Comparative Study of the Antioxidants Compounds in Fresh and Thermally Processed Tomatoes Juice

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Abstract

The purpose of our study was to determine the influences of thermal processing of tomato juice, in a manner similar to that commonly used in traditional cuisine, on the concentration of antioxidant compounds: total carotenoid and their profile; concentration of lycopene; concentration of ascorbic acid and total polyphenols. The boiling of tomato juice resulted in a significant increase in the concentration of total carotenoids and lycopene. No statistically significant differences in total carotenoids and lycopene content occurred depending on the boiling time. In fresh juice, ascorbic acid had average values of 20.73 mg /100g and significant decreased depending on the boiling time. Processing of tomato juice by boiling for 15 minutes or 30 minutes causes a slight increase in the concentration of phenols. The results obtained in this study have shown that, in the case of tomato juice, cooking by boiling determines the decrease of ascorbic acid concentration, but on the other hand makes the carotenoids and phenolic compounds more available, thus being nutritionally beneficial.

Keywords: ascorbic acid, carotenoids, phenols, tomatoes, thermal processing

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops. Significant amounts are consumed fresh or in the form of processed products such as tomato juice, paste, puree, ketchup, sauce and salsa (Del Valle et al., 2006).

Tomatoes are a major source of antioxidants contributing to the daily intake of a significant amount of these molecules. Epidemiological studies have shown that consumption of raw tomato and its tomato based products is associated with a reduced risk of cancers of prostate, pancreas stomach and cardiovascular diseases. This protective effect has been mainly attributed to its valuable bioactive components with antioxidant properties (Abdul-Hammed et al., 2015; Chang et al., 2006; Pinela et al., 2012; Sanchez-Moreno et al., 2006). Tomatoes antioxidants include carotenoids, such as lycopene, β-carotene, vitamins such as ascorbic acid; tocopherols, and phenolic compounds. These compounds play an important role inhibiting reactive oxygen species responsible for many important diseases, through free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways (Hwang et al., 2012; Pinela et al., 2012). Tomatoes are an excellent source of vitamin B6, folic acid, niacin, potassium, and trace elements, i.e. selenium, copper, manganese and zinc, which are cofactors of antioxidant enzymes. It is assumed that these trace elements play a key role in the protection mechanisms by scavenging free radicals (Nour et al., 2013).

Fruits and vegetables constitute the major sources of carotenoid in human diet (Rao and Rao, 2007). Carotenoids are a family of pigmented

compounds that are synthesized by plants and micro-organisms but not by animals. In plants, they contribute to the photosynthetic mechanism and protect them against photo-damage. Carotenoids are localized in subcellular organelles (plastids), i.e. chloroplasts and chromoplasts. In chloroplasts, the carotenoids are associated with proteins and serve as accessory pigments in photosynthesis, whereas in chromoplasts they are deposited in crystalline form or as oily droplets. The colors of carotenoids depend on conjugated double bonds and the various functional groups contained in the carotenoid molecule. As a result, the color ranges from yellow, red to orange in many fruits and vegetables. Besides, esterification of carotenoids with fatty acids can also occur during fruit ripening, which may affect the color intensity (McGlasson, 2003).

Due to the unsaturated nature of the carotenoids they are subject to changes, mainly to oxidation. However, other factors such as temperature, light and pH can also produce alterations that can influence the color of foods as well as their nutritional value. In general carotenoid content of foods is not altered to a great extent by common household cooking methods such as microwave cooking, steaming and boiling but extreme heat can result in oxidative destruction of carotenoids (Rao and Rao, 2007).

Botanically, the tomato fruit is classified as a berry. The typical red color is caused by lycopene, which is usually the predominant pigment. Lycopene is more concentrated in the outer fruit wall (pericarp) than in the internal locular tissue. The distribution of β-carotene follows a reverse pattern (Jamal et al., 2017; McGlasson, 2003). Lycopene is a highly unsaturated open straight chain hydrocarbon consisting of 11 conjugated and 2 unconjugated double bonds. Unlike β-carotene (and other carotenoids containing unsubstituted β-ring) lycopene lacks the terminal β-iononic ring in its structure and thus the provitamin A activity. It is a highly stable molecule. However, it can undergo oxidative, thermal- and photo-degradation. Studies have shown lycopene to be stable under the conditions of thermal processing and storage (Rao and Rao, 2007). Lycopene has been shown to be a potent antioxidant *in vitro* and *in vivo*. Recent interest in lycopene is due to the finding of an inverse association between the dietary lycopene and the risk of some types of cancer and cardiovascular disease. Lycopene from tomatoes powder reduces the burden of oxidative stress in hyperthyroidism and led to reduction in lipid peroxidation (Abdul-Hammed et al., 2015; Andrei et al., 2008).

Phenolic compounds are the main contributors to the antioxidant activities of the fresh products. These groups of compounds, which are rather considered as unessential secondary metabolites, are formed during normal metabolism in plant tissues. It is also reported that polyphenolic compounds are able to react along with cell mediators and enzymes, and play a pivotal role in prevention of chronic diseases (Bhat, 2016). Tomatoes are an important source of phenolic compounds, such as flavonoids and hydroxycinnamic acid derivatives, with 98% of the total flavonols located in the tomato skin as conjugated forms of quercetin and kaempferol. The flavanone naringenin is present in small quantities in tomatoes in its conjugated form. Many of these phytochemicals present in tomatoes have antioxidant properties and in combination with lycopene may contribute to the numerous health benefits (Nour et al., 2013). The changes of the phenolic fraction from tomato fruits in relation to the cultivar, season and country of origin were also investigated. Moreover, the ability of these compounds as scavengers of peroxyl radicals has been well described (Zanfini et al., 2017).

In recent years there is an amplified demand by consumers for minimally processed and safer foods, with retained original organoleptic attributes. For preservation of tomato or their products (such as purees), refrigeration or thermal processing is the most popular and widely employed method. However, these processing methods can significantly compromise the overall qualities including texture, flavour, sensory quality, bioactive compounds and nutritional values (Bhat, 2016).

The purpose of our study was to determine the influences of heat processing of tomato juice, in a manner similar to that commonly used in traditional cuisine, on the concentration of the main compounds with antioxidant function. Thus, the parameters analyzed in fresh and boiled juice were the total carotenoid concentration and their profile; concentration of lycopene; concentration of ascorbic acid and total polyphenol

Materials and methods

Chemicals and reagents

All chemicals and reagents were purchased from Merck (Darmstadt, Germany), were of analytical grade and were used without further purification.

Samples

The tomatoes used were regular red tomatoes grown in village organic farm and purchased from the local supermarket. In order to obtain the juice, 100 g of tomatoes were thoroughly washed, sorted out and homogenized for 10 minutes at 20,000 rpm in a blender (Ultra-Turrax Miccra D-9 KT Digitronic, Germany). The samples were thermally processed by boiling 15 minutes respectively for 30 minutes. Six samples of each category were analyzed: fresh tomato juice (FTJ); tomato juice boiled 15 minutes (BTJ15) and tomato juice boiled 30 minutes (BTJ30).

Determination of carotenoid profile, total carotenoid concentration and lycopene concentration

Total carotenoids were extracted from 10 g tomato juice (fresh and thermally processed) with 150 mL mixture consisting of hexane: ethanol (4:1, v:v) for 24 hours, in the dark and under stirring, after which 40 mL of acetone was added. The extracts were partitioned in a separation funnel, successively with water, diethyl ether and saturated saline solution. The ether phase was evaporated to dryness under vacuum. For spectrophotometric quantification, the residue was dissolved in diethyl ether. The total carotenoid content was estimated by reading the sample absorbance at 470 nm, using 2500 as the specific absorption coefficient (Britton, 1995). All results were expressed as mg/100g juice.

The carotenoid residue was transferred quantitatively in Ethyl acetate, filtered through PTFE 0.45 µm filters and subjected to HPLC-PDA analysis. Separation of carotenoids was performed using a Shimadzu LC20 AT high performance liquid chromatograph (HPLC) with a SPD-M20A diode array detector. An YMC C30 column (24 cmx4.6 mm, 5 µm) and a gradient consisting in methanol (solvent A) and BTME (solvent B). The linear gradient was: at 0 min - 100 % A (0 % solvent B) to 60 min - 0 % A (100 %) 62 min 100 % A, followed by equilibration of column 10 min (Carillo-Lopez and Yahia, 2014). The flow rate was fixed at 1.0 mL/min and the DAD detector was set at 470 nm.

The identification of carotenoids in samples was carried out by comparing the retention time and the UV-Vis absorption spectrum of each compound with those of available standard compounds. In order to perform a quantitative analysis, for each chromatogram peaks integration was performed and the % of each pigment was calculated from the total. Taking into account the data obtained from total carotenoid dosing and from peak integration, lycopene concentration was determined. The results were expressed as mg lycopene/100g juice.

Determination of ascorbic acid concentration

Ascorbic acid content was quantitatively determined according to the spectrophotometric determination described by Kapur et al., (2012), based on the oxidation ascorbic acid to dehydroascorbic acid by bromine water in the presence of acetic acid. After coupling with 2,4-dinitrophenylhydrazine (DNPH) a red complex was produced and absorbance of that complex was photometrically measured at 521 nm.

Determination of total polyphenol concentration

Total polyphenol concentration was analyzed photometrically, using the modified Folin–Ciocalteu method. Each sample (1 g) was extracted with 5 mL ethanol 40% for 30 minutes, then the ethanolic extract was diluted 1:5 (v/v) with distilled water. A volume of 1 mL of the extracts obtained previously was mixed with 5 mL of Folin–Ciocalteu reagent and 4 mL Na_2CO_3 7.5 %. Each sample was allowed to stand for 90 min at room temperature and measured at 765 nm. Calculation of total phenols expressed in mg gallic acid GAE/100 g juice was performed using a standard curve and taking into account the dilution factors.

Statistical analysis

Results were collected as means±SD of six independent determinations. In order to determine the significant differences between the fresh and thermally processed juice, analysis of variance (ANOVA) followed by student's t-test were performed. Differences were considered significant at $P \leq 0.05$.

Results and discussions

In fresh tomato juice, the carotenoid concentration had average values of 11.74 mg/100g (range between 11.39 – 12.06 mg/100g).

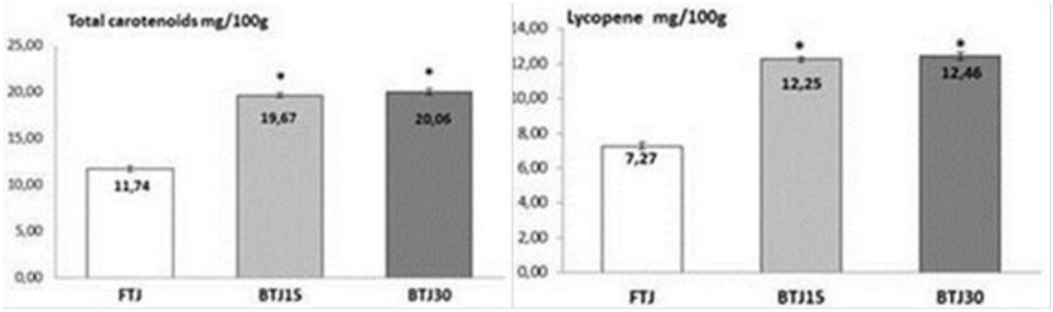

Figure 1. Total carotenoids and lycopene concentration in fresh and processed tomato juice (*p ≤ 0.05, significant)

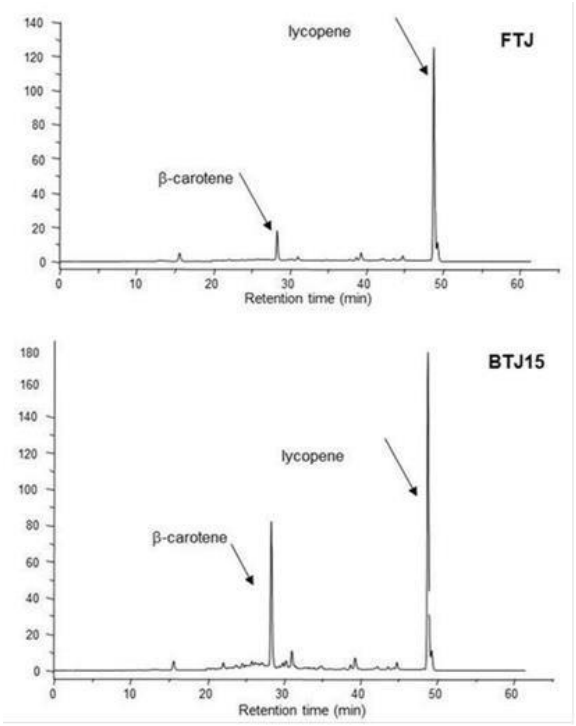

Figure 2. HPLC chromatograms of carotenoids in fresh and boiled juice

The concentration of carotenoids is influenced by the heat treatment of tomato juice. As can be seen from Figure 1, the boiling of tomato juice results in a significant increase ($p \leq 0.05$) in the concentration, the average values being 19.66 mg/100g for boiled juice 15 minutes and 20.06 mg /100g respectively for the boiled 30 minutes. It has also been observed that the boiling time does not induce significant changes in concentration ($p > 0.05$).

The data obtained are consistent with those presented by Sanchez-Moreno et al., (2006) according to which boiled tomato juice shows increases in carotenoid concentration.

For all types of samples, HPLC separation of carotenoids was performed. Figure 2 shows two of the chromatograms obtained (in the case of fresh juice FTJ and boiled for 15 minutes – BTJ15).

The main carotenoids identified in tomato juice were β-carotene (retention time - 28.4 min) and lycopene (retention time - 48.8 min). Other quantifiable carotenoids in fresh tomatoes and tomato products such as juice, paste, puree, and sauce include phytoene, phytofluene, δ-carotene, neurosporene, γ-carotene, but their concentrations are significantly lower than that of lycopene (Gama et al., 2006; Rao and Rao, 2007).

The data obtained show that the boiling of tomato juice does not change the qualitative profile of carotenoids, but there are different variations in their relative concentrations. The extractable lycopene and β-carotene amounts were affected by heating time. These results are consistent with other studies of the effect of processing on lycopene content. Hwang et al., (2012) showed that total lycopene content in the tomatoes increased with increased heating time and temperature, compared to the raw tomatoes. Oven baking at 130°C and 160°C for 10 min resulted in a 50.3% and 68.1% increase in extractable lycopene, respectively. Extractable β-carotene content also increased with heat treatment. After 5, 10, and 20 min of heating at 100°C, the extractable β-carotene increased by 17.9%, 50.2%, and 58.2%, respectively (Hwang et al., 2012).

Taking into account the data obtained from total carotenoid quantification and from peak integration, the lycopene concentration was determined and the results are presented in Figure 1. Total lycopene content in the tomatoes increases significantly ($p \leq 0.05$) with increased

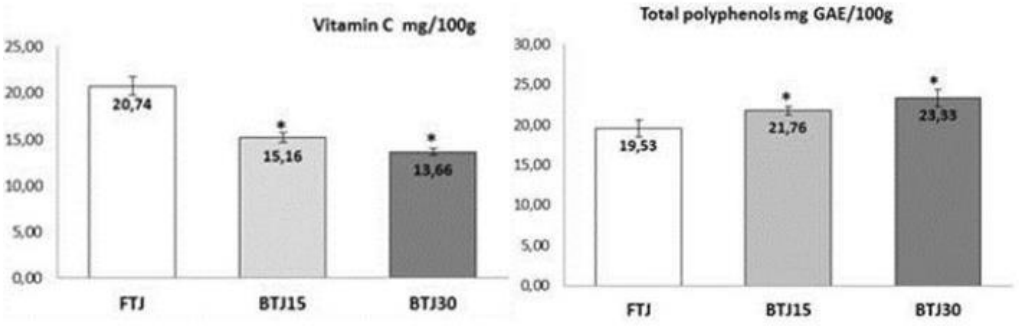

Figure 3. Vitamin C and total polyphenols concentration (*p ≤ 0.05, significant)

heating time, at 12.25 mg/100 g after 15 min and 12.46 mg/100 g after 30 min. No statistically significant differences in lycopene content occurred depending on the boiling time. A possible explanation of this increase in lycopene concentration is related to the enhanced release of this pigment from the specific matrix, making it more accessible in extraction. The quantities of lycopene in the boiled/processed samples for 15 or 30 minutes were similar, probably because the complete release of lycopene from the cell matrix by thermal processing was reached from the first boiling period. Thermal processing disrupts cell membranes and cell walls, thus facilitating the release of lycopene from tomatoes (George et al., 2011).

Conflicting data on carotenoid stability during thermal processing of red tomato can be found in the literature. In the study presented by Takeoka et al., (2001) there was an increase in lycopene concentration in boiled tomato juice. Thus, samples of juice obtained from two types of tomatoes were analyzed. In the first case, the fresh tomato juice had a content of 17.3 mg lycopene/100 g which, by boiling, increased to 19.46 mg/100 g. In the second type of fresh juice the concentration of this pigment was 20.14 mg/100g and after boiling it increased to 22.76 mg/100g. Sanchez-Moreno et al., (2006) showed that extractable lycopene content significantly increased when tomatoes were pasteurized. If in the fresh juice the concentration of this pigment was 1024 μg/100mL, it increased by pasteurization to 2449 μg/100mL. Contrarely, Sharma and LeMaguer (1996) observed a 20% decreases of lycopene in heated tomato pulp. Also, a significant decrease in the content of both lycopene and β-carotene was observed during the preparation of a tomato paste (Capanoglu et al., 2008).

In plants, the concentration of ascorbic acid is closely related to genotype, light, harvest time. The level of ascorbic acid in certain plants can be influenced, in particular, by the light level and by the feed-back inhibition of enzymatic activity (Andrei et al., 2014).

The decrease in the concentration of ascorbic acid in food occurs in the process of heat treatment (boiling). The data obtained in the present study regarding the ascorbic acid content in tomato juice are presented in Figure 3.

The boiling time does not significantly affect the decrease in ascorbic acid concentration, the values recorded at 15 minutes of boiling being close to those observed after 30 minutes of boiling. The loss of vitamin C occurs mainly through chemical degradation involving the oxidation of ascorbic acid to dehydroascorbic acid, followed by hydrolysis to 2,3-diketogluconic acid, respectively, further polymerization with the formation of nutritionally inactive products. In fact, heat is known as the main factor in accelerating the oxidation processes of ascorbic acid, the thermal processing being the one that induces the loss of vitamin C in processed fruits and vegetables (Dewanto et al., 2002; Chang et al., 2006; Sanchez-Moreno et al., 2006).

Phenolic compounds are a class of secondary metabolites present in plants, foods, plant-derived and beverages. Among the antioxidants present in the human diet, polyphenols are the most numerous. Therefore, the consumption of foods containing them may have an important role in human (Andrei et al., 2014). In the present study, the concentration of total phenols in fresh and boiled tomato juice was determined, the results

being shown in Figure 3. The total polyphenol concentration in fresh juice samples ranged between 18.2 and 20.7 mg GAE/100g, values consistent with other published data. Thus, in the study published by George et al., (2005) the concentration of these compounds in tomato juice samples ranged from 16.7 to 20.1 mg GAE/100g juice. Processing of tomato juice by boiling for 15 minutes or 30 minutes causes a slight increase in the concentration of phenols at 21.7 mg GAE/100g and 23.33 mg GAE/100g respectively. Polyphenolic compounds appear in plants as metabolic intermediates that accumulate in vacuoles. It is assumed that thermal processing processes could accelerate the release of phenolic compounds by destroying cellular components. On the other hand, destruction of cell membranes can also trigger the release of oxidative and hydrolytic enzymes, which could degrade antioxidants in fruits. However, high temperature processing could disable these enzymes and therefore lead to a slight increase in the total polyphenol concentration (Chang et al., 2006).

Conclusion

Processes of tomato juice processing, in a manner similar to that commonly used in households, influence in a different way the concentration of the main antioxidant compounds. Boiling of tomato juice does not alter the qualitative profile of carotenoids but induces increases in their relative concentrations. It is important to know the effect of thermal processing, especially since it is known that tomato lycopene has a significantly higher bioavailability than fresh vegetables.

Concentration of ascorbic acid decreases by heat treatment of juice. Boiling time does not significantly affect the decrease in ascorbic acid level, the values recorded at 15 minutes are close to those observed after 30 minutes. On the other hand, boiling juice for 15 or 30 minutes causes a slight increase in the concentration of polyphenols.

The results obtained in this study have shown that, in the case of tomato juice, cooking by boiling determines the decrease of ascorbic acid concentration, but on the other hand makes the carotenoids and phenolic compounds more readily available, thus being nutritionally beneficial.

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