PAPER

GASTROINTESTINAL STABILITY OF CAROTENOIDS FROM RAW AND FREEZE-DRIED VEGETABLES

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ABSTRACT

In this study, the gastrointestinal stability of carotenoids (lycopene and *β*-carotene) extracted from raw and freeze-dried samples of tomato, carrot and red pepper was investigated. Extracted carotenoids fractions (lycopene and *β*-carotene) were submitted to a two-phase *in vitro* digestion process using human gastrointestinal enzymes. The use of freeze-drying has a strong effect on the enhancement of the gastrointestinal stability of carotenoid, especially after simulated intestinal phase. In addition, the effect of pH on carotenoid stability is much lower in freeze-dried plant material than in raw samples of tomato, carrot and red pepper. The food matrix also plays important role in carotenoids gastrointestinal stability rate, which was found to be the most stable in red pepper.

Keywords: carotenoids, *in vitro* digestion, lycopene, *β*-carotene

1. INTRODUCTION

Environmental influences such as contamination, ultraviolet (UV) radiation, smoking, stress and improper diet may result in cell damage caused by free radicals, believed to be the cause of many degenerative diseases, such as certain types of cancer, cardiovascular disease, type 2 diabetes, age-related macular degeneration (AMD) and cataracts, among others, and even mortality caused by some of these serious diseases (MAIANI *et al*., 2009; FIEDOR and BURDA, 2014; MeyErs *et al*., 2015; NOLAN *et al*., 2015; SLUIJS *et al*., 2015). In order to protect itself, the body uses antioxidants and neutralizers of the free radicals, which are commonly provided by the diet. Carotenoids, which are considered as the most widely distributed pigments in nature (SCHWARTZ *et al*., 2008), are known to be very efficient scavengers of singlet oxygen ($^1O_2$), as well as other reactive oxygen species (ROS). Carotenoids are responsible for the attractive, yellow to red colour of fruit and vegetables, which is the first attribute that consumers evaluate. Although more than 700 different carotenoids have been identified so far, just six of them are commonly analysed in foods and blood: three hydrocarbon compounds - carotenes (*β*-carotene, *α*-carotene, lycopene) and three oxygenated forms - xanthophylls (*β*-cryptoxanthin, lutein, zeaxanthin) (OLMEDILLA-ALONSO, 2017).

The composition and the content of carotenoids in foods are dependent on different factors e.g. variety and maturity of species, cultivation practices and methods of food processing. Several reviews and databases on food sources of carotenoids, intake, stability and bioavailability have been published (HEINONEN *et al*., 1989; HART and SCOTT, 1995; LETH *et al*., 2000; MURKOVIC *et al*., 2000; O'NEILL *et al*., 2001; KIM *et al*., 2007; FERNÁNDEZ-GARCÍA *et al*., 2012; NAGAO, 2014; MEYERS *et al*., 2015; OLMEDILLAALONSO, 2017). Therefore, in order to understand the relationship between nutrition and health in humans, it is important to know not only the amounts of consumed carotenoids but to what extent they are absorbed from the different dietary sources, their bioavailability, respectively (OLMEDILLA-ALONSO 2017). In general, stability under environmental conditions, gastrointestinal stability, the bioaccessibility as well as the bioavailability of functional food ingredients represent main factors affecting usefulness of the intake of certain foodstuffs (NAGAO, 2014). Carotenoids have very low bioavailability because they are quite susceptible to conditions found in the digestive tract (temperature, pH). Likewise, they are less bioavailable due to extreme hydrophobicity, and it also depends on other factors: release from the food matrix, solubilization in the digestive tract, absorption in intestinal epithelia, and metabolism (Nagao 2014). Also, it may be due to the fact that they can be bound in carotenoproteins; in green leafy vegetables carotenoids are found bound in chloroplasts and in carrot root, *α*- and *β*-carotene are largely in crystal forms. Therefore, the carotenoids are not easily solubilized out of these tissues by the digestive process (Institute of Medicine 2000), mostly due to rigid cell walls. They are more readily released in ripe fruit as well as processed vegetables then in fresh one, which substantially improves their bioavailability (Nagao 2014). It is known that the application of a thermal treatment and/or mechanical homogenization, as well as addition of fats and oils in diet, are all techniques that enhanced the bioavailability of dietary carotenoids (FERNÁNDEZ-GARCÍA *et al*., 2012), just because of increased bioaccessibility by dispersing them in digestive tract. On the other hand, dietary fibres have been thought to decrease bioaccessibility by binding bile acids (NAGAO, 2014). Following digestive release in stomach and upper intestine, the hydrophobic components aggregate in lipid emulsion droplets, which partitioned into mixed micelles in the small intestine. The formation of micelles allows carotenoids to be soluble in the hydrophobic interior and carotenoids, which are not in that form are not typically bioaccessible and remain unabsorbed (FERNÁNDEZ-GARCÍA *et al*., 2012; NEILSON *et al*., 2017).

In order to study the rate of gastrointestinal stability of biologically active components, the use of *in vitro* digestion models procedures represents effective tool due to its simplicity, low cost and putative production of many digesta fractions. A large number of *in vitro* studies have been carried out on the bioavailability of carotenoids and their assimilation during the digestive process and a number of models are suggested to mimic *in vivo* digestion over the years (FERRUZZI *et al*., 2006; GRANADO-LORENCIO *et al*., 2007; FAILLA *et al*., 2008; COURRAUD *et al*., 2013; KOPEC *et al*., 2017), but there is a lack of studies on the stability of carotenoids under gastrointestinal conditions using human gastrointestinal juices which comprise a complex mixture of enzymes present in multiple isoforms, enzyme inhibitors and bile salts that are important for the digestion process. The aim of this study was to determine carotenoid stability after simulated gastric and duodenal phases of simulated digestion process. Raw and freeze-dried samples of tomato, carrot and red pepper, vegetables rich in carotenoids, were used for their extraction. The stability rate of carotenoids (*β*-carotene and lycopene) in relation to digestion enzymes and to the effect of pH after each simulated digestion phase was determined spectrophotometrically.

2. MATERIAL AND METHODS

2.1. Chemicals

Lycopene, *β*-carotene and BHT (butylhydroxy toluene) were purchased from Sigma – Aldrich (Basel, Switzerland). All solvents (hexane, acetone, ethanol) were of pro analysis purity and were purchased from Kemika (Zagreb, Croatia).

2.2. Samples

Raw samples of carrot, tomato and red pepper were purchased from green local market. Raw samples were processed immediately after the purchase. Samples were firstly cut in small pieces and immediately homogenized in the blender to obtain a pulp. Then, the pulp was treated with argon in order to avoid rapid oxidation process. The pulp was not stored, because for repetition of experiments only fresh samples were used. Immediately after purchasing, one part of samples was freeze-dried using Freeze-dry system (Labconco, USA) at the temperature of –50°C and the pressure of 0.2 mb, during the period of 3 days. After freeze-drying, samples were grinding into a spice grinder and immediately used for further analyses. Second part of samples were fresh (raw) and were immediately prepared for extraction in the manner that samples were cut into approximately 2 to 4 cm cubes, and then homogenized using a hand blender (Bosch Maxomixx, Germany).

2.3. Extraction of carotenoids

The extraction of carotenoids from freeze-dried and raw samples of carrot, tomato and red pepper was done using two procedures described by ALDA *et al*. (2009) and FISH *et al*. (2002). For extraction procedure, the following solvents were used: acetone, hexane and ethanol (2:1:1). Sample from homogenized raw plant material or freeze-dried plant material (1 g) was mixed with 25 mL of solvent mixture, under subdued lighting at room temperature and the bottle was treated with argon in order to protect lycopene from degradation. After solvent addition, the samples were shacked during the period of 30 min (180 rpm, room temperature). After that, 1 mL of deionized water was added and the samples were left for approximately 5 min in order to obtain two separate layers. Upper

layer (the fraction with lycopene) was separate and stored in glass, dark flasks, treated with inert gas (argon) and stored at – 20°C until analysis.

2.4. Extraction of β-carotene

Extraction of β-carotene was done according to procedure described by DAVIS *et al*. (2008). For extraction procedure, the following solvents were used: BHT in acetone (0.05%), ethanol and hexane (1:1:2). Homogenized raw plant material or freeze-dried plant material (0.6 g) was mixed with 15 mL of solvent mixture under subdued lighting at room temperature and the bottle was treated with argon to protect β-carotene from degradation. After solvent addition, the samples were shacked during the period of 10 min (180 rpm, room temperature). After that, 3 mL of deionized water was added and the samples were left for approximately 5 min in order to obtain two separate layers. Upper layer (the fraction with β-carotene) was separate and stored in glass, dark flasks, treated with inert gas (argon) and stored at –20°C until analysis.

2.5. Spectrophotometric measurement of lycopene and β-carotene

Spectrophotometric measurements were performed on UV/Vis spectrophotometer Specord 200 spectrometer (Analytik Jena GmbH, Germany) and IRAffinity-1 Fourier Transform infrared (FTIR) spectrometer (Shimadzu, Japan). IR spectra were recorded by using KBr transmision cell, in the spectral area 4000-400 cm^{-1} and with resolution 4 cm-1. Abbreviations used are for streching (*n*), deformation (*d*).
For measurement of lycopene and β-carotene, the calibration curve was done using different concentrations of lycopene and β-carotene. A 0.01 g of β-carotene and lycopene was dissolved in hexane (100 mL) to obtain the concentration of 100 μg/mL (stock solution). After that, the working solution was done (20 μg/mL) and the following concentrations were prepared: 7, 6, 5, 4, 3, 2, 1 μg/mL.
The concentration of lycopene in prepared samples was determined by UV/Vis spectrophotometer at 503 nm. Hexane was used for detection of zero. Concentration of lycopene in raw and freeze-dried samples of carrot, tomato and red pepper was determined according to following formula (FISH *et al*., 2002):

$$(A_{503} \times 31.2) / \text{g of sample}$$

Absorbance of β-carotene was determined using UV/Vis spectrophotometer at 450 nm.

2.6. Isolation of human juices

Human gastric and duodenal juices were collected from four donors (two males and two female) without known gastrointestinal pathology, and who were not taking acid secretion inhibitors or antibiotics. Gastric and duodenal juices were aspirated through the endoscope. Eight hours before the procedure, all liquid or food intake was ceased. For each patient, 3 mL of initially aspired juice were discarded and the remaining amount was collected in a sterile tube, which was centrifuged to remove mucus and cell debris. In order to reduce inter-individual variations, batches of pooled gastric and intestinal juices were prepared and then stored at –20°C until use. The approval for the collection of digestive juices was obtained from the Ethics Committee of the University Hospital Centre Split, Croatia.

2.7. Enzymatic activity of juices

The procedure described by ALMAAS *et al*. (2006) was used to determine enzymatic activity of the prepared pooled human gastric juice samples. Pepsin activity was measured using 2.5% solution of bovine haemoglobin. The solution was prepared in 0.2 mol/L phosphate buffer (pH=7.6) and then acidified (to pH=3) using H_2SO_4. In order to determine the human duodenal juice activity, casein solution (1%) dissolved in 0.2 mol/L phosphate buffer (pH=7.6) was used. A volume of 500 μL of prepared protein solutions was incubated with 5, 20 or 50 μL of gastrointestinal juice. The digestion reactions were stopped with the addition of 1 mL of 10% trichloroacetic acid (TCA). Samples were measured spectrophotometrically at 280 nm. One unit of enzyme activity (U) is defined as the amount of enzyme that causes the absorbance change of 1 between the blank and the sample, after 20 min at 37°C.

2.8. *In vitro* digestion

A two-phase digestion procedure was performed according to the method described by FURLUND *et al*. (2013). Gastric and intestinal digestion phases were performed at 37°C, in horizontal shaking bath (180 rpm). The volume of digestive juice corresponding to 1 U of enzymatic activity was 20 μL of human gastric juice and 25 μL of human duodenal juice. The pH of the samples was adjusted to pH=2.5 using 1 mol/L HCl for gastric phase, and to pH=7.5 using 2 mol/L NaOH for intestinal phase. The concentration of human juices used for this assay was 20 U per g of plant material for gastric and 62.4 U per g of plant material for intestinal phase. A 0.6 g of both fresh and freeze-dried samples were used for digestion process. The incubation period of gastric phase was 30 min, while digested intestinal samples were collected after 120 min of intestinal phase. Before spectrophotometric analyses, the digested samples were centrifuged before spectrophtometrical analyses by microcentrifuges mySpin 12 (Thermo Scientific, USA) at room temperature, during 10 min at 9000 rpm. Enzymatic reactions were stopped on ice and the samples were stored at –20°C until analyses. All digestion processes were run in duplicate. Stability rate of lycopene and β-carotene represents the ratio of their concentrations before *in vitro* digestion and after gastric or intestinal digestion phases. Samples were dissolved in *n*-hexane and according to UDDIN *et al*. (2009) and POOJARI *et al*. (2009) digestive enzymes retained their stability in non-polar solvents such as *n*-hexane. The gastrointestinal stability rate (%) of lycopene and β-carotene was calculated according to the following formula:

(Sample concentration after digestion/sample concentration before digestion) x 100

2.9. Statistics

Statistical analysis was performed using GraphPad InStat3 software (GraphPad Software Inc., San Diego, CA, USA). The relationship between the obtained parameters was described using Pearson's correlation coefficient *r*. Differences at $p<0.05$ were considered to be statistically significant

3. RESULTS AND DISCUSSION

The influence of digestion process on the carotenoids stability is not completely explored. In this study we explored the stability rate of carotenoids (lycopene and *β*-carotene) from raw and freeze-dried red carrot (*Daucus carota*), tomato (*Solanum lycopersicum*) and red pepper (*Capsicum annuum*) after gastric and duodenal simulated digestive phase using human digestive juices.

The analysis of the UV/Vis spectrum of the obtained extracts showed strong peaks at 444, 471 and 502 nm. FTIR spectral peaks, in the range of 3082 - 2835 cm-1 indicate the presence only of C-H bonds, *ie.* 3082 - 3011 cm-1 which correspond to C(sp2)-H bonds and 2965 - 2835 cm-1 to C(sp3)-H bonds stretching. Peaks are observed at 1643 *n* (C=C, alkene), 1435 and 1375 *d* (CH2, CH3, bend). The quantities of carotenoids in the extracted fractions of raw freeze-dried samples were determined spectrophotometrically by previously described methods. Although not the absorbance peak of greatest magnitude in hexane, the absorbance peak at 503 nm was used for lycopene determination in order to minimize interference from other carotenoids. If generally accepted, nominal carotenoid contents of red-fleshed watermelon, fresh red tomato, and pink grapefruit are utilized (HOLDEN *et al*., 1999) together with molar extinction coefficients at 503 nm in hexane for those carotenoids (ZECHMEISTER *et al*., 1943; ZECHMEISTER *et al*., 1943a), the potential error can be estimated if absorbance contributions by other carotenoids are ignored. Such a calculation suggests that constituent carotenoids other than lycopene will contribute to the absorbance at 503 nm 0.2% for red-fleshed watermelon, 0.4% for fresh red tomatoes, and 0.6% for pink grapefruit (FISH *et al*., 2002). Previous reports of the major carotenoids detected in the investigated material showed that the carrot is a significant source of *β*-carotene (BYSTRICKA *et al*., 2015), *S. lycopersicum* of lycopene (BARANSKA *et al*., 2006), while the unique keto carotenoids of red pepper capsanthin, capsorubin and cryptocapsin impart brilliant red colour to ripen chilly pods, while the yellow orange colour is from *β*-carotene, zeaxanthin, violaxanthin and *β*-cryptoxanthin (ARIMBOOR *et al*. 2015). Results showed that the concentration of carotenoids (lycopene) in raw and freeze-dried samples ranged from 37.73 to 53.93 mg/kg and 61.09 to 61.92 mg/kg, respectively, with the highest one in red pepper extracts. On the other hand, the absorbance peak at 450 nm was used for *β*-carotene estimation in the investigated samples. The results showed that the carotenoid (*β*-carotene) concentration in raw and freeze-dried samples ranged from 15.14 to 27.92 mg/L, and 21.43 to 56.17 mg/L, respectively.

The difference in the stability of carotenoids (lycopene and β-carotene) between raw and freeze-dried plant material as well as the difference in their gastrointestinal stability in relation to different plant matrix were detected. The high stability of carotenoids from freeze dried food rich with carotenoids is already reported by several authors (CINAR, 2005; CHEN *et al*., 2007; VASQUE-CAICEDO *et al*., 2007). However, this is the first report on the gastrointestinal stability of carotenoids from fresh and dried samples. Gastrointestinal stability of β-carotene and lycopene was evaluated using gastric and duodenal human juices. Some authors reported that colonic microbiota can maximize the bio-accessibility of carotenoids by digestion of plant cell walls (DJURIC *et al*., 2017).

Results presented in Table 1 show that the stability of carotenoids (lycopene) were significantly higher in red pepper than in carrot and tomato after simulated gastric phase. Generally, lycopene stability after simulated gastric digestion was much higher in freezedried plant material than in raw plant material. The difference in lycopene gastric stability was lower in freeze-dried plant matrix (carrot, tomato and red pepper). Interestingly, the stability rate of lycopene after simulated gastric digestion was extremely high in freezedried red pepper and tomato (96.04 and 92.09%, respectively). Lycopene was not stable after simulated duodenal digestive phase in carrot and in tomato, or its stability

was very low (27.98% in raw red pepper). The use of freeze-drying significantly improved its duodenal stability. Generally, lycopene stability in raw and in freeze-dried plant material was much lower after simulated duodenal phase in comparison with its stability after simulated gastric phase as shown in Table 2. COURRAUD *et al*. (2013) also reported high stability of carotenoids after simulated gastric incubation (in their study they used commercial digestive enzymes).

Table 1. Stability rate of carotenoids (lycopene) from a) raw, and b) freeze-dried samples of carrot, tomato and red pepper after simulated gastric phase.

		Sample		
		Carrot *(Daucus carota* L.)	**Tomato *(Solanum lycopersicum* L.)**	**red pepper *(Capsicum annum* L.)**
a)	Concentration in raw sample [mg/kg]	37.73±0.12	45.48±0.20	53.93±0.77
	Concentration after gastric phase [mg/kg]	16.35±0.84	20.12±0.94	32.80±0.19
	Stability [%]	43.00±0.23	44.14±0.47	60.81±0.45
b)	Concentration in freeze-dried sample [mg/kg]	61.09±0.24	60.45±0.55	61.92±0.41
	Concentration after gastric phase [mg/kg]	52.26±0.12	52.54±0.90	59.47±0.34
	Stability [%]	86.91±0.87	92.09±0.55	96.04±0.63

Table 2. Stability rate of carotenoids (lycopene) from a) raw, and b) freeze-dried samples of carrot, tomato and red pepper after simulated duodenal phase.

		Sample		
		Carrot *(Daucus carota* L.)	**Tomato *(Solanum lycopersicum* L.)**	**Red pepper *(Capsicu annum* L.)**
a)	Concentration in raw sample [mg/kg]	37.73±0.12	45.48±0.20	53.93±0.77
	Concentration after duodenal phase [mg/kg]	/	/	15.09±0.56
	Stability [%]	/	/	27.98±0.36
b)	Concentration in freeze-dried sample [mg/kg]	61.09±0.24	60.45±0.55	61.92±0.41
	Concentration after duodenal phase [mg/kg]	38.08±0.78	36.12±0.24	42.16±0.20
	Stability [%]	62.33±0.11	59.75±0.22	68.08±0.14

According to results presented in Tables 3 and 4 the gastrointestinal stability of carotenoids (β-carotene) differs from that of lycopene. After duodenal digestive phase β-carotene was not the most stable in red pepper, as it was the case for lycopene. In comparison with lycopene, β-carotene showed moderate stability rate after duodenal phase in both, raw and freeze-dried plant material, while lycopene was not stable in raw carrot and tomato after duodenal phase. Interestingly, the stability rate of β-carotene significantly decreased after duodenal digestive phase in freeze-dried red pepper (Table 4).

Table 3. Stability rate of carotenoids (β-carotene) from a) raw, and b) freeze-dried samples of carrot, tomato and red pepper after simulated gastric phase.

		Sample		
		Carrot *(Daucus carota* L.)	**Tomato *(Solanum lycopersicum* L.)**	**Red pepper *(Capsicum annum* L.)**
a)	Concentration in raw sample [mg/kg]	15.14±0.35	21.54±0.40	27.92±0.24
	Concentration after gastric phase [mg/kg]	3.57±0.26	10.47±0.34	20.41±0.25
	Stability [%]	23.59±0.17	48.64±0.32	73.13±0.30
b)	Concentration in freeze-dried sample [mg/kg]	21.43±0.29	33.28±0.35	56.17±0.27
	Concentration after gastric phase [mg/kg]	6.42±0.45	18.89±0.29	51.84±0.18
	Stability [%]	30.00±0.28	56.77±0.38	92.30±0.45

Table 4. Stability rate of carotenoids (β-carotene) from a) raw, and b) freeze-dried samples of carrot, tomato and red pepper after simulated duodenal phase.

		Sample		
		Carrot *(Daucus carota* L.)	**Tomato *(Solanum lycopersicum* L.)**	**Red pepper *(Capsicum annum* L.)**
a)	Concentration in raw sample [mg/kg]	15.14±0.35	21.54±0.40	27.92±0.24
	Concentration after duodenal phase [mg/kg]	5.38±0.36	12.80±0.29	13.54±0.19
	Stability [%]	35.59±0.22	59.45±0.26	48.50±0.34
b)	Concentration in freeze-dried sample [mg/kg]	21.43±0.29	33.28±0.35	56.17±0.27
	Concentration after duodenal phase [mg/kg]	11.42±0.26	22.93±0.40	11.38±0.24
	Stability [%]	53.33±0.20	68.93±0.24	20.26±0.36

In this study the influence of pH on carotenoids (lycopene and β-carotene) stability was explored (Tables 5 and 6). As it is shown in Table 5 the influence of pH on lycopene stability was stronger in raw than in freeze-dried plant material. Generally, it can be seen that carotenoids (lycopene and β-carotene) were more stable at pH 2.5 than at pH 8.0. Correlations between the stability of carotenoids in acidic and alkaline conditions were found to be significant (r=0.6926, p=0.0125) and extremely significant (r=0.9170, p<0.0001) for Tables 5 and 6, respectively. Freeze-drying technique significantly improve lycopene stability at low pH. Interestingly, concerning the stability of β-carotene there is no significant difference between raw and freeze-dried plant material.

Table 5. The influence of pH (gastric and duodenal) on the stability rate of carotenoids (lycopene) from a) raw, and b) freeze-dried samples of carrot, tomato and red pepper.

		Sample		
		Carrot *(Daucus carota* L.)	**Tomato *(Solanum lycopersicum* L.)**	**Red pepper *(Capsicum annum* L.)**
a)	Concentration in raw sample [mg/kg]	37.73±0.12	45.48±0.20	53.93±0.77
	Concentration at pH 2.5 [mg/kg]	16.06±0.89	23.76±0.22	37.61±0.87
	Stability [%]	42.56±0.78	52.12±0.47	69.73±0.87
	Concentration at pH 8 [mg/kg]	10.70±0.78	8.51±0.32	31.32±0.56
	Stability [%]	27.21±0.51	18.67±0.23	58.07±0.45
b)	Concentration in freeze-dried sample [mg/kg]	61.09±0.24	60.45±0.55	61.92±0.41
	Concentration at pH 2.5 [mg/kg]	55.03±0.44	52.47±0.70	57.30±0.66
	Stability [%]	90.65±0.54	86.79±0.10	92.53±0.53
	Concentration at pH 8 [mg/kg]	8.44±0.20	39.30±0.24	38.52±0.30
	Stability [%]	13.81±0.12	65.01±0.17	62.20±0.78

Table 6. The influence of pH (gastric and duodenal) on the stability rate of carotenoids (β-carotene) from a) raw, and b) freeze-dried samples of carrot, tomato and red pepper.

		Sample		
		Carrot *(Daucus carota* L.)	**Tomato *(Solanum lycopersicum* L.)**	**Red pepper *(Capsicum annum* L.)**
a)	Concentration in raw sample [mg/kg]	15.14±0.35	21.54±0.40	27.92±0.24
	Concentration at pH 2.5 [mg/kg]	6.80±0.12	12.06±0.26	17.58±0.35
	Stability [%]	44.97±0.54	56.02±0.22	62.98±0.26
	Concentration at pH 8 [mg/kg]	4.41±0.29	4.54±0.30	16.75±0.28
	Stability [%]	29.13±0.18	21.12±0.22	60.02±0.56
b)	Concentration in freeze-dried sample [mg/kg]	21.43±0.29	33.28±0.35	56.17±0.27
	Concentration at pH 2.5 [mg/kg]	12.18±0.15	21.04±0.29	46.22±0.52
	Stability [%]	56.87±0.45	63.23±0.17	82.29±0.13
	Concentration at pH 8 [mg/kg]	5.38±0.18	7.74±0.42	33.93±0.25
	Stability [%]	25.12±0.20	23.27±0.09	60.42±0.15

4. CONCLUSIONS

Results of this study showed that the use of freeze-drying greatly improved gastrointestinal stability of carotenoids (lycopene and *β*-carotene) from carrot, red pepper and tomato in comparison with raw plant material, especially after intestinal digestive phase. In addition, the effect of pH on the stability of carotenoids is lower in freeze-dried plant material. Also, carotenoids stability depends on the food matrix (carotenoids were the most stable in red pepper).

This article contains a study with human digestive juices. The approval for the collection of digestive juices was obtained from the Ethics Committee of the University Hospital Centre Split (11/09/2014).

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