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Effects of cooking and extra virgin olive oil addition on bioaccessibility of carotenes in tomato sauce

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Abstract: In this study, the effects of cooking and the addition of extra virgin olive oil on the in vitro bioaccessibility of carotenoids in tomato sauce were evaluated. The results indicated that total antioxidant activity, α-tocopherol, lycopene, β-carotene, lutein, and chlorophyll a contents were positively affected by the combination of cooking and addition of extra virgin olive oil. Moreover, after in vitro digestion, for intestinal phase, lycopene and β-carotene were observed in all samples, whereas α-tocopherol and chlorophyll a were not detected in any of the samples. Lycopene (≈8.5-fold) and β-carotene (≈5-fold) were significantly improved ($P < 0.05$) by the effect of cooking and addition of extra virgin olive oil to the samples compared to control tomato sauce (without oil and cooking).

Key words: Carotenoids, extra virgin olive oil, food matrix, antioxidant, in vitro bioaccessibility

1. Introduction

Consumption of fruits and vegetables in diet is strongly and inversely associated with the incidence of coronary heart disease and cancer. Diets poor in plant foods double the risk of several cancer types and considerably increase the risk for coronary heart disease (Visioli, 2000). The Mediterranean diet is a dietary regime rich in fruits, vegetables, and unsaturated fatty acids, which are important sources of antioxidant compounds (Berendsen et al., 2017). Long life of populations in the countries of the Mediterranean region and relatively low incidence of coronary heart disease and cancer in these areas have been linked to this dietary pattern (Turati et al., 2015).

Tomatoes and olive oil are considered an important part of the Mediterranean diet since they are used together in many food preparations. The positive effects of regular consumption of tomato products on human health have been reported in several studies (Pannellini et al., 2010; Ghavipour et al., 2015). Possible benefits of tomato-rich diets have been linked to the high amount of carotenoids, mainly lycopene (Riccioni et al., 2008). Lycopene has a lipophilic nature and it is recognized as an effective antioxidant compound that has antioxidant activity 2-fold higher than β-carotene and 10-fold higher than vitamin E (Colle et al., 2010; Periago et al., 2013).

Lately, several studies have focused on understanding and improving the bioaccessibility and bioavailability of bioactive compounds. Many factors, such as food matrix, type and extent of food processing, interactions with other food components (fiber, lipids, etc.) can influence the bioaccessibility of lycopene and other carotenoids (Arranz et al., 2015). Within the processing factors, heating and mechanical processing (Capanoglu et al., 2008; Kamiloglu et al., 2014) seem to be the most significant factors affecting the content and bioaccessibility of carotenoids in tomato and its products. On the other hand, the structure of carotenoid is also known to be an important factor in bioaccessibility. Although *trans* isomer is the most common form present in fresh tomato, it has been reported that after cooking all-trans-lycopene is converted to cis-lycopene which is more absorbable due to higher solubility of cis isomers in oils and bile acid micelles (Ahuja et al., 2006; Mutsokoti et al., 2017). Moreover, various studies suggest that consumption of dietary carotenoids through

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oil-based or oil containing tomato products results in higher absorption since they are lipid soluble compounds (Huo et al., 2007; Failla et al., 2014).

Most of the studies investigating the bioavailability of carotenoids in tomato and tomato products focus only on lycopene. However, very little information is available in the literature with respect to the absorption and bioavailability of lycopene and other carotenoids. The primary objective of this study was to investigate the effect of cooking and extra virgin olive oil addition on the carotenoid profile of tomato sauces. Furthermore, the effect of in vitro simulated gastrointestinal digestion on carotenoid content was also evaluated.

2. Materials and methods

2.1. Materials

Tomato sauce samples were collected from a tomato sauce (TS) factory in Turkey. The main processing steps for the commercial sauce production were washing, cold breaking (73 °C, 10 min), evaporating (11 °Bx, 73 °C), and pasteurization (110 °C, 90 s). The final tomato sauce had 11 °Bx with a pH of 4.4. Commercial extra virgin olive oil was used as the oil and added at two different ratios (5% and 10% w/w) to the tomato sauce. After the addition of 5% and 10% oil, the tomato sauces were homogenized at 25,000 × *g* using a homogenizer (IKA T18 basic, Germany). These samples were named 5O (homogenization with 5% extra virgin olive oil) and 10O (homogenization with 10% extra virgin olive oil), respectively. Afterwards, portions of 5O and 10O were subjected to cooking by heating at 94–96 °C in a water bath for 45 min, considering the information reported by Vallverdú-Queralt et al. (2015). These samples were named C5O and C10O, respectively. Both types of tomato samples were ground in a laboratory scale grinder and stored at –80 °C until analysis. A schematic overview of the experimental setup is presented in Figure 1.

2.2. Extraction of the samples

For each tomato sample, three independent extractions were performed according to the method of Vallverdú-Queralt et al. (2015). Briefly, 0.5 g of each sample were extracted with 5 mL ethanol:n-hexane (4:3, v/v) in a cooled ultrasonic bath for 5 min and subsequently centrifuged at 4000 × *g* at 4 °C for 15 min; the supernatants were collected. This extraction procedure was repeated once again, two supernatants were pooled to a final volume of approximately 10 mL and evaporated under nitrogen flow. Finally, the residue was reconstituted with 1 mL of methyl tert-butyl ether and filtered through a 25 mm, 0.45 µm PTFE filter (Waters, Mildford, MA, USA). All extracts were stored at –20 °C until analysis.

2.3. Total antioxidant capacity

The total antioxidant capacity of the extracts was determined using DPPH method. The DPPH (1,1-diphenyl-2-picrylhydrazyl) method was evaluated as previously described by Kumaran and Karunakaran (2006). Trolox was used as a standard and total antioxidant activity of extracts was expressed as mg of Trolox equivalent (TE) per 100 g of FW (fresh weight) of sample.

2.4. Targeted HPLC analysis

Carotenoid, tocopherol, and chlorophyll contents of sample extracts were identified and quantified using HPLC coupled with photodiode array (PDA) detector according to the method of Capanoglu et al. (2008). Extracts were passed through a 0.45 µm membrane filter and then injected into the system. Lycopene, β-carotene, lutein, α-tocopherol, and chlorophyll a were separated in a YMC-Pack C30 column with a gradient flow of methanol and tert-butyl ether, and detected at 450 nm. Absorbance spectra and retention times of eluting peaks were compared with those of available standards. All analyses were performed in triplicate and the results were expressed as mg per g FW of sample.

2.5. In vitro digestion of the samples

Standardized static in vitro digestion was carried out according to the procedure described by Minekus et al. (2014). Briefly, this protocol simulates the intestinal phases using gastrointestinal fluids which are prepared as described in detail in the protocol (Minekus et al., 2014). Briefly, 5 g of tomato sauce sample were mixed with 3.5 mL of salivary juice, 0.5 mL of α-amylase solution, 25 µL of 0.3 M $CaCl_2$, and 0.975 µL of distilled water to achieve a final volume of 5 mL. The reaction mixture was incubated at 37 °C for 2 min with continuous shaking. After the oral digestion step, 6 mL of gastric juice, 1.28 mL of pepsin solution, 4 µL of 0.3 mol/L $CaCl_2$ were added to the remainder of the mixture from the buccal phase and the pH was adjusted to 3.0 with 1 M HCl. The total volume was adjusted to 8 mL by adding distilled water. The mixture was incubated in a shaking water bath at 37 °C for 2 h. After the simulated stomach digestion, 7.7 mL of the intestinal juice, 3.5 mL of pancreatin, 1.75 mL of 160 mmol/L bile, and 28 µL of 0.3 mol/L $CaCl_2$ were added to the remaining gastric chyme. After adjusting the pH to 7.0 with 1 M NaOH, the total volume was adjusted to 14 mL with distilled water and the fluid mixture was incubated at 37 °C for 2 h with continuous shaking. To eliminate interferences from digestion fluids, a blank with no sample was also incubated under the same conditions. The fractions were kept at –20 °C until further analysis.

2.6. Statistical analysis

All analyses were performed in triplicate. The mean values and standard deviations of the experimental data were calculated and variance analysis (ANOVA) was performed using SPSS Software (version 19.0 for Windows). Duncan's test was used for further comparison of mean values and

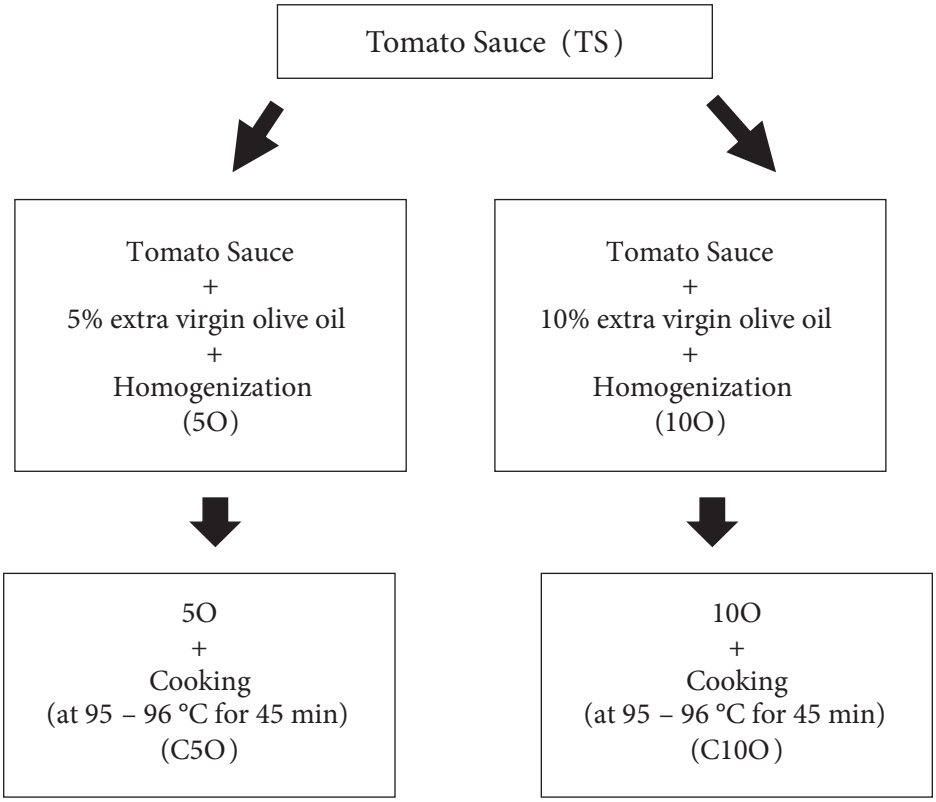

Figure 1. Diagram flowchart of the processes.

differences were considered to be statistically significant when $P < 0.05$. All data were reported as mean ± standard deviation.

3. Results

3.1. Changes in the total antioxidant capacity of tomato samples

The antioxidant capacity of samples using DPPH assay are shown in Figure 2. Total antioxidant capacity of TS was 424.9 mg TE/100 g FW. The mean values revealed that total antioxidant capacity increased from 424.9 to 471.8 mg TE/100 g FW with increasing concentration of extra virgin olive oil. By adding extra virgin olive oil, significant increases of 5% and 11% were observed in the total antioxidant capacity values of 5O and 10O, respectively, as compared to control TS ($P < 0.05$). Moreover, the total antioxidant capacity values of C5O and C10O were found to be significantly higher as compared to TS, with ratios of 2.1-fold and 2.0-fold, respectively ($P < 0.05$). The total antioxidant contents of both C5O (2.0 times higher compared to 5O) and C10O (1.8 times higher compared to 10O) were significantly higher than those obtained by only adding extra virgin olive oil ($P < 0.05$). The results indicated that C5O exhibited the highest total antioxidant capacity followed by C10O, 10O, and 5O, which were significantly different ($P < 0.05$).

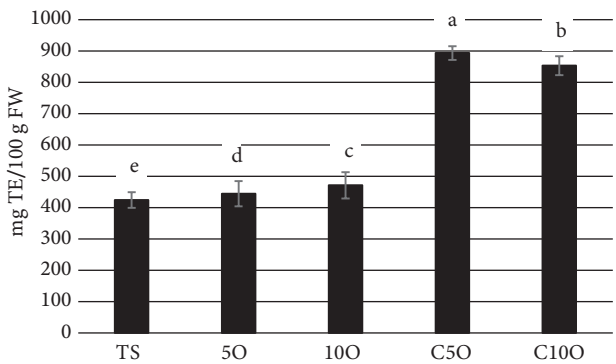

Figure 2. Changes in the total antioxidant capacity of tomato samples[a]. [a] Results are given as average values ± standard deviation of three independent samples. Different letters above bars within each sample represent statistically significant differences ($P < 0.05$). Total antioxidant activity expressed in mg TE/100 g FW.

3.2. Changes in lycopene, β-carotene, lutein, α-tocopherol, and chlorophyll a of tomato sauces

The changes observed in α-tocopherol, lycopene, β-carotene, lutein, and chlorophyll a with the addition of

extra virgin olive oil in tomato sauce are shown in Table. Lycopene, β-carotene, lutein, α-tocopherol, and chlorophyll a contents were significantly increased with increasing concentration of extra virgin olive oil in tomato sauces ($P < 0.05$). 5O led to significant increases in α-tocopherol (1.5-fold higher), lycopene (1.3-fold higher), β-carotene (1.4-fold higher), lutein (1.4-fold higher), and chlorophyll a (3.6-fold higher) contents ($P < 0.05$) as compared to TS ($P < 0.05$). Moreover, 10O showed an increase of 2.7-, 1.7-, 1.9-, 2.3-, and 6.1-fold in α-tocopherol, lycopene, β-carotene, lutein, and chlorophyll a, respectively, as compared to TS ($P < 0.05$). Furthermore, α-tocopherol was 10.9- and 6.7-fold, lycopene content 2.3- and 1.0-fold, β-carotene content 4.1- and 2.7-fold, lutein content 4.3- and 3.3-fold, and chlorophyll a content 10.0- and 7.9-fold higher in C5O and C10O, respectively, compared to TS ($P < 0.05$). In parallel with the total antioxidant activity results, the highest values for these bioactive compounds were recorded for C5O and the lowest values were observed in TS as shown in Table. α-tocopherol, lycopene, β-carotene, lutein, and chlorophyll a contents were positively affected by the combination of cooking and extra virgin olive oil addition.

3.3. In vitro gastrointestinal digestion

The impact of gastrointestinal digestion on α-tocopherol, lycopene, β-carotene, lutein, and chlorophyll a of samples is shown in Table and Figure 3. After simulated intestinal digestion, lycopene and β-carotene were observed in all samples, whereas α-tocopherol and chlorophyll a were not detected in any samples in the in vitro intestinal phase. On the other hand, lutein was detected only in C5O (0.5 mg/100 g FW) and C10O (0.6 mg/100 g FW) after intestinal digestion. According to the results, in the intestinal phase of the formulations containing extra virgin olive oil (5O, 10O), the levels of lycopene and ß-carotene were 4.6 and 4.0 times higher, respectively ($P < 0.05$). Moreover, the addition of extra virgin olive oil and cooking (both C5O and C10O) resulted in a significantly higher lycopene (≈8.5-fold) and β-carotene (≈5.0-fold) bioaccessibility compared to TS in the in vitro intestinal phase ($P < 0.05$). The results showed that lycopene, β-carotene, and lutein bioaccessibility increased with the addition of extra virgin olive oil and cooking together, compared to both TS and 5O and 10O samples ($P < 0.05$). In addition, no significant differences in the bioaccessibility of lycopene and β-carotene were observed between 5O and 10O samples. In the TS, 5O, 10O, C5O, and C10O samples, the percentage in vitro bioaccessibility of lycopene was 0.5%, 1.9%, 1.5%, 1.9%, and 4.7%, respectively. Furthermore, the percentage in vitro accessibility of β-carotene varied between 3.4% and 10.4%, the lowest value being found in TS and the highest in 5O, respectively. The bioaccessibility of lutein in C5O was 3.9% and reached a value of 6.1% in C10O.

Table. Changes in the carotenoid profile of tomato sauce samples during in vitro digestion[a].

Compounds (mg/100 g FW)	Initial	Intestinal phase
α-tocopherol		
TS	0.54 ± 0.01 e	n.d.
5O	0.79 ± 0.21 d	n.d.
10O	1.45 ± 0.03 c	n.d.
C5O	5.87 ± 1.12 a	n.d.
C10O	3.64 ± 0.41 b	n.d.
Lycopene		
TS	32.5 ± 0.30 d	0.17 ± 0.08 c
5O	41.6 ± 3.80 c	0.78 ± 0.13 b
10O	53.8 ± 3.70 b	0.80 ± 0.11 b
C5O	75.1 ± 6.50 a	1.45 ± 0.16 a
C10O	31.1 ± 5.46 e	1.47 ± 0.30 a
β-carotene		
TS	0.88 ± 0.06 e	0.03 ± 0.001 c
5O	1.19 ± 0.25 d	0.12 ± 0.007 b
10O	1.68 ± 0.11 c	0.11 ± 0.005 b
C5O	3.6 ± 0.64 a	0.16 ± 0.01 a
C10O	2.4 ± 0.21 b	0.14 ± 0.01 b
Lutein		
TS	2.98 ± 0.31 e	n.d.
5O	4.21 ± 1.51 d	n.d.
10O	6.96 ± 0.24 c	n.d.
C5O	12.9 ± 2.12 a	0.5 ± 0.11 a
C10O	9.9 ± 0.60 b	0.6 ± 0.15 a
Chlorophyll a		
TS	0.14 ± 0.01 e	n.d.
5O	0.51 ± 0.18 d	n.d.
10O	0.85 ± 0.04 c	n.d.
C5O	1.4 ± 0.19 a	n.d.
C10O	1.1 ± 0.04 b	n.d.

[a] Data represent average quantities ± standard deviation of 3 independent samples. Different letters in the columns represent statistically significant differences ($P < 0.05$).
n.d.: not detected.

4. Discussion

Mechanical homogenization, thermal processing, and the addition of oil can affect the tomato cellular matrix where carotenes are embedded. Moreover, these treatments have

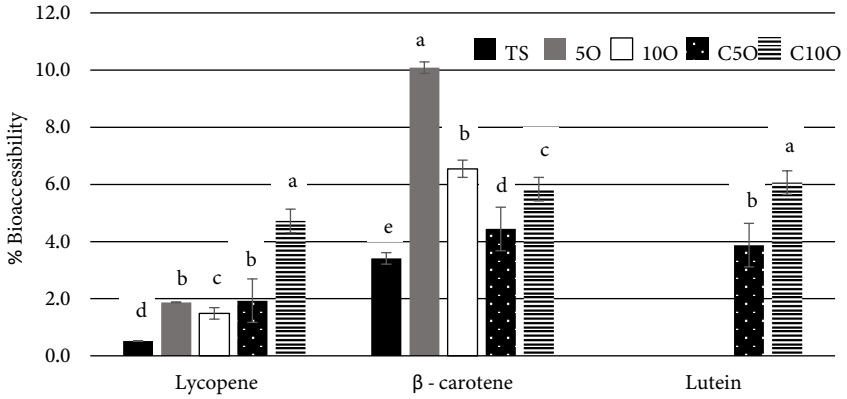

Figure 3. Percentage in vitro bioaccessibility (%). Significant differences within one group are indicated with different letters.

been reported to alter the bioaccessibility of carotenoids (Parada and Aguilera, 2007; Colle et al., 2013). In our study, bioaccessibility levels of lycopene and β-carotene were significantly raised by increasing the amount of oil (from 5% to 10%) compared to the control tomato sauce sample (without the addition of oil) ($P < 0.05$). This result is in accordance with the study of Colle et al. (2013) who showed that lycopene bioaccessibility of tomato pulp was enhanced when lipids were added before processing. Moelants et al. (2012) observed a remarkable increase in the micellarisation of lycopene from tomato and β-carotene from carrot particles as a result of the addition of olive oil during in vitro digestion. The results of these studies clearly showed that lipids have a positive effect on carotenoid bioaccessibility (Lemmens et al., 2014). The higher bioaccessibility of carotenoids containing both 5% and 10% oil can be explained by several mechanisms: I) the hydrophobic nature of lipids provides for easier solubilization of carotenoids and facilitates extraction; II) biliary secretion and micelle formation are stimulated by the lipids (Desmarchelier and Borel, 2017). On the other hand, our results also indicated that C5O exhibited the highest antioxidant activity and had higher contents of lycopene, β-carotene, lutein, α-tocopherol, and chlorophyll contents compared to C10O. Similarly, Colle et al. (2012) reported that when olive oil was added at percentages varying between 1% to 10%, the highest lycopene bioaccessibility was observed when 2% of olive oil was used. In another study, the bioaccessibility of lycopene in an oleoresin was also negatively affected by the increase in the amount of oil added (Fernández-García et al., 2007; Colle et al., 2012). These results indicate that the contents and bioaccessibility levels of carotenoids depend also on the quantity of oil in the sample. Thus, optimized levels should be used in order to provide the maximum benefit.

It has been reported that bioaccessibility of carotenoids also varies with respect to their structure, and xanthophylls, such as lutein, are more bioaccessible than carotenes due to their polarity (van het Hof et al., 1999). In contrast to these findings, in our study, lutein, α-tocopherol, and chlorophyll a were not detected after in vitro intestinal phase in tomato sauce samples containing oil. This discrepancy can be described by the fact that the bioaccessibility of carotenoids is not only affected by their chemical structure, but also by their interactions with other components. For example, Reboul et al. (2007) observed that carotenoids (lycopene and β-carotene) and naringenin ($P < 0.05$) impair lutein uptake. Similarly, Kostic et al. (1995) reported a decrease in plasma lutein levels when β-carotene was added. Additionally, our study showed that for more bioaccessible lycopene, β-carotene, and lutein, cooking and addition of extra virgin olive oil together were found to be much more efficient than only adding extra virgin olive oil to the tomato sauce ($P < 0.05$). According to Hedren et al. (2002), while 3% and 21% of the total β-carotene content were released from raw carrots in pieces and homogenized carrots (pulped), respectively, cooking the pulp and addition of cooking oil to the cooked pulp increased the bioaccessibility up to 27% and 39%, respectively. Similarly, a study by Hornero-Méndez and Mínguez-Mosquera (2007) indicated that addition of olive oil during cooking provided better carotenoid extraction and micellarisation in a dose dependent manner. Moreover, the in vivo study of Fielding et al. (2005) supports this observation, as they reported that the addition of olive oil to diced tomatoes during cooking greatly increased the absorption of lycopene. These results indicate that combination of cooking and olive oil addition improves the release of carotenes. A more efficient release of carotenoids from the food matrix can be linked to the destructive effect of cooking on the integrity of the cell wall, cell cluster, and chromoplast membranes where carotenoids are located, so that the digestive enzymes may work more efficiently by liberating

carotenoids from the food matrix into oil droplets (Hedren et al., 2002; Hornero-Méndez and Mínguez-Mosquera, 2007; Colle et al., 2013, Lemmens et al., 2014, Barba et al., 2017). Furthermore, heat enables protein denaturation to promote the disruption of protein-carotenoid complexes, which helps the release of carotenoids (Ortak et al., 2017). It is noteworthy to mention that apart from cooking and oil addition, mechanical disruption or homogenization prior to the heat treatment can also affect bioaccessibility. These treatments allow the reduction of particle size and enlargement of surface area for digestive enzymes to act, and carotenoids are more easily released from the food matrix (Hedren et al., 2002; Bengtsson et al., 2010).

To the best of our knowledge, the effect of oil addition and cooking on the carotenoid content of tomato sauce has never been reported in the literature. According to our results, both cooking and addition of extra virgin olive oil significantly improved carotenoid bioaccessibility in tomato sauce. Therefore, consumption of tomato sauce cooked with extra virgin olive oil may improve the bioavailability of carotenoids in humans. Further investigations on carotenoids, perhaps coupled to cellular models such as Caco-2, are warranted.

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