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ANTIOXIDANT ACTIVITY OF EIGHT TOMATO (*LYCOPERSICON ESCULENTUM* L.) VARIETIES GROWN IN ALGERIA

Bachir Bey Mostapha¹ --- Louaileche Hayette² --- Mouhoubi Zina³

1²²³Laboratoire de Biochimie Appliquée, Faculté des sciences de la nature et de la vie, Université de Bejaia, Bejaia, Algérie

ABSTRACT

The harmful effects of the free radicals on human organism could be inhibited by antioxidants of fruits and vegetables such as tomato. In the present work, the antioxidant contents as well as the antioxidant activity of eight tomato varieties grown in Algeria are evaluated. Ascorbic acid and carotenoid contents are assessed using spectrophotometric methods. The phenolic compounds extracted using solvents with different polarities (methanol, 50% methanol, ethanol, and 50% ethanol) have been determined using the Folin-Ciocalteu reagent. The antioxidant activities have been evaluated using the DPPH scavenging activity and the reducing power assays. Ascorbic acid content ranged between 7 and 16.7mg/100g while carotenoid concentration varied from 5.7 to 9.57mg/100g. The phenolic content varies according to the variety and the extraction solvent; pure alcohols (ethanol and methanol) allow better extraction than the diluted ones (50% ethanol and 50% methanol). Linear correlations are noted between the antioxidant activity and phenolic, carotenoid and lycopene contents. The results indicate that the samples present variations in their antioxidant substance amounts and antioxidant activity; this could be attributed to the varietal factor. The hybridisation between Joker and Marmande varieties, which present the highest phenolic and carotenoid amounts, respectively, could give another variety with a high antioxidant activity.

Keywords: Antioxidants, Tomato, Variety, Ascorbic acid, Carotenoids, Phenolics, Extraction solvent.

Contribution/ Originality

This study documents for the first time the phytochemical content (ascorbic acid, total carotenoids, lycopene and total phenolics) and antioxidant activity (DPPH radical scavenging activity and reducing power) of some tomato varieties grown in Algeria.

1. INTRODUCTION

Food can largely influence free radical effects on the organism. Thus, for a few years, the evidence has accumulated to affirm that an increase in the fruits and vegetables consumption is a good mean of protection against the free radicals. The preventive effects of these plants are due to the presence of some molecules such as polyphenolic compounds, carotenoids and certain vitamins which are opposed to the harmful action of these reactive substances (Wootton-Beard and Ryan, 2011; Harasym and Oledzki, 2014).

The epidemiological studies revealed a weak incidence of several diseases in the Mediterranean populations. This protection is due to the Mediterranean diet, which consists of vegetables and fruits intake generally, and tomato with the olive oil particularly (Weisburger, 2002; Grosso *et al.*, 2013).

Tomato (*Lycopersicon esculentum*) is one of the most consumed foods throughout the world. 162 million tonnes are produced in the world and 0.8 million tonnes in Algeria in the year 2012 (FAO, 2012). Tomato is consumed raw, in salads, or after cooking. It is also used in the various derivative products (juice, paste,...). Dietary intake of tomato and tomato products containing lycopene has been shown to be associated with a decreased risk of chronic diseases such as cancer (breast, colon, prostate) and cardiovascular diseases (Agarwal and Rao, 2000; Willis and Wians, 2003).

Lycopene, the carotenoid which gives tomato its reddish colour, is one of the most powerful scavenger of oxygen singlet and free radicals in body, and plays an important role in many biological functions, such as the modulation of intercellular gap-junction communication, hormonal and immune systems, and metabolic pathways (Woodall *et al.*, 1997; Livny *et al.*, 2002; Blum *et al.*, 2005; Basuny *et al.*, 2009).

In addition to carotenoids, tomato contains a variety of natural antioxidants, including ascorbic acid and phenolic compounds. The antioxidant content of tomato mostly depends on both genetic and environmental factors and the ripening stage (Hart and Scott, 1995; George *et al.*, 2004; Hallmann, 2012; Nour *et al.*, 2013). To our knowledge, no information is available about antioxidant activity of Algerian tomato varieties. Therefore, the aim of this investigation is to determine the content of various antioxidant substances (ascorbic acid, total carotenoids, lycopene and total phenolics) and antioxidant activity of eight tomato varieties grown in Algeria.

2. MATERIALSANDMETHODS

2.1. Tomato Samples

Eight commercial tomato (*Lycopercicon esculentum*) varieties are used in this study. At the exception of Joker which was cultivated in open-air, all the other varieties (Marmande, Sammichele, Agora, Zahra, Tafna, Nattih and Kiti) were grown in greenhouse. The healthy fruits, freshly harvested, uniformly ripened at the red ripe stage, are analysed. 1kg of each variety is selected randomly from three Algerian localities (Bejaia, Biskra and Tipaza), to study the different antioxidant contents and to explore the antiradical activity and the reducing power. Tomato fruits are crushed in a mortar and the homogeneous mass is taken for analysis.

2.2. Ascorbic Acid Determination

Ascorbic acid (AA) is determined according to the method of Klein and Perry (1982) with slight modifications. One gram of tomato homogenate is extracted with 10 ml of 1% oxalic acid for 45 min and filtered. The filtrate (3ml) is mixed with 1 ml of 2,6-dichloroindophenol and the

absorbance is read at 515nm using an UV-visible spectrophotometer (UVmini 1240, Schimadzu, China). The AA content, expressed as mg/100g fresh matter, is calculated on the basis of the L-ascorbic acid calibration curve.

2.3. Total Carotenoid and Lycopene Determination

Carotenoids are extracted according to the method described by (Sadler *et al.*, 1990); 2g of sample are extracted with 20ml of n-hexane: acetone: ethanol (2:1:1) by shaking for 30min. The upper layer is pipetted into a 50ml flask and the extraction procedure is repeated for the bottom layer with 10ml of hexane. The absorbance of the combined extracts is measured at 420nm. Total carotenoid content is calculated according to the calibration curve of β -carotene, and expressed as milligrams per 100g of fresh matter. The lycopene content is measured in the hexanic extract at 472nm using an extinction coefficient $E^{1\%}_{1cm}$ of 3450, as reported by Martínez-Valverde *et al.* (2002).

2.4. Total Phenolics Determination

Extraction is performed twice using the modified procedure of Mau *et al.* (2005). Four solvents, methanol, 50% methanol (v/v), ethanol and 50% ethanol (v/v) are used for the extraction of phenolic compounds. Five grams of homogenized tomatoes are extracted with 25ml of solvent for 24h and the mixture is filtered. The residue is then extracted with additional 25ml of solvent as described above. The phenolic compound content in the combined extracts is determined according to Singleton and Rossi (1965) and the results are expressed as gallic acid equivalents (GAE) per 100g fresh matter.

2.5. DPPH Radical Scavenging Assay

2.5.1. DPPH Radical Scavenging Assay of Alcoholic Extracts

The scavenging activity of alcoholic extracts for the radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was determined after Brand-Williams *et al.* (1995); 1 ml of the extract is added to 2ml of methanolic DPPH solution (20µg/ml). The decolourizing process is recorded at 517nm, after 30min of reaction and compared to a blank control. The percentage of DPPH⁻ scavenging is calculated according to the equation:

% DPPH $\cdot_{scavenging} = 100 (A_c - A_s)/A_c$

 $A_c = absorbance of control, A_s = absorbance of sample.$

2.5.2. DPPH Radical Scavenging Assay of Hexanic Extracts

The antioxidant activity of carotenoids is measured in terms of radical-scavenging ability according to the modified procedure of Jiménez-Escrig *et al.* (2000); 400µl of hexanic extract are mixed with 2ml of DPPH solution. The absorbance is measured at 580nm and the percentage of DPPH scavenging is expressed as described above.

2.6. Reducing Power Determination

The reducing power of the alcoholic extracts is determined by the method described by Yen and Chen (1995); 1ml of extracts is mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml potassium ferricyanide (1%). The mixture is incubated at 50°C for 20min; 2.5ml of trichloroacetic acid (10%) is added to the mixture, which is then centrifuged at 1400g (Sigma 2-16K) for 10min. Finally, 2.5ml of upper layer solution is mixed with 2.5ml distilled water and 0.5ml FeCl₃ (0.1%); the absorbance is measured at 700nm.

2.7. Statistical Analysis

Experiments were performed in triplicate and results were expressed as means \pm standard error. Analysis of variance (ANOVA) is used and the least significant difference (LSD) at p<0.05 is calculated using the Statistica 5.5 to determine significant differences between the results.

3. RESULTS AND DISCUSSION

3.1. Ascorbic Acid Content

The ascorbic acid contents of the analysed tomato samples varied from 6.94 (Agora) to 16.70mg/100g (Nattih) (Table 1). The ascorbic acid contents of the varieties present significant differences (px0.05), except Marmande and Tafna. These results are similar to those of Toor et al. (2006) on New Zealand tomatoes. However, the investigation of Abushita *et al.* (1997) shows that the concentration of this antioxidant ranged between 22 and 48mg/100g on tomato varieties cultivated in Hungary. George *et al.* (2004) reported that ascorbic acid concentration of 12 tomato varieties ranged between 8.4 and 32.4mg/100g; Nour *et al.* (2013) reported similar contents (9.9-34mg/100g) in tomato cultivars grown in southwestern Romania.

The greenhouse grown tomatoes are found to have lower levels of ascorbic acid than those grown outdoors because of the lower light intensity in the greenhouses (Dumas *et al.*, 2003). This could explain the relative lower contents of ascorbic acid in the studied tomato varieties.

3.2. Total Carotenoid and Lycopene Contents

The highest carotenoid concentration is recorded for Marmande and Tafna varieties, followed by Kiti and Zahra. Carotenoid contents in Sammichele, Joker and Agora varieties do not present significant differences (Table 1). The carotenoid levels of the analysed tomato samples are higher than those reported by Abushita *et al.* (1997) (1.85-6mg/100g) and Zoran *et al.* (2014) (2.5-3.5mg/100g). A large variation in the carotenoid content of the varieties could mainly be attributed to factors such as environment and genotype (George *et al.*, 2004).

Lycopene concentrations in tomato varieties vary from 3.90 (Nattih) to 7.70 mg/100 g (Marmande) (Table 1). Lycopene contents of the eight analysed varieties are significantly different (p<0.05). The study performed by Martínez-Valverde *et al.* (2002) on Spanish varieties shows variations of the lycopene concentrations from 1.8 to 6.5 mg/100 g. Contents from 0.5 to 6.3 mg/100 g are obtained by Kuti and Konuru (2005). The results found in this investigation were relatively higher than those reported by Nour *et al.* (2013) for tomato cultivars grown in

southwestern Romania (1.2-4.9mg/100g) but lower than those obtained by Frusciante *et al.* (2007) and Pinela *et al.* (2012) for tomato varieties cultivated in Italy (2.33-16.9mg/100g) and Portuguese farmer varieties cultivated in homegardens (10.9-18.6mg/100g), respectively.

The lycopene constitutes 60 to 74% of the carotenoids of tomato and tomato products (Clinton, 1998). Shi and Le Maguer (2000) reported that lycopene represents 80 to 90% of total carotenoids. The lycopene content in the analysed samples varies from 64.09 to 80.48% (Table 1). These results confirm the existence of a good linear correlation between lycopene and total carotenoid contents (r = 0.94).

	Ascorbic acid	Carotenoids	Lycopene			
Variety	(mg/100g)	(mg/100g)	(mg/100g)	Percentage		
Marmande	12.64 ± 0.19^{e}	$9.57\pm0.15^{\rm a}$	$7.70\pm0.01^{\mathtt{a}}$	80.48ª		
Sammichele	$9.86\pm0.12^{\rm f}$	7.61 ± 0.20^{d}	$5.23\pm0.08^{\rm f}$	68.74°		
Agora	$6.94\pm0.16^{\text{g}}$	7.32 ± 0.14^{d}	5.37 ± 0.04^{e}	73.39 ^d		
Zahra	$15.04 \pm 0.13^{\circ}$	8.16 ± 0.15°	$6.33\pm0.07^{\text{d}}$	77.54 ^{bc}		
Tafna	$12.60\pm0.07^{\rm e}$	$9.46\pm0.10^{\mathrm{a}}$	$6.98\pm0.04^{\rm b}$	73.70 ^d		
Nattih	16.70 ± 0.21^{a}	5.18 ± 0.23^{e}	$3.90\pm0.03^{\rm h}$	75.46 ^{cd}		
Kiti	$14{,}55\pm0.02^{\texttt{d}}$	$8.56\pm0.25^{\mathrm{b}}$	$6.78 \pm 0,02^{\circ}$	79.28 ^{ab}		
Joker	$15,\!43\pm0,\!26^{\mathrm{b}}$	7.35 ± 0.11^{d}	$4.71\pm0,\!03^{\text{g}}$	64.09 ^f		

Table-1. Ascorbic acid, carotenoid and lycopene contents of tomato varieties

Different letters in the same column indicate significant differences (p<0.05)

3.3. Total Phenolic Contents

The extraction of phenolics from plant tissue poses several problems, including the presence of different enzymes (e.g. polyphenol oxidases) that can oxidize the phenolics. Drying is a good method to remove the enzymatic activity, but it could cause decrease of the polyphenol contents (Ribéreau-Gayon, 1968). For this reason, fresh tomatoes were used. As polyphenols have variable polarities, different solvents were used in this study (methanol, 50% methanol, ethanol and 50% ethanol). Several authors have used water for extraction (Odabasoglu *et al.*, 2004). However, water could dissolve undesired molecules such as proteins and polysaccharides, especially if the extraction is carried out at high temperatures (Shi *et al.*, 2003). Mixtures alcohol/water are widely used for phenolic extractions (Chu *et al.*, 2000; Martínez-Valverde *et al.*, 2002).

The phenolic compound concentrations of tomato varieties are presented in Fig. 1. The phenolic contents in methanolic extracts (pure methanol) of the samples varied from 20.64mg/100g (Marmande) to 49.55mg/100g (Joker). The Sammichele, Agora, Zahra and Tafna varieties contain closer concentrations, approximately 24mg/100g. The extraction by 50% methanol resulted in contents ranging between 17.96 and 37.80mg/100g. The quantities of phenolic compounds extracted by this solvent are significantly different, except Agora and Sammichele.

As indicated in Fig. 1, the extraction with absolute ethanol gives a contents in phenolic compounds ranging between 22.53 (Marmande) and 51.58mg/100g (Joker). Agora and Zahra have similar phenolic levels; however, phenolic contents of the other varieties are significantly

different (p<0.05). The concentrations from 20 to 30.25 mg/100g were found for 50% ethanol extracts. Joker and Nattih varieties have similar contents; the values of Marmande and Zahra are also similar.

The solvents used in our investigation allow the extraction of phenolic compounds with significant differences (p<0.05). The best solvent of extraction is ethanol then methanol followed by 50% methanol and finally 50% ethanol.

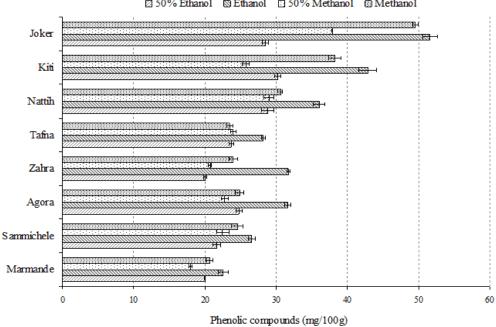


Fig-1. Total phenolic contents of the tomato varieties ☑ 50% Ethanol ◎ Ethanol ◎ Methanol ◎ Methanol

Bars represent the standard error of the mean.

The phenolic contents in the tomato varieties of this work are comparable with several findings of other studies. Similar concentrations are reported by Martínez-Valverde *et al.* (2002) in Spanish tomato. The phenolic content reported by Kaur and Kapoor (2002) in Indian tomato is 68mg/100g. The Italian tomato varieties, analysed by Minoggio *et al.* (2003), contain 2.25 to 25.84mg/100g. Nour *et al.* (2013) reported concentrations of 30-55.9mg/100g in cultivars grown in southwestern Romania. For tomato Portuguese cultivars, Pinela *et al.* (2012) and Vinha *et al.* (2014) have obtained phenolic levels of 24.5-31.3mg/100g and 22.6-79.3mg/100g, respectively.

In the present investigation, the highest content of phenolic compounds is obtained in Joker variety. This could be explained by the field grown of this variety compared to other varieties greenhouse grown. Therefore, field grown tomato, which receive higher amount of light have been reported to contain a higher amount of phenolics than greenhouse grown tomatoes (Hunt and Baker, 1980; Davies and Hobson, 1981). Light increases the biosynthesis of phenolic compounds in plants by increasing the activities of enzymes, especially phenylalanine ammonia-

lyase (PAL). The PAL converts phenylalanine into coumaric acid, which is the precursor of molecules involved in the synthesis of phenolic components (Smith, 1973).

3.4. DPPH Radical Scavenging Activity

3.4.1. DPPH Radical Scavenging Effect of Hexanic Extracts

To test the scavenging ability of hexanic extracts, the absorbance is measured at 580nm, using this wavelength, the carotenoid chromophoric system did not produce any interference with DPPH radical (Jiménez-Escrig *et al.*, 2000), and the volume of hexanic extract mixed with methanolic DPPH solution must be lower.

The DPPH[•] scavenging activity of the non-polar extracts is illustrated in Fig. 2a. Marmande variety presents the highest activity (29.17%) followed by Tafna and Zahra which have similar activities.Nattih variety exhibits the weakest antiradical activity (3.42%). The scavenging power of the hexanic extracts shows linear correlation with total carotenoid and lycopene contents; the correlation coefficients are 0.8 for carotenoids and 0.78 for lycopene.

Jiménez-Escrig *et al.* (2000) measured the scavenging capacity of several carotenoids by the DPPH method and indicate that lycopene is the carotenoid which presents the best effect. These authors suggest that the antiradical activity increases with the number of double bounds and the presence of the functional groupings as in the xanthophyll class.

3.4.2. DPPH Radical Scavenging Effect of Alcoholic Extracts

The scavenging ability of the analysed tomato extracts against the DPPH radical varied depending on the variety and to the extraction solvent (Fig. 2b). The extraction with pure methanol reveals that Joker variety presents the greatest activity with 63.44%. The studied varieties present significant differences (p0.05), except Agora and Sammichele. Marmande exhibits the weakest scavenging power (20.18%).

The extraction with 50% methanol showed that the antiradical activity ranged between 10.06% (Sammichele) and 41.27% (Joker). The scavenging activities of Agora and Zahra do not present a significant difference; Marmande and Sammichele have also almost the same scavenging activity.

The scavenging activity of the ethanolic extract varies from 65.09% (Joker) to 24.23% (Marmande); Tafna, Nattih and Zahra samples have similar activities. The extraction with 50% ethanol shows that Joker and Kiti varieties have the strongest activities with 32.29 and 31.40%, respectively. Tafna, Marmande, Zahra and Sammichele have closer activities (~14%).

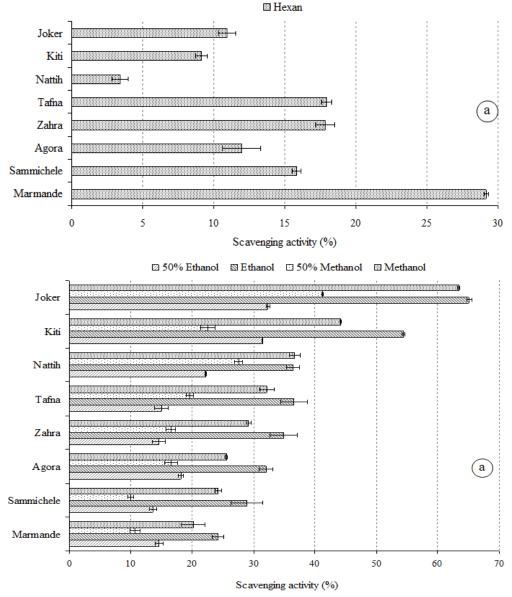


Fig-2. DPPH radical scavenging in hexanic (a) and alcoholic (b) extracts of the tomato varieties

Bars represent the standard error of the mean.

For the four solvents used, Joker variety presents the greatest scavenging activity followed by Kiti, Nattih then Tafna samples, and Marmande has the weakest activity. The extraction with pure alcohol gives a higher scavenging power than that of diluted alcohol. The extraction solvent which gives the best scavenging power is the ethanol. The extracts prepared with 50% methanol and 50% ethanol present similar activities.

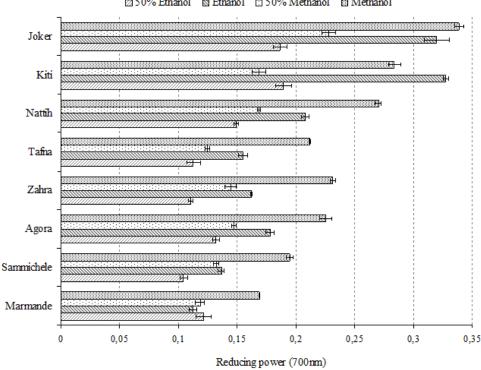
According to Borguini et al. (2013), DPPH scavenging potential of alcoholic extract of tomatoes cultivated under conventional system (19.52) was higher than that of water extract

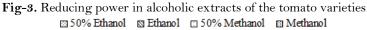
(11.33%). However, findings of Shahzad *et al.* (2014) revealed that tomato water extracts scavenge more efficiently DPPH radical (44.63) than methanol extracts (37.68).

The obtained results indicate the existence of a strong linear correlation between the phenolic compound content and the antiradical activity of the extracts (Table 2) and are in agreement with the data of Minoggio *et al.* (2003). However, Martínez-Valverde *et al.* (2002) and Hdider *et al.* (2013) noticed a weak correlation for tomato phenolics extracted with methanol 80% (r = 0.42) and for high-pigment tomato cultivars grown in Italy (r=0.35).

3.5. Reducing Power

The methanolic extracts present a reducing power ranging between 0.17 (Marmande) and 0.34 (Joker). When 50% methanol is used as the extraction solvent, the values varied from 0.12 (Marmande) to 0.23 (Joker). The reducing power obtained in the ethanolic extracts ranged between 0.11 and 0.32 while with 50% ethanol extracts, the values varied from 0.12 to 0.19 (Fig. 3).





Bars represent the standard error of the mean.

The studied varieties have reducing powers significantly different (p < 0.05). Joker presents the greatest value. The best reducing power is obtained with ethanol followed by methanol. The reducing power of 50% methanol and 50% ethanol extracts does not present a significant

difference. The results of reducing power show a good linear correlation with the phenolic compound content of all the extracts (Table 2).

Ascorbic acid extraction is better in an aqueous acid medium. Nevertheless, this vitamin is extracted using alcohol such as methanol (Mau *et al.*, 2005). Ascorbic acid contents measured in the tomato varieties show no obvious relationship (Table 2) with the scavenging activity and reducing power of the alcoholic extracts. This could be explained, on the one hand, by the instability of the ascorbic acid in the alcoholic extracts, on other hand, by the presence of other molecules, such as phenolics, having considerable scavenging and reducing properties, which can mask those of this vitamin.

The scavenging activity and the reducing power, respectively use two mechanisms of action of antioxidants molecules on the free radicals which are the transfers of hydrogen and electron. The scavenging ability on DPPH radical measured in different extracts (methanol, 50% methanol, ethanol and 50% ethanol) is correlated with reducing power; the correlation coefficients are 0.95, 0.93, 0.95 and 0.99, respectively.

Table-2. Correlation	coefficients	between	phenolic	and	ascorbic	acid	contents	and	antioxidant
activity of alcoholic ex	tracts								

	Antiradical power		Reducing power			
Solvent	Phenolic compounds	Ascorbic acid	Phenolic compounds	Ascorbic acid		
Methanol	0.97^{a}	0.55^{ab}	0.94 ^a	0.54 ^a		
Methanol 50%	0.95^{a}	0.57^{a}	0.93ª	0.46^{b}		
Ethanol	0.96^{a}	0.48^{bc}	0.95^{a}	0.44^{b}		
Ethanol 50%	0.88^{b}	0.45 ^c	0.87^{b}	0.44 ^b		

Different letters in the same column indicate significant differences (p<0.05).

In conclusion, this study indicates that Algerian tomatoes have the same biological properties, are a good source of various antioxidants such as ascorbic acid, phenolic compounds and carotenoids, in particular lycopene, as tomatoes of the other countries. Total phenolic concentrations of the analysed tomatoes differ depending on the variety and the extraction solvent. Pure alcohols (ethanol and methanol) allow better extraction than the diluted ones (50% ethanol and 50% methanol). Positive correlations were found between phenolic, carotenoid and lycopene contents and the antioxidant activities. Using a reduced volume of hexanic extract and measuring the absorbance at 580nm, the DPPH method could be applied to determining the antioxidant activity of carotenoids. Antioxidant amounts and the antioxidant activity of the tomato varieties show differences which could be due to varietal factors. The hybridisation between Joker and Marmande varieties, which present the highest phenolic and carotenoid amounts respectively, could give another variety with a high antioxidant activity. More work is needed to determine the effects of cooking and storage conditions in the bioactive contents of the studied tomato varieties.

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