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LYCOPENE CONTENT AND ANTIOXIDANT CAPACITY OF PORTUGUESE WATERMELON FRUITS

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ABSTRACT

Red flesh watermelon is one of the main food sources with lycopene as the most abundant carotenoid and has been associated with a lowered risk of prostate cancer. In order to assess health benefits of Portuguese watermelon, five accessions from the Portuguese Bank of Germplasm were chosen for antioxidant quantification and total solids content. Two of the accessions were further characterized for lycopene content. Due to its lycopene and antioxidant contents, accession 6185 may be considered a valuable germplasm for breeding programmes. The intake of one to three wedges of 6185 watermelon accession would provide between 6 to 18 mg of lycopene and 0.18 to 0.54 mmol TEAC (Trolox Equivalent Antioxidant Capacity), which might protect from prostate cancer and other oxidative stress related diseases.

KEYWORDS

Portuguese Watermelon, Lycopene, Antioxidant.

INTRODUCTION

Since long that food has been considered to provide more than the basic nutrients for the human organism, due to the presence of bioactive substances, especially in food of plant origin. The term “nutraceutical” was proposed to define any substance that may be considered a food or part of a food and provides medical or health benefits including the prevention and treatment of disease [1]. Another widely recognized, and perhaps more accepted, term is “functional food”. Although there is no universal accepted definition of functional food, all the definitions provided by several organizations clearly state that a functional food should provide health benefits beyond basic nutrition, when consumed as part of the usual diet [2, 3, 4].

Unmodified whole foods, especially fruits and vegetables, may be regarded as the simplest functional foods. A large number of foods of plant origin have since long been shown to have a beneficial effect in health or a protecting effect in the development of some chronic diseases, mainly atherosclerosis and cardiovascular diseases, some types of cancers and macular degeneration [3, 5, 6, 7]. These protecting and/or beneficial effects have been in many cases correlated with the presence of phytochemicals with antioxidant properties, such as carotenoids, polyphenolic acids, sulfides and flavonoids [8, 9, 10].

Although initial studies have been conducted with isolated bioactive phytochemicals, there is now scientific evidence suggesting the existence of synergistic effects in mixtures of bioactive components [11]. Lycopene, a red-pigmented carotenoid with antioxidant activity has been associated with a lowered risk of prostate, digestive tract, bladder, breast and cervical cancers, as well as with cardiovascular disease [12, 13]. Some studies have suggested that multiple tomato components including other carotenoids and polyphenols potentiate the action of lycopene in chemopreventive activity [14, 15]. Watermelon is a fruit rich in lycopene and with a total antioxidant capacity similar to tomato [16]. In Portugal, consumption of fruits has increased about 31% since 1990, being, however, still lower than the dietetic recommendations [17]. Although the major fruits eaten throughout the year are oranges and apples, watermelon is a significant culture in the country, being appreciated in the summer.

In this study, five accessions of watermelon from the Portuguese Bank of Germplasm were chosen for lycopene and antioxidant quantification after an initial screening of thirty one accessions, in order to evaluate the health benefit capacities of Portuguese watermelon landraces.

MATERIALS AND METHODS

Plant material

In 2002, thirty-one watermelon (*Citrullus lanatus* Thumb Mansf) accessions from the Portuguese Bank of Plant Germplasm were characterized in the experimental fields of *Escola Superior Agrária de Santarém*. Just five of them showed to be morphologically uniform and the main traits of their fruits are presented in Table 1. From these five accessions, two fruits of each were sampled for total antioxidants and total soluble solids.

The five accessions were grown in 2003, in isolated plots, for multiplication. Taking into account the availability of fruits and former analytical results, fruits of accessions 3354 and 6185 were sampled for further antioxidants and lycopene analysis. In 2004 the two accessions were grown in single row plots, with ten plants per row, for further analysis.

In each year, tillage, planting, fertilization, irrigation, weed and pest control were done according to common production practices.

For each accession, two fruits were analyzed. A portion of the flesh was removed from different locations, pooled and used to prepare extracts in triplicate. In order to minimize carotenoid oxidation, all extracts were immediately analyzed.

Table 1: Major fruit traits of *Citrullus lanatus* morphologically uniform accessions

Accession	Fruit shape	Fruit size	Rind colour	Flesh colour	Flesh flavour
3354	Round	Small	Striped green	Dark red	Very sweet
4870	Oval	Medium	Striped green	Light red	Very sweet
5124	Elongate	Large	Wide striped green	Red	Very sweet
6185	Elongate	Small	Wide striped light green	Light red	Very sweet
7793	Round	Medium	Striped green	Red	Sweet

Antioxidant analysis

Extraction of antioxidants and TEAC (Trolox Equivalent Antioxidant Capacity) assay was performed according to Dzuric and Powell [16]. Briefly, 0.5g of sample was homogenized with 500 μ L of water and centrifuged for 2 minutes at 14 000 x g. The pellet was washed two times with 250 μ L of water and all the supernatants were pooled to yield the aqueous fraction. To extract the organic fraction, the pellet was then washed three times with 500 μ L of acetone/methanol (7:3) and the resulting supernatants pooled. For the TEAC assay, 12 μ L of either aqueous or organic fraction were mixed with 485 μ L of PBS buffer, 36 μ L of metmyoglobin 70 μ M and 300 μ L of 500 μ M ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)). The reaction was started by adding 167 μ L of 450 μ M H₂O₂. Tubes were incubated at 30° C for 6 min and absorbance was measured at 734 nm. Each extract was analyzed in triplicate. Separate calibration curves were done with Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) for analysis of the aqueous and the organic fraction.

Lycopene quantification

The carotenoid extraction technique of Barua [18] was modified using tetrahydrofuran (0,6mL/g fresh weight) until the extractant was colorless. The homogenate was centrifuged for 2 min at 1300 x g at room temperature and the supernatants were pooled and evaporated at 37 °C on a rotary evaporator to a final volume of about 2.5 mL and an appropriate dilution was done with the mobile phase-solvent mixture (acetonitrile: tetrahydrofuran: methanol: 1% ammonium acetate; 65:25:6:4) as described by Nierenberg and Nann [19]. Prior to HPLC analysis, samples were filtered through a 0.45 μ M filter (Millex ®-LH, PTFE, 4 mm). The HPLC system consisted of a high pressure pump (Waters 600E), a U6K manual injector valve equipped with a 2 mL injection loop, an analytical column (Nova-Pak, C18, 60 Å, 4 mm) and an absorbance detector (Waters 486 Absorbance Detector). System control and data acquisition was performed by Millennium 32 Chromatography Manager software. The mobile phase for elution consisted of acetonitrile: tetrahydrofuran: methanol: 1% ammonium acetate (65:25:6:4); the flow rate was 0.5 mL/min, injection volume was 25 μ L and detection was carried out at 484 nm. For each extract triplicates of injections were done. Peak identification was made on the basis of retention time and quantification was based on external calibration curves using chromatographic areas of at least eight standard solutions between 2.5 and 100 ng/ μ L. Lycopene standard was obtained from SIGMA (Tomato 90-95%).

Total soluble solid content (TSSC)

A 0.5 mL sample of freshly homogenized watermelon heart tissue prepared as referred in 2.1 was placed on an automatic digital refractometer (ATAGO SMART-1) calibrated with a 10% sucrose solution and results were given in °Brix. The analyses were done in triplicate.

RESULTS AND DISCUSSION

Of the thirty-one accessions of *Citrullus lanatus* under characterization only those that showed no morphological differences in the fruits (table 1) were sampled for analysis of total antioxidants and total soluble solid content (table 2). Total antioxidants varied widely between accessions. Total soluble solid content of mature fruits were similar to what was found for other studied cultivars [20], ranging from 10.0 to 11.6 °Brix. Since the commercial value is related with sweetness and the nutritional quality and potential health benefits are related to total antioxidants, the two accessions showing high values of antioxidants, as well as high total soluble solids (3354 and 6185) were chosen for further analysis the following year.

Table 2: Screening of *Citrullus lanatus* accessions for total antioxidants (TEAC) and total soluble solid content (TSSC). TEAC values are the sum of aqueous and organic fractions. All values are indicated as the mean of replicates \pm standard deviation. TEAC and TSSC were analyzed in triplicates.

Accession	TEAC (mM)	TSSC (°Brix)
3354	0.89 \pm 0.02	11.17 \pm 1.44
4870	0.26 \pm 0.01	11.60 \pm 1.06
5124	0.94 \pm 0.02	10.63 \pm 1.12
6185	1.31 \pm 0.02	11.10 \pm 0.96
7793	0.65 \pm 0.01	10.00 \pm 0.40

Lycopene content of accessions 3354 and 6185 are shown in table 3. Identification of the major HPLC peak as lycopene was done by comparison of retention times with the standard (11.575 min) and a calibration curve ($R^2 = 0.9976$) was used for quantification.

The 6185 accession had lycopene content ($40 \mu\text{g g}^{-1}$ FW) similar to the average content of lycopene in watermelon fruits reported in the USDA Nutrient Database [21], which is $45.32 \mu\text{g g}^{-1}$ FW. Accession 3354 had lower lycopene content ($21 \mu\text{g g}^{-1}$ FW), but proximate to the minimum of the range reported for watermelon (23.0 to $72.0 \mu\text{g g}^{-1}$ FW) [12]. Variations in lycopene contents among different red flesh watermelon cultivars were found [22], seedless types seeming to have higher contents (60.9 to $112.4 \mu\text{g g}^{-1}$ FW) than hybrid and seeded types (35.2 to $76.1 \mu\text{g g}^{-1}$ FW). Recently, values of $35.35 \mu\text{g g}^{-1}$ FW for redfleshed watermelon and 21.19 for pink watermelon were reported [23].

Table 3: Lycopene content of accessions 3354 and 6185. Values are means of three extracts \pm standard deviation.

Retention time (Rt) of lycopene standard was 11.575 min			
Accession	R _t Peak 1 (min)	Lycopene	Lycopene $\mu\text{g g}^{-1}$ FW
3354	11.804 \pm 0.08		21.22 \pm 0.90
6185	11.766 \pm 0.41		40.32 \pm 4.54

Antioxidant values were also analyzed in the following year. Both accessions showed higher values in the aqueous fraction (table 4). Dzuric and Powell [16] screened several tomato products and

watermelon for antioxidant content and found that TEAC values tended to be higher in the aqueous fractions of the foods, although for watermelon the difference between the content of aqueous and the organic fraction was not significant. Comparing the total antioxidant values obtained (table 4) with the results found for the same accessions in year before (table 2), it can be noticed that there is a difference between fruits of two consecutive years, but accession 6185 is still the one with the higher values. The total antioxidant contents (aqueous plus organic) in mmol/100g of fresh weight (table 4) of both accessions are in the range of values found in other cultivars tested by other authors (table 5).

Table 4: Antioxidant contents of accessions 3354 and 6185. Values are means of three extracts \pm standard deviation

Accession	Aqueous TEAC (mM)	Organic TEAC (mM)	Total TEAC (mM)	Total TEAC (mmol/100g)
3354	0.33 \pm 0.01	0.16 \pm 0.00	0.49 \pm 0.01	0.06 \pm 0.01
6185	0.74 \pm 0.01	0.29 \pm 0.01	1.03 \pm 0.01	0.12 \pm 0.01

Table 5: Comparison of antioxidant content in watermelon and tomato obtained with different antioxidant assays.

Method	Reference	watermelon	tomato
FRAP (mmol Fe ²⁺ /100 FW)	[24]	0.06	0.31
	[25]	0.11	0.51
TRAP (mmol Trolox/100g FW)	[26]	-	0.34
	[25]	0.05	0.13
TEAC (mmol Trolox/100g FW)	[27]	0.10	0.20
	[25]	0.07	0.16
	[16]	0.14	0.28
ORAC (mmol Trolox/100g FW)	[26]	-	0.26
	[25]	-	0.42
	[28]	0.14	0.37

FRAP – Ferric-reducing ability of plasma; TRAP – Total radical trapping antioxidant parameter; TEAC – Trolox equivalent antioxidant capacity; ORAC – Oxygen radical absorbance capacity.

CONCLUSIONS

The most common carotenoids in human diet are β -carotene, α -carotene, β -cryptoxanthin, lycopene, lutein and in less amounts, phytoene and phytofluene [29]. Tomato and red flesh watermelon are the two main food sources with lycopene as the most abundant carotenoid.

It has been observed that consumption of 20 mg/day of lycopene from either fresh frozen watermelon juice or canned tomato juice, resulted in a 100 to 200% increase in human plasma lycopene, thus showing that lycopene is bioavailable from non heat processed tomato and watermelon [30]. Recently, it was reported a rise in lycopene plasma levels after consumption of 21.9 mg of lycopene in tomato juice for eight weeks, as well as in lycopene metabolites such as apolycopenals [31]. These metabolites are thought to have bioactivity and potentially mediate some of the health promoting effects of carotenoid rich foods [32]. Doses higher than 20 to 30 mg of lycopene per day are not as effective as lower doses since absorption becomes limiting [8].

Other carotenoids like β -carotene and phytoene are also bioavailable from watermelon, although in much lower amounts than lycopene [30].

It has been shown that consumption of carotenoid rich fruits, including watermelon, is inversely associated with prostate cancer risk [33]. In the case of watermelon, decreased risk was associated to consumption of 25.7 to 82.2 g/day in a dose-response relationship. Also, a decreased risk was found with intakes of more than 5 mg of lycopene per day.

Assuming a medium sized wedge of 286g, with 52% of edible portion [21] the consumption of one to three wedges of the 6185 accession (about 148 to 446g of edible portion) would provide

between 6 and 18 mg of lycopene, suggesting that a single wedge of watermelon each day may be effective in offering protection against prostate adenocarcinoma.

It is evident from the analysis of table 5 that there is a wide variation in total antioxidant content when different methods are used; FRAP assays tending to give higher results than TEAC assays. Wide variations are also observed even when the same method is used by different authors. For example, for the TEAC assay, watermelon values were 0.07 and 0.14. For tomato a lower variation was found, values being 0.16, 0.28 and 0.26. The observed wide variation in antioxidant content can be explained by natural variability of the plant material. In watermelon, it has been shown that cultivar, growth conditions, state of maturity and storage time and conditions influence lycopene content of fruits [20, 22, 23, 34, 35]. Since lycopene is the main carotenoid in watermelon, accounting for 84 to 97% of total carotenoids [22, 23], and is the carotenoid with higher relative antioxidant potential [36] it is expected that the total antioxidant content of fruits also varies in the same conditions.

Accession 6185 has similar antioxidant content to apple, 0.13mmol TEAC/100 FW for yellow Golden and 0.16 for red Delicious [25], one of the most consumed fruits in Portugal. Other commonly consumed fruits have higher antioxidant content, for example, 0.87 mmol TEAC/100g FW for orange, 0.38 for black grape and 0.25 for white grape [25]. Fruits that are rich in flavonoids are the ones with most potent antioxidant capacity, especially blackberry and raspberry, which have 2.0 and 1.7 mmol TEAC/100g FW, respectively [25]. Even higher antioxidant capacities have been reported for non traditional brazilian fruits, up to 9.66 mmol TEAC/100g FW [37]. However, these fruits are not normally consumed in Portugal.

It was shown in a recent study that consumption of 150 mL of watermelon juice resulted in potent antioxidant effects in human plasma, as well as other fruit juices like apple, orange, grape, peach, plum and kiwi [38]. The contents of lycopene and TEAC of accession 6185 make it a valuable germplasm for breeding programmes, since the intake of one to three wedges of 6185 watermelon accession would provide between 6 to 18 mg of lycopene and 0.18 to 0.54 mmol TEAC, which might protect from prostate cancer and other oxidative stress related diseases.

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