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Effect of dietary fibre addition in tomato sauce on the *in vitro* bioaccessibility of carotenoids

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RESEARCH ARTICLE

Abstract

The effect of inulin addition (5 and 10%) on the total antioxidant capacity, α-tocopherol and carotenoid contents, and *in vitro* gastrointestinal digestion of tomato sauces was investigated. In this study, α-tocopherol and carotenoids were quantified using HPLC. Moreover, the total antioxidant activity of the ethanol/hexane extracts was determined by two different *in vitro* assays. A negative linear relationship was observed between carotenoids and dietary fibre. Total antioxidant capacity (13-52%), lycopene (56-62%), β-carotene (32-59%), lutein (28-56%), and α-tocopherol (59-67%) decreased significantly ($P<0.05$) in all tomato samples enriched with inulin. Moreover, carotenoid bioaccessibility was adversely affected by the addition of dietary fibre.

Keywords: carotenoids, dietary fibre, food matrix, antioxidant, *in vitro* bioaccessibility, gastrointestinal digestion

1. Introduction

Diet is an important and modifiable factor influencing the functions of the body and it has been recommended as one of the key elements of prevention strategies for age-related chronic diseases and functional decline (Assmann *et al.*, 2017). Increased consumption of vegetables and fruit or a diet rich in antioxidants has been shown to reduce systemic inflammation (Ozawa *et al.*, 2017). The so-called 'Mediterranean diet' which is rich in fruits, vegetables and unsaturated fatty acids is a frequently studied dietary pattern. Greater adhesion to this diet has been linked to a lower rate of cognitive decline in a large number of observational and interventional studies (Berendsen *et al.*, 2017).

Tomatoes and processed tomato products are key components of the Mediterranean diet. Tomato and its products are being comprehensively studied based on their content of various health-related antioxidants, such as carotenoids, tocopherols and flavonoids (Melendez-Martinez *et al.*, 2010). Especially, tomatoes are mentioned as one of the main sources of dietary carotenoids, particularly α-carotene, β-carotene, lycopene, lutein and cryptoxanthin. Carotenoids prevent photo-damage in plants and they contribute to the photosynthetic mechanism. Carotenoids have a significant role in diet with being a primary source of vitamin A, owing to bioconversion of β-carotene (provitamin A) into retinol (vitamin A) (Del Giudice *et al.*, 2017). Lycopene is a lipophilic antioxidant carotenoid and a natural pigment giving the red colour to tomatoes. Tomatoes are seen as the main source of lycopene, which has a superior capability in quenching singlet oxygen among the other carotenoids. Epidemiological studies suggest that consumption of tomato and tomato-based products reduces the risk of bad cholesterol which is defined as increased levels of low density lipoprotein in blood (Friedman *et al.*, 2000), oxidative stress (Lindshield *et al.*, 2007) and several diseases, such as obesity (Ghavipour *et al.*, 2015; Young *et al,.* 1998), coronary heart disease (Klipstein-Grobusch *et al.*, 2000) and cancer (Polivkova *et al.*, 2010).

Dietary fibre is defined as the part of plant foods that are resistant to hydrolysis by human digestive enzymes. They include polysaccharides and lignin, as well as oligosaccharides, such as inulin, and resistant starches (Jones *et al.,* 2006). They are complex carbohydrates composed of soluble and insoluble components. The soluble dietary fibres can be fermented by intestinal microbiota, resulting with physiologically active by-products. On the other hand, the insoluble fibres have important bulking properties and decrease the intestinal transit (Mudgil and Barak, 2013). Dietary fibres are well known to play an important part in many physiological processes and in the prevention of several diseases. Dietary fibre consumption is associated with lower incidences of coroner heart disease, stroke, and peripheral vascular disease (Merchant *et al.*, 2003). Also, hypertension, diabetes, obesity, and dyslipidaemia, and colon cancer are also less prevalent in dietary fibre consuming individuals (Dahl and Stewart, 2015; Lairon *et al.,* 2005).

Such beneficial effects of dietary fibres have given rise to the development of a market which has a rapidly growing potential for fibre-rich products. However, food matrix could have an important effect on the utilisation of such bioactive components. Dietary fibre has been shown to reduce the bioavailability of macronutrients, notably lipids, and several minerals as well as trace elements in the human diet. In addition, it is suggested that dietary fibres have an effect on the absorption of carotenoids, α-tocopherol and polyphenolic compounds (Palafox-Carlos *et al.*, 2011).

The aim of the present work was to reveal the changes in the carotenoid profile and antioxidant activity of tomato sauces in the presence of inulin. Furthermore, the effect of inulin was also investigated in terms of its effect on *in vitro* simulated gastrointestinal digestion.

2. Materials and methods

Materials

A commercial tomato sauce was collected from a tomato sauce factory in Turkey. As dietary fibre source, inulin obtained from chicory was used, which was provided from the market and added at two different ratios (5 and 10% w/w) to the tomato sauce. After the addition of 5% (5I) and 10% (10I) inulin, the tomato sauces were homogenised at 25,000×*g* for 5 min using a homogeniser (IKA T18 basic, Staufen, Germany). An aliquot of each sample was stored at -20 °C until the day of the analysis. All tomato samples were ground in a laboratory scale grinder and stored at -80 °C until analysis. A schematic overview of the experimental set-up is presented in Figure 1.

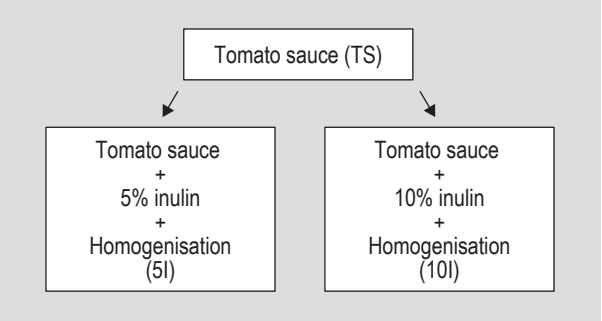

Figure 1. Diagram flowchart of the processes.

Extraction of carotenoids

For each tomato sample, three independent extractions were performed according to the method of Vallverdú-Queralt *et al.* (2012). Briefly, after extracting 0.5 g of each sample with 5 ml ethanol: n-hexane (4:3, v/v) in a cooled ultrasonic bath (Azakli, Turkey) for 5 min., the samples were centrifuged (Hettich Zentrifugen Universal 32R, Andreas Hettich GmbH, Tuttlingen, Germany) at 4,000×*g* at 4 °C for 15 min, and the clear upper phases were collected. The remained pellet was extracted once more with the same procedure, and two supernatants were combined to a final volume of 10 ml. They were then evaporated under nitrogen flow. Finally, the residue was reconstituted with methyl tert-butyl ether up to 1 ml and filtered through a 25 mm, 0.45 µm PTFE filter (Waters, Mildford, MA, USA). These extracts were stored at -20 °C until analysis.

Spectrophotometric assays

The total antioxidant activity of the extracts was determined by two different *in vitro* assays: The DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Kumaran and Karunakaran, 2006) and the CUPRAC (copper(II) reducing antioxidant capacity) method (Apak *et al.*, 2004) using a UV-visible spectrophotometer (Optima SP-3000 nano; Optima Corp., Tokyo, Japan). For the DPPH assay, 100 µl of extract was mixed with 2 ml of 0.1 mM DPPH in methanol. After 30 min of incubation, absorbance was measured at 517 nm (Kumaran and Karunakaran, 2006). For the CUPRAC assay, 100 µl of extract was mixed with 1 ml of 10 mM copper (II) chloride, 7.5 mM neocuproine, and 1 M ammonium acetate. Subsequently, 1 ml of distilled water was added to the mixture so as to bring the final volume to 4.1 ml. After 30 min of incubation at room temperature, absorbance was measured at 450 nm (Apak *et al.*, 2004). In both assays, trolox was used as a standard and total antioxidant activity of extracts was expressed as mg of trolox equivalent (TE) per 100 g of DW of sample.

Targeted HPLC analysis

Individual carotenoids, tocopherols and chlorophylls of sample extracts were identified using HPLC (Thermo Scientific, Waltham, MA, USA) by comparing their retention times with those of reference compounds and using calibration curves for quantification (Capanoglu *et al.*, 2008). Extracts were filtered by a 0.45 µm membrane filter and then injected into the system. Solvent systems were A (ultra-pure water:methanol, 20:80 v/v, 0.2% ammonium acetate in ultra-pure water), B (*t*-butyl methyl ether), and C (methanol) for carotenoids with a flow rate of 1 ml/min. Separation of compounds in the extracts was conducted in a 60 min run. Lycopene, β-carotene, lutein, and α-tocopherol were separated on a YMC-Pack C30 column (Kyoto, Japan) with a gradient flow of methanol and tert-butyl ether and detected at 450 nm (Figure 2). A photodiode array detector was used to detect the eluting compounds.

In vitro digestion of the samples

Standardised 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 were prepared as described in detail in the protocol (Minekus *et al.*, 2014). Briefly, 5 g of tomato sauce sample was mixed with 3.5 ml of salivary juice, 0.5 ml of α-amylase solution, 25 µl of 0.3 mol/l $CaCl_2$ 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 remaining mixture from the buccal phase and the pH was adjusted to 3.0 with 1 mol/l HCl, and the total volume was adjusted to 8 ml by adding distilled water. Then, the mixture was incubated in a shaking water bath (Memmert SV 1422, Memmert GmbH and Co. Nürnberg, Germany) at 37 °C for 2 h. After simulated stomach digestion, 7.7 ml of intestinal juice, 3.5 ml of pancreatin, 1.75 ml 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 mol/l NaOH, the total volume was completed to 14 ml with distilled water and the fluid mixture was incubated at 37 °C for 2 h with continuous shaking. At the end of the procedure, 2 ml aliquots were taken from each sample. The same procedure under the same conditions was applied without adding sample and used as a blank. The fractions were kept at -20 °C until further analysis.

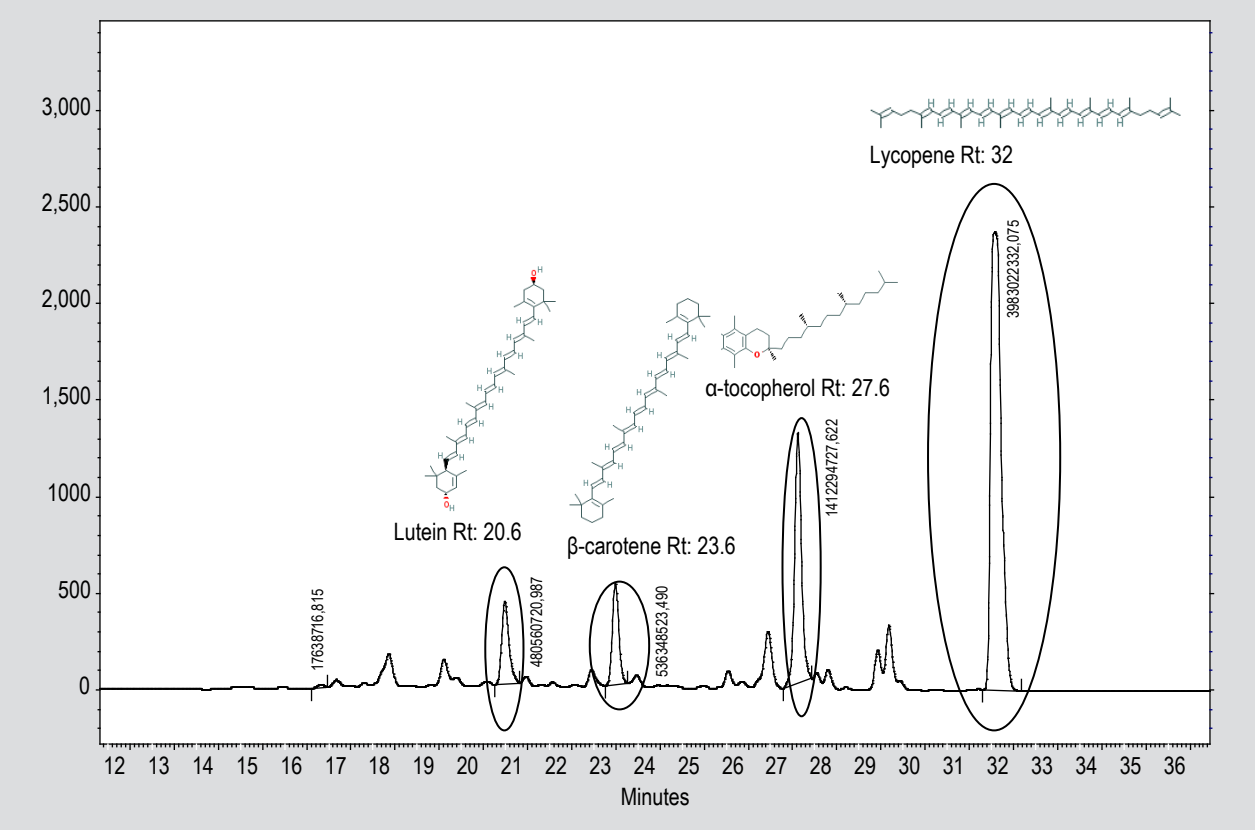

Figure 2. HPLC chromatogram of tomato sauce.

Statistical analysis

Statistical analyses were performed using the data of three independent replicates. Data were subjected to statistical analysis using SPSS software (SPSS, Chicago, IL, USA) for the analysis of variance (ANOVA). Pairwise comparisons between the treatments were done using Duncan's test with a 95% confidence level and all data were reported as mean ± standard deviation. The correlation coefficients (R^2) for spectrophotometric assays were calculated using the Microsoft Office Excel 2016 software (Microsoft Corporation, Redmond, WA, USA).

3. Results and discussion

Changes in antioxidants in tomato sauce enriched with inulin

The effect of inulin addition on the antioxidant capacity of tomato sauces analysed by DPPH and CUPRAC assays are shown in Table 1. Total antioxidant capacity decreased significantly (P<0.05) in the extracts of all tomato samples enriched with inulin. Total antioxidant values analysed by DPPH assay decreased from 3,863.4 to 3,348.3 mg TE/100 g DW in tomato sauces containing 5% inulin and from 3,863.4 to 2,956.1 mg TE/100 g DW in sauces containing 10% inulin (P<0.05). In addition, the same pattern was observed in the CUPRAC assay. CUPRAC assay values reduced slightly from 10,727.3 mg TE/100 g DW to 8,631.8 mg TE/100 g DW or to 5,123.4 mg TE/100 g DW in sauces containing 5% or 10% inulin, respectively. The results showed that tomato sauce containing 10% of inulin had the lowest total antioxidant capacity compared to the tomato sauce (P<0.05). It has been reported that the addition of inulin affects the carotenoid content and consequently the antioxidant capacity, adversely. This could be related to the physical and chemical interactions between inulin and carotenoids which prevent their action as antioxidants (Saura-Calixto, 2011; Palafox-Carlos, 2011). Based on these findings, inulin may convey appreciable amounts of lipophilic compounds through the gastrointestinal tract. Notably, according to our results the total antioxidant capacity values determined by the CUPRAC method were higher than the values obtained by the DPPH method (Table 1). This apparently high CUPRAC values can be well explained with the fact that CUPRAC reagent is selective, stable, and Cu(I) ion emerging as a product of the CUPRAC cannot act as a prooxidant (chelated state [i.e. Cu(I)-Nc]) (Apak *et al.*, 2013). Furthermore, CUPRAC and DPPH showed a positive linear relationship with a high correlation coefficient of R^2=0.951 indicating that CUPRAC and DPPH were well correlated with each other.

Table 1. Changes in the total antioxidant capacity of tomato samples containing 5 or 10% inulin.[1,2,3]

Samples	DPPH	CUPRAC
TS	3,863.4±98.9 a	10,727.3±98.0 a
5I	3,348.3±99.5 b	8,631.8±91.1 b
10I	2,956.1±113.0 c	5,123.4±80.0 c

[1] Results are given as the average values ± standard deviation of three independent samples. Different letters in the columns represent statistically significant differences (P<0.05).
[2] Total antioxidant activity expressed in mg Trolox equivalent/100 g dry weight.
[3] CUPRAC = copper(II) reducing antioxidant capacity; DPPH = 1,1-diphenyl-2-picrylhydrazyl; TS = tomato sauce (control); 5I and 10I = tomato sauce with 5 and 10% added inulin, respectively.

Effect of dietary fibre addition on tocopherols and carotenoids and their *in vitro* bioaccessibility

The changes observed in α-tocopherol, lycopene, β-carotene, and lutein with the addition of inulin into tomato sauce are shown in Table 2. The contents of α-tocopherol, lycopene, β-carotene, and lutein significantly decreased by 67, 56, 32 and 28% in the sauce containing 5% inulin as compared to the tomato sauce, respectively (P<0.05). Similarly, after the addition of 10% inulin, α-tocopherol, lycopene, β-carotene, and lutein contents decreased by

Table 2. Changes in the carotenoid profile of tomato sauce during *in vitro* digestion.[1,2]

Compounds (mg/100 g DW)	Initial	Intestinal phase
α-tocopherol		
TS	4.9±0.006^a	n.d.
5I	1.6±0.4^c	n.d.
10I	2.0±0.003^b	n.d.
Lycopene		
TS	295.1±0.3^a	1.5±0.08^a
5I	130.0±0.5^b	n.d.
10I	111.0±1.9^c	0.5±0.01^b
β-carotene		
TS	8.0±0.06^a	0.3±0.01
5I	5.4±0.01^b	n.d.
10I	3.3±0.03^c	n.d.
Lutein		
TS	27.1±0.3^a	n.d.
5I	19.4±0.1^b	n.d.
10I	11.9±0.03^c	n.d.

[1] Data represent average quantities ± standard deviation of three independent samples. Different letters in the columns represent statistically significant differences (P<0.05).
[2] TS = tomato sauce (control); 5I and 10I = tomato sauce with 5 and 10% added inulin, respectively. n.d. = not detected; DW = dry weight.

59, 62, 59 and 56%, respectively, compared to the tomato sauce (P<0.05). Overall, for α-tocopherol, lycopene, β-carotene, lutein, and total antioxidant capacity, levels in sauce containing 5% or 10% inulin were significantly lower compared with the tomato sauce (P<0.05). The results showed that tomato sauce containing 10% inulin had the lowest lycopene, β-carotene, lutein, and total antioxidant capacity compared to the tomato sauce (P<0.05). The low amount of carotenes and α-tocopherol in sauce samples containing inulin is probably associated with the fact that there are interactions between these compounds and dietary fibre which may prevent their release.

Carotenoid bioavailability is dependent upon a number of factors which are grouped in the mnemonic SLAMENGHI: Species of carotenoids, intermolecular Linkages, Amount of carotenoids consumed in a meal, the effect of Matrix in which carotenoids are located, Effectors balancing the absorption and bioconversation, Nutrient condition of the host, Genetic variability, Host-related factors, and Interactions among these variables (Castenmiller and West, 1998). Furthermore, studies on humans, animals, and *in vitro* have clearly shown that dietary fibres have a negative effect on the absorption of carotenoids (Desmarchelier and Borel, 2017; Hoffman *et al.,* 1999; Palafox-Carlos *et al.*, 2011; Riedl *et al.,* 1999; Rock and Swendseid, 1992; Verrijssen *et al.*, 2014).

In vitro digestion results for the intestinal phase are shown in Table 2. Lycopene content was found to be 1.5 mg/100 g DW in the *in vitro* intestinal phase of tomato sauce. After the addition of 10% inulin, lycopene content decreased by ~3-fold when compared to the tomato sauce in the *in vitro* intestinal phase (P<0.05). Moreover, β-carotene content was found to be 0.3 mg/100 g DW in the *in vitro* intestinal phase of tomato sauce. However, in the *in vitro* intestinal phase, α-tocopherol, β-carotene and lutein were not detected at all in sauces containing 5 and 10% inulin. After digestion α-tocopherol and lutein were not also identified in the control tomato sauce samples. These findings were similar to the results of Hoffman *et al.* (1999) who reported that the addition of pectin to chow or test meal decreased the bioavailability of β-carotene in chicks and humans. Riedl *et al.* (1999) also showed that soluble fibres and insoluble fibres significantly reduced the bioavailability of lycopene and lutein (40-74%) in humans. However, only soluble fibre was found to greatly decrease the bioavailability of β-carotene (33-43%). In a more recent study, Sriwichai *et al.* (2016) indicated that the carotenoid bioaccessibility was negatively correlated to the pectin contents of the leafy vegetables. Another more recent study conducted by Al-Yafeai and Böhm (2018) indicated that adding pectin to tomato paste significantly reduced the bioaccessibility of lycopene derivatives; (*all-E*)-lycopene to 13%, (*Z*)-lycopene to 25%, and (*all-E*)-β-carotene to 44% compared with untreated samples.

A possible explanation could be that carotenoid absorption depends not only on the release from the food matrix but also on the subsequent solubilisation by bile acids and digestive enzymes, culminating in their incorporation into micelles (Parada and Aguilera, 2007). However, soluble fibres are known to bind bile acids, thereby avoiding micelle formation with carotenoids. Sequestration of micelle formation could result subsequently in decreased absorption of carotenoids. Moreover, dietary fibres delay gastric emptying and interfere with micelle formation necessary for the absorption of carotenoids (Desmarchelier and Borel, 2017; Palafox-Carlos *et al.*, 2011; Priyadarshani, 2017; Rock and Swendseid, 1992). The results are in line with the study of Yonekura and Nagao (2009) which investigated the effects of soluble fibres on the micellization of β-carotene and lutein throughout the *in vitro* digestion, and carotenoid uptake of Caco-2 cells from mixed micelles. The study results revealed that soluble fibres restrict carotenoid uptake primarily by increasing viscosity, and accordingly decreasing the micelles' diffusion rate in the medium (Yonekura and Nagao, 2009). Another explanation could be that delayed gastric emptying and other factors may promote the oxidative cleavage of carotenoids to other compounds (Rock and Swendseid, 1992).

Inulin has several physico-chemical functions, such as water binding and alteration of viscosity. It can effect passage rate, rheological and colloidal state of digesta, and interactions with digestive enzymes and bile salts in the stomach and small intestine. Based on these findings, we have speculated that the behaviour of inulin in the gastrointestinal tract can be linked with these mechanisms: (1) inulin increases the viscosity of food digesta. Thus, an increased digesta viscosity may also impair the efficiency of emulsification in the small intestine which reduces the bioavailability of lipophilic compounds; (2) physical entrapment of lipophilic compounds within inulin can decrease the bioaccessibility; (3) the binding of inulin with lipophilic compounds and bile salt may avoid micelle formation with them (Capuano, 2017; Padayachee *et al.*, 2017; Roberfroid, 1993).

It is also worthwhile emphasising that dietary fat in the diet plays a vital role in increasing carotenoid bioaccessibility by micelle production which would promote the quantity of carotenoids solubilised in micelles and hence available for absorption (Desmarchelier and Borel, 2017). For example, Arranz *et al.* (2015) reported that the consumption of tomato juice with oil enhances postprandial absorption of lycopene isomers in plasma. However, in our study, the tomato sauce formulation does not contain oil, and as a result the extremely low amount of bioaccessibility obtained for carotenoids and tocopherols appear to be associated with the absence of lipid fraction. Furthermore, for the most consumed processed tomato products (paste, sauce, puree, juice, ketchup, soup, etc.) the total daily lycopene intake for very apolar carotenoids such as lycopene were reported

to be in the range of 2.1 to 9.1% (Agarwal *et al.,* 2001), whereas in the present work levels of bioaccessibility were found to be lower. These results also indicated that different processing techniques can affect the bioaccessibility of carotenoids differently.

Our study showed that in the tomato sauce, lycopene and β-carotene were detected whereas lutein was not detected in the *in vitro* intestinal phase. This can be well explained by the fact that some polyphenols (e.g. naringenin) and carotenoids may reduce the carotenoid bioaccessibility. Reboul *et al.* (2007) reported that carotenoids (lycopene and β-carotene) and naringenin impair lutein uptake. Our previous study indicated that the flavonoid naringenin was 20-fold higher in the industrial tomato sauce compared to the fruit (Tomas *et al.,* 2017). Possibly, this high level of naringenin could adversely affect the *in vitro* bioaccessibility of lutein. Additionally, all the non-absorbed carotenoids and dietary fibre travel through the gastrointestinal tract passing to colon, where carotenoids may exert their antioxidant activity in the colon environment (Palafox-Carlos *et al.*, 2011). Moreover, animal and human studies have shown that some dietary bioactives (i.e. β-cryptoxanthin) may be absorbed through the colon where they or their metabolites (i.e. apo-carotenoids) may exert bioactivity (Granado-Lorencio *et al.*, 2017; La Frano *et al.*, 2013).

4. Conclusions

Our work highlighted that the addition of inulin affects adversely not only lycopene (56-62%), β-carotene (32-59%), lutein (28-56%), and α-tocopherol (59-67%) contents, and total antioxidant capacities (13-52%) of tomato sauce, but also their *in vitro* bioaccessibility. It can be also concluded that inulin is physically sequestering carotenoids by strong interactions, affecting their bioaccessibility. Still, more research is necessary to investigate the interaction of different fibre sources and their mixtures with carotenoids.

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