounds justifies the labeling of tomatoes as functional foods.[3]

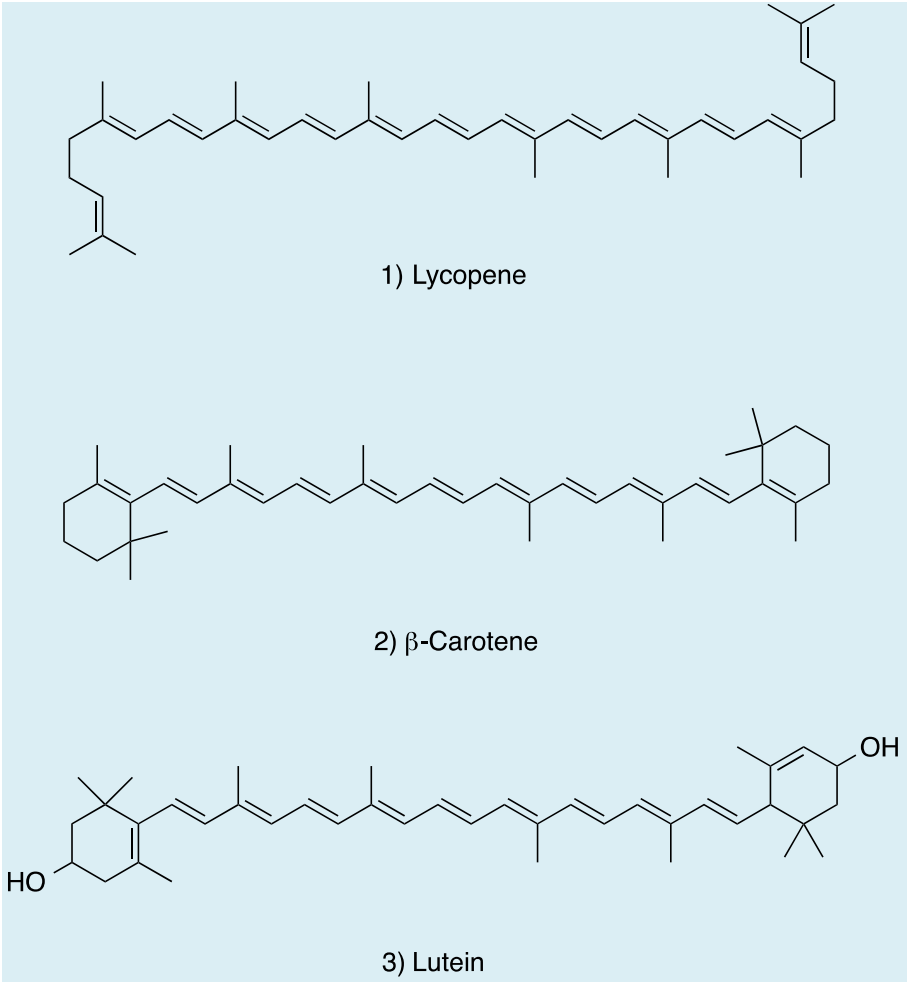

Figure 1. Chemical structures of tomato carotenoids

Actually, there are several studies on nutritional value and antioxidant capacity regarding tomato varieties from different locations, which were designed for different purposes. Nevertheless, until now exist few reports in relation to national cultivars, moreover on local varieties with naturally increased amounts of lycopene.[4]

A special emphasis is given to lycopene in this article because this compound is recognized to have the strongest antioxidant activity among 600 carotenoids from natural origin. The ability of lycopene to react with free radicals is two and ten times higher in relation to other antioxidant compounds such as β-carotene and β-tocopherol, respectively. Therefore, lycopene helps to protect macromolecules against oxidative damage, regulating expression of genes, modulating immune responses and improving the antioxidant status of human plasma.[5]

The demand for functional foods is an actual matter of great relevance since: (a) they represent a great commercial niche due to their visual and nutritional properties, (b) the awareness of food/health relationship by consumers is increasing and (c) this food type is widely used from the industrial point of view in formulations of food additives/supplements. In this context, new tomato cultivars with increased levels of lycopene were developed by methods of plant breeding in order to attend this crescent demand of functional foods.[6]

The antioxidant capacity of several tomato varieties has been also determined through different assay types. It was concluded that there is a great dependence between antioxidant activity of tomato extracts and

tomato cultivar/employed assay. Lycopene, along with caffeic and ferulic acids, showed a significant positive correlation with antioxidant capacity from tomato samples.[7] However, these relations were not tested nor confirmed in Brazilian tomatoes.

Therefore, this work aims at the determination of lipophilic antioxidant activity and total lycopene content of several Brazilian tomato cultivars, one of them (BRS Zamir) being a local high-lycopene sample type.

2. Materials and Methods

The experimental sections of this article follow the fluxogram which is showed below:

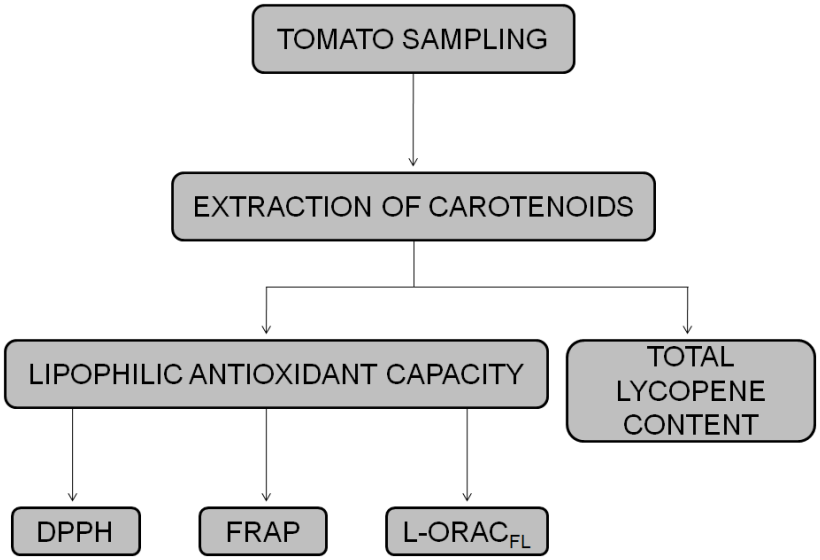

Figure 2. Fluxogram of experimental procedures

2.1. Sampling

Tomatoes of BRS Zamir cultivar, produced by the Brazilian Agricultural Research Corporation (EMBRAPA), were studied in the present work, together with dried Goji berries and the following tomato cultivars: "Sweet Grape" "Sweet Cherry", "Honey Grape", "Italiano", "Holandês" and "Longa Vida". It is important to mention that every sample, with exception to BRS Zamir tomatoes, was acquired from the local commerce. All tomatoes were planted and harvested in the 2014 growing season (February-June). All samples were acquired and processed on the same month (May). For every cultivar type, fruits were submitted to the pooling process and 2 kg of selected samples, visually free of bruises and other injuries, were taken for further processing. After this step, samples were diced and homogenized in a domestic blender for 2 min and submitted for analyte extraction.[1] The moisture content of every sample was determined using AOAC Official Method 930.15.[8] It is also important to mention that, with exception to dried goji berry, only fully ripe samples were chosen for this work because the ripening stage, along with cultivar type, greatly affects, in tomato fruits, the amounts of the compounds which were described in the introduction of this article.[9] Besides, peel and seeds were not removed from samples, since they are important contributors to the overall antioxidant capacity and lycopene content of tomatoes, especially in cherry type tomatoes (which is the case for BRS Zamir, Sweet Cherry, Sweet Grape and Honey Grape cultivars).[10]

According to Szuvandzsiev *et al.*,[11] most of the lycopene content in tomatoes is concentrated in their peels, followed by fruit wall, placenta and seed.

2.2. Extraction of Carotenoids

Approximately 5 g of previously prepared tomato/goji berry sample was measures into a 200 mL amber colored erlenmeyer wrapped with aluminum foil and homogenized with a mixer. A 1:1 (v/v) hexane-ethanol mixture was added to the flask and submitted to continuous sonication, until the sample did not possess any color, on an ultrasonicator (from Cristofoli brand, with the following dimensions: 26.4 cm (length), 16.4 cm (width), 8 cm (depth), 42 KHz (frequency) and 160 watts (power)). This ultrasound assisted extracton method was chosen because it demands less amounts of time and solvents regarding conventional liquid-liquid solvent extraction.[12] The extract was isolated in a separatory funnel with addition of 5 mL distilled water (to force system separation into two distinct layers, one polar and the other being nonpolar). The non polar phase containing the compounds of interest (carotenoids, especially lycopene) was concentrated through rotary evaporation at 30 °C until 150 mL.[1]

2.3. Lipophilic Antioxidant Capacity Analysis of Extracts Through Frap Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was executed according to the steps which were described in the article of Kaur *et al.*[1] The FRAP reagent was prepared by mixing 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol L^{-1} TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol L^{-1} HCl and 20 mmol L^{-1} $FeCl_3$ in a 10:1:1 (v:v:v) ratio. Approximately 3 mL of FRAP reagent was mixed with 100 µL of sample extract in an amber colored erlenmeyer wrapped with aluminum foil. The system was mixed in a magnetic stirrer and incubated at 37 °C for 30 min in an appropriate water bath. The reduction of ferric-TPTZ complex to ferrous-TPTZ through the action of antioxidant compounds from sample extract was measured in a Genesys 10 uv UV-vis spectrophotometer (from Thermo Scientific brand) at 593 nm. Methanolic solutions of Trolox ((±)-6-hydroxi-2,5,7,8-tetramethylcromane-2-carboxylic acid) with different concentrations were used as a standard to achieve a calibration curve and the antioxidant capacity was expressed in mmol of Trolox equivalent (TE) g^{-1} of fresh sample. The following calibration curve (equation 1) was used:

$$y = 0.001x + 0.0321 \ (r^2 = 0.9999) \qquad (1)$$

2.4. Lipophilic Antioxidant Capacity Analysis of Extracts Through DPPH Assay

The lipophilic antioxidant capacity of extracts was also measured through the DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical capture method, according to Brand-Williams, Cuvelier and Berset,[13] with some modifications.[14] Solutions of extracts with concentration of 2 mg mL^{-1} were prepared, then a 25.0 µL aliquot was mixed with 2.0 mL of a DPPH $6.25x10^{-5}$ mol L^{-1} methanolic solution. The mixture was stored away from any light source for 30 min and the absorbance was measured at 517 nm in the same spectrophotometer (model Genesys 10 uv) which was used in the FRAP assay. Methanolic solutions of Trolox with different concentrations were also used to achieve an appropriate calibration curve and the antioxidant capacity was expressed in mmol of Trolox equivalent (TE) g^{-1} of fresh sample. The following calibration curve (equation 2) was used:

$$y = -0.0005x + 0.8278 \ (r^2 = 0.9983) \qquad (2)$$

2.5. Lipophilic Antioxidant Capacity Analysis of Extracts Through L-ORAC$_{FL}$ assay

Lipophilic antioxidant capacity (LAC) was determined according with Huang *et al.*,[15] using a spectrofluorimeter of PerkinElmer brand, VictorTMX4 model.

Twenty microliters of extract were added to the microplates. For blank and calibration curve, 20.0 µL of a 1:1 (v/v) hexane-ethanol mixture and Trolox solutions with different concentrations were used, respectively. Two hundred microliters of fluorescein 4.0 mmol L^{-1} in phosphate buffer (pH=7) were added.

The microplate was inserted on the spectrofluorimeter and heated to 37 °C. Then, 75.0 µL of an AAPH (2,2-azobis(2-aminopropane) dihydrochloridre) 17.2 mg mL^{-1} solution were added for L-ORAC analysis. After all these additions, reading on the spectrofluorimeter was immediately started until completion of 30 cycles with intervals of 1 min. The employed emission and excitation wavelengths were 515 and 485 nm, respectively.

Results were expressed in mmol of Trolox equivalent (TE) g^{-1} of fresh sample. The following calibration curve (equation 3) was used:

$$y = 0.1957x + 1.8651 \ (r^2 = 0.9856) \quad (3)$$

Where: x = ORAC value in µmol g-1 TE; y= area below fluorescence decay curve (AUC) of sample or standard minus area below fluorescence decay curve of blank. AUC can be calculated through equation 4:

$$AUC = (1+f1/f0 + f2/f0+...fn+1/f0) \quad (4)$$

Where f0 is initial intensity of fluorescence and fn is the fluorescence intensity on time n.

2.6. Total Lycopene Content

Total Lycopene content from the sample lipophilic extracts was determined and quantified according to the method developed by Fish *et al.*[16] This same author states that conventional spectrophotometric assays for this type of compound are simple, cheap, fast and reliable. Besides, normal analytical quantification of lycopene is complicated and demands a lot of time.[11] In order to achieve such quantification, the absorbance from all extracts was measured at 503 nm in a spectrophotometer model Genesys 10 uv (from Thermo Scientific brand), since lycopene, unlike other carotenoids, has a maximum absorbance in this particular wavelength. Total lycopene was calculated according to equation 5:

$$Lycopene = (A_{503} \times 0.0312)/m \quad (5)$$

Where Lycopene is quantified in mg kg^{-1} of sample, m refers to sample mass in kg and A_{503} is the absorbance of the sample extract which was read in a cuvette made of quartz, with a path length of 1 cm, at 503 nm.

All data was acquired in triplicate. Variance analysis (ANOVA) was applied to every result and means were submitted to comparison through the Tukey test with the Statistica 7.0 software. The significance level which was employed for rejection of null hypothesis was 5% ($p < 0.05$). Pearson correlation coefficients (R) used to determine the relationships between the obtained results were calculated by using Microsoft Office Excel 2007 software.

3. Results And Discussion

Table 1 shows the results of lipophilic antioxidant capacity, total lycopene content and moisture values. Regarding moisture values, they were higher than 90% for every

sample, with exception for goji berries (15.55%). For tomatoes, the obtained values are in accordance with the reported data for this sample type.[4,7,17]

Table 1. Results of antioxidant capacity by DPPH, FRAP and L-ORAC$_{FL}$ assays (mmol TE g^{-1}), lycopene (mg kg^{-1} sample) and moisture (g 100g^{-1} sample)

Cultivar	DPPH	FRAP	L-ORAC$_{FL}$	Lycopene	Moisture
Sweet Cherry	0.76±0.10^{e}	0.15±0.03^{e}	2.4±0.15^{e}	3.37±0.28^{d}	92.95±0.07^{c}
Sweet Grape	2.24±0.31^{cd}	1.41±0.02^{de}	20.21±0.95^{d}	38.99±0.75^{b}	92.89±0.36^{c}
Longa Vida	1.45±0.15^{de}	2.56±0.68^{cd}	2.8±0.13^{e}	30.87±0.36^{c}	94.31±0.02^{b}
Italiano	2.14±0.20^{cd}	2.75±0.28^{cd}	4.51±0.85^{e}	28.73±0.12^{c}	95.64±0.03^{a}
Goji	2.95±0.20^{c}	2.62±0.25^{cd}	34.94±1.25^{c}	0.14±0.01^{d}	15.55±0.03^{d}
Holandês	2.60±0.47^{c}	3.49±0.45^{bc}	46.56±2.01^{b}	40.15±2.0^{b}	95.03±0.01^{ab}
Honey Grape	4.22±0.45^{b}	4.54±0.12^{b}	50.01±1.23^{b}	43.39±1.52^{b}	92.71±0.41^{c}
BRS Zamir	10.31±0.82^{a}	17.08±1.05^{a}	85.39±2.15^{a}	144.02±3.75^{a}	92.78±0.42^{c}

Results expressed as mean ± standard deviation for analysis in three replicates. Means followed by different superscript letters (^{a}) in same column are significantly different by Tukey´s test ($p<0.05$).

The BRS Zamir cultivar showed the greatest LAC values, regardless of employed assay, followed by honey grape and holandês cultivars. The worst tomato, in terms of antioxidant capacity, was sweet cherry.

The DPPH and FRAP LAC values of every sample of this work were superior regarding the following tomatoes which were studied in New Delhi, India: Taiwan, Balkan, Pusa Gaurav, Pusa Ruby, Roma, Pusa Uphar, Pusa sadabahar, Avikash, Pusa cheetal and Chiku.[1] According to the table 2, the LAC values from Indian samples through DPPH method ranged from 0.00021 to 0.00024 mmol TE g^{-1} sample, while total antioxidant capacity values from Indian samples through FRAP method ranged from 0.00225 to 0.00252 mmol TE g^{-1} sample.[1] It is very important to note that originally Kaur *et al.*,[1] published their results in µmol TE g^{-1} sample, but all relevant data was converted to mmol TE g^{-1} sample in order to standardize the numbers in table 2 and, therefore, allow a correct comparison of data. Since our procedures for sample extraction and LAC determination were similar in relation to what was proposed by these authors,[1] it is likely that the differences between results may be explained through the different chemical compositions of samples, which are consequences of the particular edaphoclimatic factors and post-harvest storage conditions which are applied to tomato cultivars.[10]

L-ORAC$_{FL}$ results were higher than the obtained through DPPH and FRAP methods, probably because it responded to a greater number of antioxidant compounds in relation to the other LAC methodologies. Some authors[18] state that other antioxidant capacity assays, despite being simpler and cheaper in relation to L-ORAC, give underestimated results regarding foods or beverages of a complex composition, while Cao and Prior[19] noted that the ORAC method had greater specificity and was more versatile for reacting with different classes of antioxidant compounds than other antioxidant capacity methods. Other authors also report that carotenoids show low responses through FRAP assay.[1,20] As discussed in the introduction, the lipophilic fraction of tomato extracts have many carotenoids into its composition. Besides, along with data of Kaur *et al.*,[1] it can be

concluded that DPPH and FRAP assays are the least recommended for measuring antioxidant activity of tomato extracts.

The analyzed Longa Vida cultivar showed a total lycopene value of 30.87±0.36 mg kg^{-1}, higher than the established for Eroe F1 salad tomatoes from Italy by Zanfini *et al.*[3] (around 8.5 mg kg^{-1}). However, the BRS Zamir cultivar showed a total lycopene value which is higher than the determined for Naomi F1 cherry tomatoes by the same authors cited above (around 112.7 mg kg^{-1}).

Table 2. Comparison of antioxidant capacity results with data from Kaur *et al.*,[1] (mmol TE g^{-1} sample)

Cultivars from this work	DPPH LAC values	FRAP LAC values	Cultivars studied by Kaur *et al.*,[1]	DPPH[1] LAC values	FRAP[1] TAC values
Sweet Cherry	0.76±0.10^{e}	0.15±0.03^{e}	Taiwan	0.00023±0.0003^{c}	0.00247±0.00039^{c}
Sweet Grape	2.24±0.31^{cd}	1.41±0.02^{de}	Balkan	0.00021±0.0001^{c}	0.00245±0.00030^{c}
Longa Vida	1.45±0.15^{de}	2.56±0.68^{cd}	Pusa Gaurav	0.00024±0.0002^{c}	0.00233±0.00011^{c}
Italiano	2.14±0.20^{cd}	2.75±0.28^{cd}	Pusa Ruby	0.00024±0.0008^{c}	0.00267±0.00022^{c}
Goji	2.95±0.20^{c}	2.62±0.25^{cd}	Roma	0.00017±0.0001^{c}	0.00248±0.00037^{c}
Holandês	2.60±0.47^{c}	3.49±0.45^{bc}	Pusa Uphar	0.00012±0.0005^{c}	0.00225±0.00018^{c}
Honey Grape	4.22±0.45^{b}	4.54±0.12^{b}	Pusa sadabahar	0.00021±0.0001^{c}	0.00252±0.00030^{c}
BRS Zamir	10.31±0.82^{a}	17.08±1.05^{a}	Avikash	0.00019±0.00015^{c}	0.00233±0.00023^{c}
			Pusa cheetal	0.00022±0.0001^{c}	0.00234±0.00039^{c}
			Chiku	0.00017±0.0001^{c}	0.00250±0.00026^{c}

Results expressed as mean ± standard deviation for analysis in three replicates. Means followed by different superscript letters (^{a}) in same column are significantly different by Tukey´s test ($p<0.05$).

In general, according to Zanfini *et al.*,[3] cherry type tomatoes have higher lycopene content and antioxidant capacity than other types of this fruit. In our work, the sweet grape, honey grape and BRS Zamir showed the highest LAC values, and these three are all cherry-type tomatoes. The exception accounts for the Holandês cultivar.

In relation to the lycopene contents which were determined, most of the samples showed values which are inside the range for many *Solanum lycopersicon L* fruits (18 to 90 mg kg^{-1} sample).[1,4,21] The notorious differences were found in Sweet Cherry and BRS Zamir cultivars. A low lycopene value was already expected for Sweet Cherry tomato, since it possesses a yellow hue (an indicative that this carotenoid exists in low amounts in this particular tomato sample).

In relation to BRS Zamir, the observed trend for it was also expected, since the BRS Zamir cultivar was especially created with emphasis on high lycopene contents. Besides, the lycopene content of BRS Zamir cultivar was also superior regarding to tomatoes which were studied by Kaur *et al.*[1] in New Delhi, India: Taiwan, Balkan, Pusa Gaurav, Pusa Ruby, Roma, Pusa Uphar, Pusa sadabahar, Avikash, Pusa cheetal and Chiku.

The lycopene values from Indian samples ranged from 43.1 to 59.7 mg kg^{-1} sample.[1]

Rambo, Senior, Ramillete, Liso, Pera, Canario, Durina, Daniella, and Remate varieties from a Spanish supermarket showed lycopene values ranging from 18.60 to 64.98 mg kg^{-1}.[21] The literature also reports lycopene values regarding four different tomato farmer' varieties from northeastern Portugal (Amarelo, Batateiro, Comprido and Coração) of approximately 50.2, 94.9, 81.0 and 92.2 mg kg^{-1}, respectively. Although most of the analyzed tomates from this study did not show results far from these numbers, all these values are below in relation to what was determined for BRS Zamir.[4]

However, despite being a high-lycopene tomato cultivar, its content of this carotenoid is inferior regarding other samples which were grown for the same end, such as HLY02, HLY13 and HLY18 from southern Italy.[6] This difference is inherent of the genetic procedures which were applied from every cultivar, in addition to edaphoclimatic factors. However, these procedures are particular data of the enterprises which produced such tomatoes.

Table 3 shows the correlations between LAC and lycopene values. The greatest correlation was obtained with both DPPH and FRAP methods. This trend was expected, since these methods possess the same reaction mechanism (electron transference). An explanation about why a perfect correlation was not achieved relies on the fact that different solvents are used for solubilization of radicals during execution of LAC assays. The DPPH-ORAC and FRAP-ORAC correlation values were lower in relation to DPPH-FRAP. Zulueta *et al.*[18] justifies these facts through the different kinetics and reaction mechanisms of the various antioxidants present: while DPPH and FRAP methods are based on electron transference, the ORAC assay is based on hydrogen atom transference.

Table 3. Pearson correlation values (r) between results

	r	Equations of curves
DPPH-FRAP	0.9643	y = 1.741x - 1.4763
DPPH- L-$ORAC_{FL}$	0.8098	y = 8.7924x + 1.546
FRAP-L-$ORAC_{FL}$	0.7124	y = 4.6513x + 10.727
DPPH-Lycopene	0.8609	y = 0.0623x + 0.7657
FRAP-Lycopene	0.9094	y = 0.1135x - 0.3514
L-$ORAC_{FL}$-Lycopene	0.6151	y = 0.5145x + 9.65

All LAC data, with exception to ORAC, showed good correlation values with total lycopene content, despite the fact that carotenoids do not have ferric reducing ability, therefore they should not react in FRAP method. However, Ilahy *et al.*[22] obtained an excellent correlation value (0.820) between FRAP and lycopene after analyzing several high lycopene tomato fruits. Nevertheless, from the literature[23] it can be concluded that LAC of Brazilian tomato fruits is mainly attributed to the presence of carotenoids, particularly lycopene.[5]

4. Conclusion

For the set of samples which were analyzed, all LAC/lycopene assays showed defined trends: BRS Zamir, Honey Grape and Holandês cultivars showed the highest LAC/lycopene values while Sweet Cherry variety gave the lowest. The L-$ORAC_{FL}$ assay was proven to be the most suitable for determination of LAC from tomato and goji berry samples. The BRS cultivar possesses a

lycopene amount which is superior regarding many tomato types which were previously analyzed by other authors, but inferior to other High-lycopene cultivars.

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