Changes in functional properties and in vitro bioaccessibilities of β -carotene and lutein after extrusion processing

Melda Ortak¹ · Cagla Caltinoglu¹ · Ilkay Sensoy¹ · Sibel Karakaya² · Behic Mert¹

Revised: 6 August 2017 / Accepted: 15 August 2017 / Published online: 4 September 2017
© Association of Food Scientists & Technologists (India) 2017

Abstract In this research, carrot pulp was added to traditional snack made from corn-grit. As the biological activity of carotenoids in the body depends on their bioaccessibility, change in carotenoid bioaccessibility during extrusion processing was investigated. In addition, phenolic content, antioxidant activity, β -carotene and lutein contents were investigated before and after the extrusion process. Two different temperature profiles were used for extrusion process. In-vitro bioaccessibilities of β -carotene and lutein increased by extrusion, β -carotene at both temperature profiles while lutein only at higher temperature profile. Extrusion decreased the antioxidant activity, total phenolic, β -carotene and lutein contents. Results suggest that even though amount of functional components decrease, in vitro bioaccessibility could be enhanced by extrusion. Therefore, carrot pulp can successfully be added as a functional ingredient to extruded.

Keywords Functional food · Antioxidant activity · Total phenolic content · Food matrix · Food structure

Introduction

During the past few decades, snack consumption has significantly increased in all over the world and has become very important part of the diet for all age groups

(Caltinoglu et al. 2014). In the cereal based snacks produced by extrusion, the most commonly used ingredients are corn flour and corn grits due to their good expansion ability as a result of high starch composition (Dar et al. 2014). However, high glycemic index and their unsatisfactory nutritional value became a public concern when corn used as a single ingredient; which could be solved by fortifying corn based snacks with fruits or vegetables (Caltinoglu et al. 2014).

Extrusion cooking is technologically convenient for the fortification or nutritional enrichment of the carbohydrate based snacks with functional ingredient addition. Carrots (*Daucus carota* L.) can be a good functional ingredient because of their rich phytochemical content and bioactive compounds with antioxidant properties (Kaisangsri et al. 2016).

Certain amount of carotenoid is needed for utilization in physiological functions or to be stored in the human body. As human body is not capable of synthesizing carotenoids; it is required to be taken from food sources. However, all the consumed amount of carotenoid does not enter the systematic circulation. Therefore, fraction of absorbed amount of carotenoid is more important than the consumed amount within the food. Food structure or physical changes occurred during processing can affect the release in digestive system. Furthermore, digestive enzymes may not be effective enough to break cellular structure that acts as a barrier around the phytochemicals such as carotenoids. For example, raw carrots' strong cell structure could be an obstacle for the release of β -carotene in the digestive system. For that reason, effective delivery of phytonutrients in the digestive system requires new generation of foods designed with the understanding of the relationship between food matrix, processing, and bioaccessibility or bioavailability.

✉ Ilkay Sensoy
isensoy@metu.edu.tr

¹ Department of Food Engineering, Middle East Technical University, 06800 Ankara, Turkey

² Department of Food Engineering, Ege University, 35100 Izmir, Turkey

There are a lot of parameters affect the stability of carotenoids: mainly oxidation, prolonged exposure to light or heat and also enzymatic degradation and exposure to acidic environment for a long time (Rodriguez-Amaya 2002; Rodriguez-Amaya and Kimura 2004). However, information related to the effect of extrusion process on degradation and in vitro bioaccessibility of carotenoids is limited. Therefore, the aim of this study was to investigate the effect of food extrusion process on in vitro bioaccessibility and on the selected carotenoids found in carrot tissue and corn grit. Change in the β -carotene and lutein content of the carrot pulp added extrudates and their in vitro bioaccessibilities before and after extrusion were investigated. During extrusion process two different extrusion zone temperature profiles and two different screw speeds were used to compare the effects of processing conditions on the functional components. Effect of carrot pulp addition on the quality of the extrudates was also investigated.

Materials and methods

Materials

Corn grit and carrots obtained from Teknik Tarım (Manisa, Turkey) and from local groceries (Ankara, Turkey), respectively. Carrot pulp was obtained by pulping the whole carrots after removing the stems (PRJ-3060, Premier, P.R.C). Feed samples, except the control, were prepared by adding carrot pulp to corn grit at a ratio of 1:4 to the final moisture content of $25.34 \pm 0.06\%$. The control, which has no carrot pulp, was prepared by mixing corn grit with distilled water to the moisture content of $24.68 \pm 0.16\%$. The moisture contents of both the ingredients (corn grit and carrot pulp) and the feed samples were measured with a halogen moisture analyser at 160°C (MX-50, AND, Japan). Prepared feed samples were allowed to equilibrate at room temperature for 2 h before the extrusion process, which were stored in plastic bags at 4°C , overnight.

Extrusion

The extruder (Feza Gıda Muh. Makine Nakliyat and Demir Tic. Ltd. Sti., Istanbul, Turkey) used in this study was a laboratory scale extruder with co-rotating intermeshing twin screw, which was equipped with computer control and data acquisition system (Fig. 1). The die diameter and the barrel length to diameter ratio (L:D) were 3 mm and 25:1, respectively. Computerized data acquisition system was used to control the barrel zone temperatures (four heating zones) by the help of electrical heating and water cooling.

The twin screw volumetric feeder, which was built into the extruder system, was used to feed the extruder.

Flow rate of the feed was kept constant at 36 ± 1 g/min for all samples, during extrusion. Functional components were investigated by comparing effect of processing conditions (profiles 1 and 2) on antioxidant activity and phenolic content (Table 1). β -Carotene and lutein contents and their in vitro bioaccessibility analysis were also completed on profiles 1 and 2, but at constant screw speed at 225 rpm. Temperature profile 3 only used for quality parameter analysis since it showed the best expansion characteristics among the studied range (Table 1).

Extrudate samples were taken only when actual measured barrel zone temperatures and die temperatures varied $\pm 2^\circ\text{C}$ from the set temperatures. Samples used for chemical analysis (antioxidant activity, total phenolic content, β -carotene and lutein contents and in vitro bioaccessibility) were stored in plastic bags at -20°C until the analysis. Samples that were analysed physically (expansion characteristics, color, texture, water absorption and water solubility indexes) were cut into 5 cm and dried at $50 \pm 0.1^\circ\text{C}$ for 5 h in an oven (ES500, Nüve, Turkey).

The final moisture contents of the dried samples were 6.73 ± 0.08 and $6.73 \pm 0.23\%$ for the samples with and without carrot pulp, respectively. The dried samples were kept in a vacuum jar at room temperature until the analysis.

Methods

Total phenolic content

The procedure described by Anton et al. (2009) was used with some modifications. 10 ml of 80% acetone was mixed with the 0.4 g of grounded sample and left for extraction on a rotary shaker (JeioTech-Multichannel Stirrer, MS-52 M) at 250 rpm for 2 h. Extracted solution was centrifuged at $4000\times g$ for 10 min and then 200 μl of filtered supernatant was allowed to react with 1.5 ml of freshly tenfold diluted Folin–Ciocalteu reagent (Merck, Germany) for just 5 min. Following the addition of 1.5 ml of Na_2CO_3 (60 g/l) solution incubated for 90 min and absorbance was measured at 725 nm (Shimadzu, UV-1700 Pharma Spec, Japan). The calibration curve was prepared with gallic acid (Sigma-Aldrich, Germany) in the range of 0–600 mg/l. The results were converted to per dry sample weight, due to known amount of sample weight in the extraction step, and the results were expressed as gallic acid (mg)/dry sample weight (g).

Antioxidant activity

The same procedure in the total phenolic content determination method was followed for the extraction of

Fig. 1 Twin screw extruder used in the study **Table 1** Temperature profiles and screw speeds during extrusion process

	Barrel zone temperature (°C)				Die temperature (°C)	Screw speed (rpm)
	T1	T2	T3	T4		
Profile 1	80	90	100	130	120	125, 225
Profile 2	80	105	130	160	130	125, 225
Profile 3	80	90	115	145	125	175

antioxidants. After the filtration of extract, 200 μ l of aliquot was reacted with 3.8 ml of DPPH (Sigma-Aldrich, Germany) solution (6.34×10^{-2} mM in methanol). The solution was incubated at dark for an hour and then absorbance was measured at 517 nm. For the calibration, Trolox (Sigma-Aldrich, Germany) curve was used in the range of 0–16 μ M and, the results were expressed as Trolox equivalent (μ mol)/dry sample weight (g).

β -Carotene and lutein content

HPLC analysis was used for the quantification of β -carotene and lutein. Carotenoid standards were obtained from Sigma Aldrich (Germany). All the solvents used in the analysis obtained from Merck (Germany).

Extraction of carotenoids Extraction of carotenoids was carried out according to the method by Cemeroglu (2010). 15 g grinded sample was mixed with 45 ml water and kept +4 °C for 8 h for hydration. 0.2 g CaCO_3 and 80 ml of extraction solvent, that was composed of acetone:hexane:tetrahydrofuran (50:30:20, v/v/v) and 0.1% BHT, was added on 5 g homogenated sample. After extraction for

20 min at 250 rpm (JeioTech-Multichannel Stirrer, MS-52 M) samples were centrifuged (Sigma, 2-16 PK, Germany) at $9500 \times g$ at +4 °C for 20 min to separate the supernatant layer, hexane phase. Hexane was evaporated under nitrogen evaporator (Biotage, TurboVap-LV, USA) at 38 °C prior to HPLC analysis.

HPLC analysis Carotenoid separation was performed by reverse phase HPLC system with C-18 column (250 $\times$ 4.6 mm, 5 μ m particle size, Inertsil ODS-2, GL Sciences Inc., USA) by using combined method of O'Connell et al. (2007) and O'Sullivan et al. (2010). Samples dried under nitrogen evaporator were solubilized with a mixture of 250 μ l tetrahydrofuran (THF) and dissolved in a predetermined amount of mobile phase. Prior to injection to HPLC (Thermo Scientific, Finnigan Surveyor), samples filtered through 0.45 μ m filter (Syringe Filter, PTFE 25 mm). Mixture of acetonitrile:methanol:dichloromethane (75:20:5, v/v/v) containing 4.5 mmol/l BHT, 10 mmol/l ammonium acetate and 3.6 mmol/l triethylamine was used at 1 ml/min flow rate as the mobile phase. Column temperature and auto-sampler temperature were adjusted to 20 and 4 °C, respectively. 25 μ l of sample was injected in duplicate. Detection

was carried out at 450 nm. Chromatograms were analyzed by ChromQuest 4.2.34 software system.

In-vitro bioaccessibility

In-vitro bioaccessibility experiments were conducted at the Food Engineering Department of Ege University (İzmir, Turkey) according to the method of Minekus et al. (2014). For 5 g of crushed sample, 500 µl simulated salivary fluid (Table 2) and human salivary α -amylase (Sigma A 1031) was added and vortexed. The pH was adjusted to 7.0 and then 2000 U/ml pepsin solution (Sigma P 7000) and 10 ml simulated gastric fluid (Table 2) was added, respectively. Prior to incubation (Memmert, Type SV 1422, Schwabach, Germany) at 37 °C for 2 h, pH was adjusted to 3.0. Then, dialysis membranes (Spectra/Por 6 8000 MWCO, Spectrum Lab. Inc.) containing simulated duodenal fluid (Table 2) were placed inside the tubes and pH was elevated to 7.0. Eight g pancreatin (Sigma P1750; 100 TAME U/ml) and 24 g bile (Sigma-Aldrich B8631, USA) was dissolved in a pancreatic juice and 2.0 ml of resulted pancreatin-bile solution was placed into the tubes. Simulated digestion was completed by holding the pH at 7.0 during the incubation period at 37 °C for 2 h.

Extraction of carotenoids, β -carotene and lutein, from digested raw and extruded samples were completed with the same previous carotenoid analysis. HPLC conditions, extraction solvent and mobile phase used in bioaccessibility analysis were same as the ones used in the HPLC runs of carotenoid analysis for the non-digested feed and extrudates.

Expansion characteristics (SEI, VEI, LEI)

The extrudates used for the following analysis were randomly selected among the extrudates which was previously cut and dried. Sectional expansion index (SEI), volume expansion index (VEI) and longitudinal expansion index (LEI) was calculated according to Caltinoglu et al. (2014).

Bulk density (apparent density)

Bulk densities of the extrudates were determined by using the Archimedes' principle according to Caltinoglu et al. (2014). Five measurements were taken and averaged.

True density (particle density)

True density analysis were performed at Middle East Technical University Central Laboratory by using helium pycnometer (Quantachrome Ultrapycnometer 1000, Florida, USA), according to Caltinoglu et al. (2014).

Porosity

Porosity was calculated according to the following formula. Results were given as the average of five samples

$$\text{Porosity} = 1 - \frac{\rho_{(\text{extrudate})}}{\rho_{(\text{true})}}$$

where $\rho_{(\text{extrudate})}$ is bulk density of the extrudate (g/cm^3) and $\rho_{(\text{true})}$ is true density of the extrudate (g/cm^3).

Table 2 Composition of digestive fluids (Minekus et al. 2014)

	Stock concentration (mol/l)	Simulated salivary fluid (pH 7)		Simulated gastric fluid (pH 3)		Simulated duodenal fluid (pH 7)	
		Volume of Stock (ml)	Concentration (mmol/l)	Volume of stock (ml)	Concentration (mmol/l)	Volume of stock (ml)	Concentration (mmol/l)
<i>Constituents</i>							
KCl	0.6	15.1	15.1	6.9	6.9	6.8	6.8
KH ₂ PO ₄	0.5	3.7	3.7	0.9	0.9	0.8	0.8
NaHCO ₃	1	6.8	13.6	12.5	25	42.5	85
NaCl	2	–	–	11.8	47.2	9.6	38.4
MgCl ₂	0.15	0.5	0.15	0.4	0.1	1.1	0.33
(H ₂ O) ₆							
(NH ₄) ₂ CO ₃	0.5	0.06	0.06	0.5	0.5	–	–
<i>pH adjustment</i>							
NaOH	1	–	–	–	–	–	–
HCl	6	0.09	1.1	1.3	15.6	0.7	8.4

Water absorption index (WAI) and water solubility index (WSI)

The water absorption index and water solubility index were determined according to Caltinoglu et al. (2014).

Color

A colorimeter (CR-10, Konica Minolta, Japan) was used to determine color values of the grounded extruded samples in terms of lightness, redness and yellowness (L^* , a^* and b^* values) as described in Caltinoglu et al. (2014).

Texture

Hardness, the peak force of the first compression of the product, was measured with a texture analyzer (TA.XT Plus, Stable Micro Analyzer, UK) according to procedure given in Caltinoglu et al. (2014). The extruded sample (4 cm long) was placed on three-point bend rig that has two adjustable supports, which were fixed 1 cm away from the center. Texture analyzer was operated in the compression mode with a sharp testing blade (0.12 cm thick, 8 cm wide). The analyzer head moved the probe down at a rate of 1.00 mm/s until it broke the extrudates. Break sensitivity was 10.0 g, trigger force was 5.0 g. Calibration was conducted before the analysis.

Statistical analysis

All the results were analyzed by one-way analysis of variance (ANOVA) and differences between the means were determined by Duncan Multiple Comparison Test. Differences at $p \leq 0.05$ were considered as significantly difference.

Results and discussion

Effect of extrusion on the total phenolic content and antioxidant activity

Extrusion process caused a significant reduction ($p \leq 0.05$) in both total phenolic content and antioxidant activity (Table 3). However, change in the extrusion parameters, temperature and screw speed, did not affect the total phenolic content and antioxidant activity significantly within the studied range. Several researchers indicated a reduction in total phenolic and antioxidant content after extrusion (Gujral et al. 2012; Sarawong et al. 2014). Anton et al.

(2009) and Dlamini et al. (2007) indicated a correlation between phenolic content and antioxidant activity.

Shear stress and barrel temperature are the two main parameters responsible for reduced phenolic content and antioxidant activity of extrudates. High shear stress arising from extrusion conditions may partially break down antioxidant derivatives and rupture the molecular structure of phenolics (Brennan et al. 2011; Sharma et al. 2012; Zielinska et al. 2007). During extrusion process barrel temperature was over 80 °C and exposure to high temperature might alter or damage the physical structure of heat sensitive phenolics (Brennan et al. 2011; Sharma et al. 2012; Zielinska et al. 2007). The other possible effect of high barrel temperature in extrusion is the polymerization reactions. These reactions induced during extrusion cooking might decrease the extractability of phenolic compounds causing reduced values (Brennan et al. 2011; Sarawong et al. 2014; Sharma et al. 2012).

Effect of extrusion on β -carotene and lutein content

Carotenoid content was determined before and after extrusion (Table 4). Extrusion process with temperature profile 1 reduced the β -carotene content whereas extrudates formed at temperature profile 2 was not significantly different from feed in terms of its β -carotene content ($p > 0.05$). Extrusion at temperature profiles 1 and 2 reduced the lutein content to some extent ($p \leq 0.05$).

During extrusion, because of high barrel temperature and screw speed, thermal and mechanical stress formed during cooking. Combined effect of these two stresses supports the extensive loss of β -carotene and lutein content as a result of their unsaturated structure. In addition, thermal stress promotes the oxidation and isomerization reactions, which is linked to the degradation of carotenoids (Carbonell-Capella et al. 2014). Reduction of β -carotene content by thermal (140 °C last zone extruder temperature) and mechanical degradation (100–175–250 rpm screw speed) during extrusion process was also investigated by Kaisangsri et al. (2016).

The reason of the β -carotene reduction at temperature profile 1 while it was kept stable at profile 2 might be explained by the isomerization of β -carotene from its natural more stable trans form to cis configuration. In raw carrot tissue most of the β -carotene is in trans form, however during cooking higher extrusion temperature at profile 2 might promote isomerization to cis form. Cis- β -carotene is more polar than its trans-form, which makes it more soluble. Enhanced solubility of β -carotene might compensate the loss at temperature profile 2 (Carbonell-Capella et al. 2014; Sharma et al. 2012).

Table 3 Effect of extrusion process on functional properties of extrudates

	Total phenolic content		Antioxidant activity	
	Gallic acid equivalent/dry weight (mg/g)	% loss	Trolox equivalent/dry weight ($\mu\text{mol/g}$)	% loss
Feed	18.15 $\pm$ 2.83 ^a		6.80 $\pm$ 1.02 ^a	
Extrudate (Profile 1, 125 rpm)	10.26 $\pm$ 2.27 ^b	43.50	5.34 $\pm$ 0.49 ^b	21.49
Extrudate (Profile 1, 225 rpm)	9.45 $\pm$ 0.94 ^b	47.96	4.91 $\pm$ 0.76 ^b	27.76
Extrudate (Profile 2, 125 rpm)	9.60 $\pm$ 1.49 ^b	47.16	5.20 $\pm$ 0.55 ^b	23.55
Extrudate (Profile 2, 225 rpm)	10.01 $\pm$ 0.36 ^b	44.86	5.09 $\pm$ 0.68 ^b	25.18

Results are mean $\pm$ SD (n = 3); values of the same column, followed by the different letters (a, b) are statistically different ($p \leq 0.05$)

Table 4 Effect of extrusion on the β -carotene and lutein content

	Prior to in vitro digestion (μg/g dry weight)	After in vitro digestion		Relative in vitro bioaccessibility (%)
		Dialyzed portion (μg/g dry weight)	Not dialyzed portion (μg/g dry weight)	
<i>β-carotene content</i>				
Feed	11.13 ± 2.43 ^a	2.47 ± 0.32 ^a	ND	22.55 ± 2.33 ^b
Extrudate (Profile 1 and 225 rpm)	4.23 ± 0.98 ^b	1.69 ± 0.44 ^b	ND	39.84 ± 5.93 ^a
Extrudate (Profile 2 and 225 rpm)	9.26 ± 2.02 ^a	3.16 ± 0.33 ^a	ND	34.72 ± 4.36 ^a
<i>Lutein content</i>				
Feed	2.66 ± 0.47 ^a	0.20 ± 0.04 ^b	ND	7.50 ± 0.77 ^b
Extrudate (Profile 1 and 225 rpm)	1.91 ± 0.28 ^b	0.18 ± 0.04 ^b	ND	9.54 ± 2.37 ^b
Extrudate (Profile 2 and 225 rpm)	1.38 ± 0.30 ^b	0.34 ± 0.05 ^a	ND	24.91 ± 0.89 ^a

Results are mean $\pm$ SD (n = 3); values of the same column, followed by the different letters (a, b) are statistically different ($p \leq 0.05$)

ND not detectable level

Effect of extrusion on in vitro bioaccessibility of β -carotene and lutein

Relative in vitro bioaccessibility of β -carotene increased significantly ($p \leq 0.05$) with extrusion (Table 4). Reasons for increased bioaccessibility linked to the thermal stress during extrusion which caused the disrupted cell wall structure and protein-carotenoid complexes. Due to disrupted, thus softened structure of cell wall; higher release of β -carotene from food matrix could be expected (Hornero-Mendez and Mínguez-Mosquera 2007; Southon and Faulks 2003). Moreover, heat induced protein denaturation promotes the disruption the protein-carotenoid complexes, which helps carotenoid molecules to escape from protein bonding and results into formation of free β -carotene

molecules that can easily be liberated (Mulokozi et al. 2004; Parker 1996). In addition to the thermal stress, mechanical stress could have also played a role in improved bioaccessibility as crushing between extruder screws causes breaking food material down into smaller pieces. Reduced particle size creates more surface area for digestive enzymes to work on (Hedren et al. 2002; Southon and Faulks 2003; Donhowe and Kong 2014).

Relative in vitro bioaccessibility of lutein was higher ($p \leq 0.05$) for the extrudates processed at higher temperature (Table 4). The reason of the increased in vitro bioaccessibility of lutein at temperature profile 2, while it was similar to feed at profile 1, could be explained by the severity of thermal stress. Thermal stress at profile 1 was not severe enough to break the protective cell wall of

lutein. Thus, did not cause any significant change on in vitro bioaccessibility ($p > 0.05$). The increased relative bioaccessibility at profile 2 was associated with higher processing temperature which enhanced the release of lutein by breaking the cell wall. Similar results were obtained in the study of Hornero-Mendez and Mínguez-Mosquera (2007) where effects of heat processing on bioavailabilities of α and β carotene in carrot tissue were studied.

In the literature there are several studies stating the negative effect of thermal or mechanical stress on carotenoid content, in the meantime their positive effect on in vitro bioaccessibility, which was explained by the disruption of food matrix or structure by heat or pressure (Courraud et al. 2013; Hornero-Mendez and Mínguez-Mosquera 2007; Veda et al. 2010).

Expansion characteristics

Porous structure was obtained within the extrudate due to the air cells generated. The size, number and distribution of these air cells are primary determinative factors for the expansion characteristics of the extrudates (Lue et al. 1990; Moraru and Kokini 2003). Rheological properties of the melt influence the size, number and distribution of the bubbles, hence, the expansion characteristics of the extrudates. It is known that expansion characteristics depend on the degree of gelatinization. Starch is the main component in expansion phenomena and other ingredients such as protein, sugars, fats, and fiber act as diluents (Case et al. 1992; Moraru and Kokini 2003; Thakur et al. 2017).

Analysis of variance showed no statistically difference ($p > 0.05$) on any of the expansion characteristics of the corn grit extrudates with and without carrot pulp (Table 5).

This may be attributed to the insignificant contribution of the added carrot pulp which was not enough to have a significant effect on the rheological properties of the melt.

Water absorption and water solubility indexes (WAI and WSI)

The water absorption index (WAI), which can be used as an index of gelatinization, measures the volume occupied by the granule or starch polymer after swelling in excess water. On the other hand, the water solubility index (WSI) measures the degradation of starch, the amount of free polysaccharide or polysaccharide released from the granule on the addition of excess water (Sriburi and Hill 1999; Thakur et al. 2017). In this study, carrot pulp addition had no statistically significant effect on WAI or WSI ($p > 0.05$) (Table 5), which may be attributed to the fact that the concentration of the fiber was not enough to make changes in the concentration of the starch, or dilute starch.

Color

Color is used as an index of quality in food materials and people expect a certain taste of flavour, based on color. Being directly related to the acceptability of the food products, color become an important characteristic of a food material to be reported. Statistically significant decrease ($p \leq 0.05$) was observed in L^* values with the addition of carrot pulp while there were a significant increase ($p \leq 0.05$) in a^* and b^* values of the corn grit extrudates (Table 5). The reduction in the lightness (L^* value) of the corn grit extrudates with pulp addition can be explained by the carotenoid content of carrot tissue. Increase in redness (a^* value) and yellowness (b^* value)

Table 5 Effect of carrot pulp addition on the quality of extrudates

	Corn grit extrudate	Carrot pulp added corn grit extrudate
SEI	6.38 $\pm$ 1.00 ^a	5.96 $\pm$ 0.83 ^a
LEI	0.87 $\pm$ 0.04 ^a	0.87 $\pm$ 0.07 ^a
VEI	5.55 $\pm$ 0.28 ^a	5.18 $\pm$ 0.44 ^a
Bulk density (g/cm ³)	0.273 $\pm$ 0.014 ^a	0.296 $\pm$ 0.007 ^a
Porosity	0.82 $\pm$ 0.01 ^a	0.80 $\pm$ 0.01 ^a
WAI (g/g)	4.49 $\pm$ 0.05 ^a	4.38 $\pm$ 0.17 ^a
WSI (%)	12.93 $\pm$ 0.67 ^a	13.53 $\pm$ 1.31 ^a
L^*	81.19 $\pm$ 0.33 ^a	79.60 $\pm$ 0.45 ^b
a^*	2.09 $\pm$ 0.35 ^a	3.01 $\pm$ 0.17 ^b
b^*	35.75 $\pm$ 1.34 ^a	39.64 $\pm$ 0.63 ^b
Hardness (N)	10.55 $\pm$ 1.74 ^a	10.40 $\pm$ 1.68 ^a

SEI results are mean $\pm$ SD (n = 30); VEI and LEI results are mean $\pm$ SD (n = 5); bulk density and porosity results are mean $\pm$ SD (n = 5); WAI and WSI results are mean $\pm$ SD (n = 6); L^* , a^* , b^* results are mean $\pm$ SD (n = 10); hardness results are mean $\pm$ SD (n = 25); values of the same row followed by the different letters (a, b) are statistically different ($p \leq 0.05$)

were due to the presence of carotenoids in carrot, which are mainly α -carotene and β -carotene (Simon and Wolff 1987).

Texture

Hardness values, reported, indicate the maximum force (peak force) required for a probe to penetrate the extrudate, whose high values are not desirable property for snacks (Mendonca et al. 2000; Yanniotis et al. 2007). According to the results, addition of carrot pulp showed no statistically significant difference in hardness values ($p > 0.05$). This could be due to the fact that fiber contribution of the pulps in the studied range was low.

Conclusion

Results indicated that carrot pulp could be added as a functional ingredient to the extrudates because quality parameters were not affected significantly. Extrusion resulted in the decreased level of β -carotene and lutein contents as well as antioxidant activity and total phenolic content. However; as the amount of absorbed carotenoid is more important than their consumed amount, in this study emphasis was placed on the bioaccessibility of carotenoids. In-vitro bioaccessibilities of β -carotene and lutein increased by extrusion, β -carotene at both extrusion temperature profiles while lutein only at higher extrusion temperature profile. Thus; it could be stated that although extrusion process decreased the quantity of carotenoid intake, the percent carotenoid ready for body utilization was increased. Even though some favourable effect of extrusion was stated in the present study on the in vitro bioaccessibility of carotenoids, further research on in vivo studies are needed to understand the behaviour of carotenoids in human digestive system. In conclusion, observed data could be used to design functional foods with the help of added in vivo research.

Acknowledgements This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK 110 O 792).

References

Anton AA, Gary Fulcher R, Arntfield SD (2009) Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (*Phaseolus vulgaris* L.) flour: effects of bean addition and extrusion cooking. *Food Chem* 113(4):989–996

Brennan C, Brennan M, Derbyshire E, Tiwari BK (2011) Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. *Trends Food Sci Technol* 22(10):570–575

Caltinoglu C, Tonyali B, Sensoy I (2014) Effects of tomato pulp addition on the extrudate quality parameters and effects of

extrusion on the functional parameters of the extrudates. *Int J Food Sci Technol* 49(2):587–594

Carbonell-Capella JM, Buniowska M, Barba FJ, Esteve MJ, Frigola A (2014) Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: a review. *Compr Rev Food Sci Food Saf* 13:155–171

Case SE, Hamann DD, Schwartz SJ (1992) Effect of starch gelatinization on physical properties of extruded wheat and corn-based products. *Cereal Chem* 69(4):401–404

Cemeroğlu B (2010) Gıda Analizleri. Nobel Yayınları, Ankara

Courraud J, Berger J, Cristol JP, Avallone S (2013) Stability and bioaccessibility of different forms of carotenoids and vitamin A during in vitro digestion. *Food Chem* 136(2):871–877

Dar AH, Sharma HK, Kumar N (2014) Effect of extrusion temperature on the microstructure, textural and functional attributes of carrot pomace-based extrudates. *J Food Process Preserv* 38(1):212–222

Dlamini NR, Taylor J, Rooney LW (2007) The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chem* 105(4):1412–1419

Donhowe EG, Kong F (2014) β -Carotene: digestion, microencapsulation, and in vitro bioavailability. *Food Bioprocess Technol* 7(2):338–354

Gujral HS, Sharma P, Kumar A, Singh B (2012) Total phenolic content and antioxidant activity of extruded brown rice. *Int J Food Prop* 15(2):301–311

Hedren E, Diaz V, Svanberg U (2002) Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *Eur J Clin Nutr* 56(5):425–430

Hornero-Mendez D, Mínguez-Mosquera MI (2007) Bioaccessibility of carotenes from carrots: effect of cooking and addition of oil. *Innov Food Sci Emerg Technol* 8(3):407–412

Kaisangsri N, Kowalski RJ, Wijesekara I, Kerdchoechuen O, Laohakunjit N, Ganjyal GM (2016) Carrot pomace enhances the expansion and nutritional quality of corn starch extrudates. *LWT Food Sci Technol* 68:391–399

Lue S, Hsieh F, Peng IC, Huff HE (1990) Expansion of corn extrudates containing dietary fiber: a microstructure study. *LWT Food Sci Technol* 23(2):165–173

Mendonca S, Grossmann MVE, Verhe R (2000) Corn bran as a fibre source in expanded snacks. *LWT Food Sci Technol* 33(1):2–8

Minekus M, Alminger M, Alvito P, Balance S, Bohn T, Bourlieu C, Carriere F, Boutrou R, Corredig M, Dupont D, Dufour C, Egger L, Golding M, Karakaya S, Kirkhus B, Le Feunteun S, Lesmes U, Macierzanka A, Mackie A, Marze S, McClements DJ, Menard O, Recio I, Santos CN, Singh RP, Vegarud GE, Wickham MSJ, Weitschies W, Brodkorb A (2014) A standardised static in vitro digestion method suitable for food—an international consensus. *Food Funct* 5(6):1113–1124

Moraru CI, Kokini JL (2003) Nucleation and expansion during extrusion and microwave heating of cereal foods. *Compr Rev Food Sci Food Saf* 2(4):147–165

Mulokozi G, Hedren E, Svanberg U (2004) In vitro accessibility and intake of β -carotene from cooked green leafy vegetables and their estimated contribution to vitamin A requirements. *Plant Foods Hum Nutr* 59(1):1–9

O'Connell OF, Ryan L, O'Brien NM (2007) Xanthophyll carotenoids are more bioaccessible from fruits than dark green vegetables. *Nutr Res* 27(5):258–264

O'Sullivan L, Jiwan MA, Daly T, O'Brien NM, Aherne SA (2010) Bioaccessibility, uptake, and transport of carotenoids from peppers (*Capsicum* spp.) using the coupled in vitro digestion and human intestinal Caco-2 cell model. *J Agric Food Chem* 58(9):5374–5379

Parker RS (1996) Absorption, metabolism, and transport of carotenoids. *FASEB J* 10(5):542–551

- Rodriguez-Amaya DB (2002) Effects of processing and storage on food carotenoids. *Sight Life Newsl* 3:25–35
- Rodriguez-Amaya DB, Kimura M (2004) HarvestPlus handbook for carotenoid analysis. International Food Policy Research Institute. <https://assets.publishing.service.gov.uk/media/57a08cbae5274a31e00013d4/tech02.pdf>. Accessed 27 October 2016
- Sarawong C, Schoenlechner R, Sekiguchi K, Berghofer E, Ng PK (2014) Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. *Food Chem* 143:33–39
- Sharma P, Gujral HS, Singh B (2012) Antioxidant activity of barley as affected by extrusion cooking. *Food Chem* 131(4):1406–1413
- Simon PW, Wolff XY (1987) Carotenes in typical and dark orange carrots. *J Agric Food Chem* 35(6):1017–1022
- Southon S, Faulks R (2003) Carotenoids in food: bioavailability and functional benefits. In: Johnson I, Williamson G (eds) *Phytochemical functional foods*. Woodhead Publishing Limited, Abington, pp 107–127
- Sriburi P, Hill SE (1999) Extrusion of cassava starch with either variations in ascorbic acid concentration or pH. *Int J Food Sci Technol* 35(2):141–154
- Thakur S, Singh N, Kaur A, Singh B (2017) Effect of extrusion on physicochemical properties, digestibility, and phenolic profiles of grit fractions obtained from dry milling of normal and waxy corn. *J Food Sci* 82(5):1101–1109
- Veda S, Platel K, Srinivasan K (2010) Enhanced bioaccessibility of β -carotene from yellow–orange vegetables and green leafy vegetables by domestic heat processing. *Int J Food Sci Technol* 45(10):2201–2207
- Yanniotis S, Petraki A, Soumpasi E (2007) Effect of pectin and wheat fibers on quality attributes of extruded cornstarch. *J Food Eng* 80(2):594–599
- Zielinska D, Szawara-Nowak D, Zielinski H (2007) Comparison of spectrophotometric and electrochemical methods for the evaluation of the antioxidant capacity of buckwheat products after hydrothermal treatment. *J Agric Food Chem* 55(15):6124–6131