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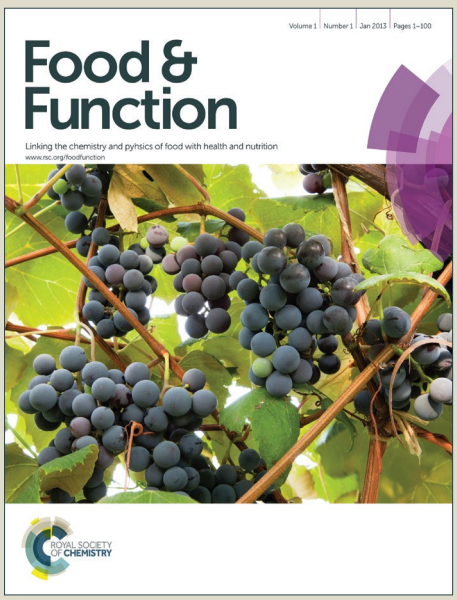

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EFFECT OF EXTRUSION ON THE FUNCTIONAL COMPONENTS AND *IN VITRO* LYCOPENE BIOACCESSIBILITY OF TOMATO PULP ADDED CORN EXTRUDATES

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ABSTRACT

Effect of processing on functional ingredients and their *in vitro* bioaccessibility should be investigated to develop better food products. Tomato pulp was added as a functional ingredient to the extrudates. Effects of extrusion on the functional properties of the extrudates and *in vitro* bioaccessibility of lycopene were investigated. Two different temperature set were applied during extrusion: 80 °C, 90 °C, 100 °C and 130 °C and 80 °C, 100 °C, 130 °C and 160 °C. Screw speed and feed rate were kept constant as 225 rpm and 36 ± 1 g/min, respectively. Feed moisture content was adjusted to 30 ± 1 % by mixing tomato pulp to the corn grit. Antioxidant activity and total phenolic content decreased after the extrusion process. High performance liquid chromatography (HPLC) analysis indicated that lycopene content decreased after extrusion process when feed and extrudates were compared. *In vitro* bioaccessibility of lycopene for the extruded samples with 160°C last zone treatment temperature was higher than the feed and extruded samples with 130°C last zone treatment temperature. Results indicate that extrusion affects the food matrix and the release of functional components.

Keywords: Extrusion; Phenols; DPPH; Bioaccessibility; Lycopene; Food matrix.

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1. Introduction

Extruders work at high temperature and short time with high shear.[1] They produce products in different size, shape or texture.[2] Extruded products are popular among the consumers because they are ready to eat and have long shelf life. These products are usually rich in starch.[3]

Fruits and vegetables are known to be rich in bioactive compounds known as phytochemicals as well as vitamins, minerals and fibers. Phytochemicals that are found in vegetables and fruits have the role of preventing diseases. Polyphenols and carotenoids are some examples.[4,5]

Tomatoes are rich in lycopene and β-carotene as well as fiber, protein, vitamin A, C, and potassium. [6,7] The color of tomato (*Solanum lycopersicum*) is due to red color of lycopene and orange-yellowish color of β-carotene. [8,9] Lycopene has high antioxidant properties and acts like a free radical scavenger.[10] Intake of lycopene in moderate amounts is found to decrease the risk of cardiovascular disease, prostate cancer and gastrointestinal tract cancer.[3,11-13] In order for body to use the antioxidants, phenols or carotenoids, these compounds should be released from the food matrix they are trapped in. [14] Nutrients could be bound to plant organelles like carotenoids in carrots or they could be trapped in a food matrix of protein and starch such as isoflavones in baked products.[15] It is known that destruction of natural food matrix and increasing the oil content increases the absorption of carotene in the body.[16,17] For example, beta-carotene enters the blood plasma faster if carrots are consumed as carrot juice or cooked carrots.[18] Chewing and enzymes in the digestion system may not be enough to break the structure of the carrot's strong cell structure to release beta carotene.

Bioaccessibility is defined as the amount of an ingested nutrient that is available for absorption after digestion in the digestive system.[19-21] Bioavailability is defined as the fraction

of ingested nutrients that is available for utilization in the body and also includes nutrient accessibility.[20,22,23] Bioavailability of a nutrient is more important than the total amount present in the original food. Design of new generation foods for better nutrient delivery in the digestive system requires knowledge of how processing affects the bioaccessibility or bioavailability. Hence, it is important to know how extrusion affects the accessibility of the nutrients.

Therefore, the aim of the study was to investigate the effect of extrusion on the *in vitro* bioaccessibility of lycopene and the effect of extrusion on the functional properties of the tomato pulp added extrudates. Tomato pulp was added as a functional ingredient to corn grits.

2. Materials and methods

2.1 Ingredients

The corn grit of feed material was obtained from Teknik Tarım (Manisa, Turkey). The tomatoes used in the analyses were purchased from local groceries (Ankara, Turkey). Before the mixing process, the seed and stem parts were separated from the tomatoes. The tomato pulp (95.27 ± 0.13 % moisture) and corn grit (12.23 ± 0.06 % moisture) were mixed in the amounts as the final feed having 30 ± 1 % moisture content. The mixed feed material was stored at 4°C overnight. Samples were allowed to equilibrate at room temperature before the extrusion process.

Chemicals

All chemicals used were analytical grade except for HPLC analysis where HPLC grade were used.

2.2 Extrusion Process

A laboratory scale co-rotating twin-screw extruder (Feza Gıda Müh. Makine Nakliyat and Demir Tic. Ltd. Şti. İstanbul, Turkey) with computer control and data acquisition system was used for the study. The die diameter and the barrel length to diameter ratio (L/D) were 3 mm and 25:1, respectively. The screw configuration of extruder was shown below, Table 1.

The extruder had four heating zones controlled by electrical heating and water cooling. The barrel zone temperatures and rotor speed were controlled by the computerized data acquisition system. The feed was fed to the extruder with a twin-screw volumetric feeder that was built into the extruder system. Flow rate of the feed was 36 ± 1 g/min. The screw speed was kept constant at 225 rpm where the barrel temperature zones were set at 80 °C, 90 °C, 100 °C and 130 °C (die: 116 °C) and 80 °C, 100 °C, 130 °C and 160 °C (die: 139 °C). Samples were taken only when the actual measured barrel zone temperatures and die temperatures varied only ± 2°C from the set temperatures.

2.3 Total Phenolic Content and Antioxidant Activity Analyses

Extraction

The extraction step of total phenolic content and antioxidant activity was conducted according to Caltinoglu *et al.*[24]. The extrudates were grounded (KSW 445 CB, Bomann, Germany). The feed and extrudate samples were mixed with acetone:water mixture (4:1) (v/v). Following to two hours stirring step, the mixture was centrifuged at 3000xg for 12 minutes (Sigma, 2-16 PK, Germany). The obtained supernatant was filtered through 0.45 μm syringe type filter (Syringe Filter, PTFE 25 mm).

Total Phenolic Content

The total phenolic content procedure was conducted according to the procedure from Caltinoglu *et al.*[24]. Folin-Ciocalteau (Merck, Germany) reagent was used in the analysis. The

absorbance of the samples was read at 725 nm by using UV-Visible spectrophotometer (Shimadzu, UV-Visible Spectrophotometer, UV-1700, Japan). The results were expressed in mg gallic acid (3,4,5-Trihydroxybenzoicacid, Sigma–Aldrich, Germany) equivalent (GAE)/gr dry weight of sample.

Antioxidant Activity

Antioxidant activities of the feed and extrudates were determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma–Aldrich, Germany) as described in Caltinoglu *et al.*[24]. The absorbance of samples which were reacted with DPPH, was read at 517 nm with UV-Visible spectrophotometer. A standard curve was prepared with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid from Sigma–Aldrich, Germany), a synthetic, hydrophilic vitamin E analogue, as an external standard with a range of concentrations from 10μM to 100 μM. The results were expressed as trolox equivalent (TE) /gr dry weight of sample.

2.4 *In Vitro* Bioaccessibility Analysis

***In Vitro* Digestion Analysis**

In vitro digestion analyses were performed at the Food Engineering Department of Ege University, Izmir, Turkey according to the method of Minekus *et al.*[25]. Five grams of samples were weighted and put into the centrifuge tubes. 5 mL of simulated salivary fluid (Table 2) that is prepared according to Minekus *et al.*[25] with minor adjustment to KCI concentration (0.6 M) was added on the samples and on the blank sample tube. Later, human salivary α-amylase (Sigma A 1031) was added in the sample tubes. The pH of the tubes were checked, adjusted to 7 and incubated for 2 minutes at 37°C. 2000 U/ml pepsin solution (Sigma P 7000) and 10 ml of simulated gastric fluid (Table 2), were added respectively. The content of the

tubes were adjusted to pH 3. The tubes were incubated for 2 hours in a shaking water bath (Memmert, Type SV 1422, Schwabach, Germany) at 37°C. The tubes were removed from the orbital shaker and the gastric digestion step was completed. The dialysis membranes (SPECTRA/POR 6 8,000 MWCO, Spectrum Laboratories, Inc.) containing simulated duodenal fluid were placed into each tube. The tubes' pH were adjusted to pH 7. The solutions of porcine pancreatin (Sigma P1750; 100 TAME U/ml) and 10 mM bile (Sigma-Aldrich B8631, USA) were placed into tubes. Again, the tubes were left at orbital shaker at 37°C for 2 hours. At the end of the incubation period the dialysis membranes were taken from the tubes. The membranes were opened and the inside matter was transferred to another tube which were frozen at -20 °C until the HPLC analysis. The solution outside of the dialysis membranes were also put into new tubes and frozen at -20 °C until the HPLC analysis.

Extraction of Carotenoids

The extraction method was based on the method by O'Connell *et al.*[26]. The extrudates were grounded (KSW 445 CB, Bomann, Germany) and sieved from a mesh sieve, 425 microns (200 M.M. B.S., Endecotts Ltd, London) prior to analysis. 10 gram of homogenized samples from both extrudates and feed were taken and approximately 30 mL of distilled water was added onto them. The water added mixtures were left at 4 °C overnight. Then, samples were mixed with a homogenizer (Witeg, HG-15A, Germany) at 13500 rpm for 2 minutes for homogenization. From the homogenized samples, 4 grams were taken and 0.4 gram Calcium carbonate ($CaCO_3$) was added. The samples were mixed with 25 mL of hexane:acetone:ethanol (50:25:25) (v/v). They were stirred at magnetic stirrer at 250 rpm for 20 minutes. After 20 minutes, the supernatant part was separated from the rest and put in another beaker. The residue part was again mixed with 25 mL of hexane:acetone:ethanol (50:25:25) (v/v). The mixture was stirred at 250 rpm for 20 minutes. Afterwards, both

supernatants were combined and centrifuged at 9500xg at 4°C for 20 minutes. The hexane layer formed after centrifugation was dried under the stream of nitrogen at 37°C with nitrogen evaporator. The dried samples were immediately dissolved with predetermined volumes of THF and mobile phase and prepared for HPLC analysis.

For *in vitro* bioaccessibility analysis, the digested raw and extruded products were extracted with the same method but with minor modifications. Before the extraction step, the digested samples were thawed at the room temperature. After that, 25 mL of hexane:acetone:ethanol (50:25:25) (v/v) was added to the samples. The samples were left to stirring at 250 rpm for 20 minutes. The stirred samples were centrifuged at 9500xg at 4°C for 20 minutes to separate the hexane layer. The hexane layer was dried under the stream of nitrogen. The dried samples of digested products were immediately dissolved with predetermined volumes of THF and mobile phase and prepared for HPLC analysis.

HPLC Analysis

HPLC analysis was performed as described by O'Connell *et al.*[26]. The extracts obtained from the feed and extruded samples which were dried under nitrogen, were dissolved with 100 µL THF and then diluted with 900 µL mobile phase. Dried extracts obtained from digested feed and extruded samples were dissolved in 50 µL THF and diluted with 450 µL mobile phase. The tubes were mixed with vortex. The samples were filtered through the 0.45 µm syringe filter (Syringe Filter, PTFE 13 mm). The HPLC system (Thermo Scientific, Finnigan Surveyor) with a UV visible detector (Finnigan Surveyor, UV-Vis Plus Detector) was used for lycopene quantification. The colon was reverse phase C-18 column (Inertsil ODS-2, 4.6x250 mm, 5 µm). The column temperature was set to 20°C and the wavelength used was 450 nm. The flow rate was 1 mL/min while the injection volume was 25 µL. The mobile phase was the mixture of acetonitrile: methanol: dichloromethane (75:20:5) (v/v/v) which

contain 10 mmol/L ammonium acetate, 4.5 mmol/L buthylated hydroxytoluene and 3.6 mmol/L triethylamine. The mobile phase filtrated by 0.45 μm filter (Filtration Membranes, Membrane Disc PVDF 47 mm) and degassing was completed with ultrasonic agitation.

The standard curve was prepared with lycopene (Sigma-Aldrich,Germany, purity > 85.0 %) with a range of concentrations from 1 mg/L to 7.5 mg/L. *In vitro* bioaccessibility of feed and extrudate samples was calculated as follows:

$$\textit{In vitro}\ \text{bioaccessibility}\ (\%) = \frac{\begin{pmatrix}\text{mg lycopene in}\\ \text{dialyzed portion}\end{pmatrix} + \begin{pmatrix}\text{mg lycopene in}\\ \text{not dialyzed portion}\end{pmatrix}}{\text{mg lycopene in the sample before } \textit{in vitro} \text{ digestion}}$$

2.5 Statistical Analysis

The results were analyzed by analysis of variance (ANOVA) to determine if there was a significant difference between the samples ($p \leq 0.05$). When significant difference was observed, Duncan's Multiple Comparison Test was applied ($p \leq 0.05$) by using SAS software version 9.1 (SAS Institute Inc., NC, USA).

3. Results and discussion

3.1 Total Phenolic Content

Extrusion decreased the total phenol content (Table 3). Extrudates treated with 130°C and 160°C last zone temperature had lower total phenolic content compared to feed (Table 3). Several researches indicated a decrease in total phenolic content after extrusion.[27,28] The high temperature during the extrusion process may cause the phenolic compounds to decompose as these compounds are known to be heat sensitive especially at the temperatures above 80°C.[28] In addition, the phenols may be exposed to decarboxylation with the temperature of

the extrusion process and moisture content. This transformation can cause polymerization of phenols which means the extraction of these phenols and the activity of antioxidants would be limited.[29] In this study, extruded samples with higher treatment temperature (160°C last zone temperature) had higher phenol content compared to samples with lower treatment temperature (130°C last zone temperature). This could be due to the conversion from bound form to released form and having higher extractability after higher treatment temperatures.[2]

3.2 Antioxidant Activity

DPPH method was chosen for antioxidant activity analysis due to its fast, simple and inexpensive nature for comparison analysis. Extrusion decreased the antioxidant activity (Table 4). Extrudates with 160°C last zone temperature had higher antioxidant activity compared to extrudates with 130°C last zone temperature. The reason could be that more Maillard products might have been formed and extractability of phenols could be higher at higher temperature extrusion in our study. Similarly, in study of Masatcioglu *et al.*[2], increase in extrusion temperature caused antioxidant activity to increase significantly. The reason was attributed with the Maillard reaction, as the high temperature could result in more Maillard reaction products leading to higher activity.[2]

In the research of Sharma *et al.*[28], it was seen that the samples which were treated with extrusion had higher antioxidant activity than the raw samples. In the same study, when the temperature was increased from 150°C to 180°C, the antioxidant activity of the samples decreased significantly. Gujral *et al.*[30] stated that the extrusion caused a decrease in the antioxidant activity in the samples where three paddy cultivars were extruded. The decrease was observed at 100°C, moreover, when the temperature was raised to 120°C, the results showed more reduction. The reason of decrease in this level was attributed to the destruction due to high temperature treatment of extrusion. The antioxidant activity of extruded samples

decreased by 60-68 % as a result of the extrusion process in the study of Altan *et al.*[31] Moreover, in addition to degradation of these substances with temperature, evaporation could be another reason for the decrease during extrusion.[31]

3.3 Lycopene Content and *In vitro* Bioaccessibility of Lycopene

Absorption of carotenoids can be achieved in two ways; by harming the cell with mechanical damage or extraction to a lipophilic environment.[32] With extrusion technique, feed material was treated with both thermal and mechanical process. The thermal process has the same effect as mechanical disruption on the cells.[32]

In this study, both extrudates with the last zones treatment temperatures of 130°C and 160 °C had lower lycopene content than the feed (Table 5). The high shear and high temperature of extrusion process could destroy the lycopene in the samples, which are known to be labile to heat and mechanical disruption. It was seen that extrudates with the last zone temperature of 160°C showed higher lycopene content than the extrudates with last zone temperature of 130°C. This could be due to the destruction of cell walls and the chromoplast membrane entrapping lycopene with high temperature and enhanced extractability of lycopene from the sample.[32-34]

Even though the processing decreased the lycopene content, *in vitro* bioaccesibility of lycopene, which is a measure of % accesible lycopene content after digestion simulation, was higher for the sample with 160 °C last zone temperature compared to feed and extrudates with last zone temperatures of 130°C (Table 5). With the extrusion process fiber compounds in the cell wall structure are broken down, thus, the accessibility of lycopene can increase through the digestion process. The reason for higher *in vitro* bioaccessibility of lycopene for the samples that have last zone temperature of 160°C compared to the samples that have last zone temperature of 130°C could be that the damage to the cell matrix at higher temperature

result in more lycopene accessibility. Similarly, Colle *et al.*[19] stated that with increasing treatment temperature, *in vitro* bioaccessibility of lycopene increased for tomato pulp heat treated for 30 min, where the significant difference was observed at temperatures of 120°C and 140°C. In the study of Gartner *et al.*[32], it was concluded that the bioavailability of lycopene that was absorbed from tomato paste showed significantly higher values than the bioavailability of lycopene from fresh tomato. It was stated that the cell walls that were damaged due to cooking made lycopene more reachable.

In Dehghan-Shoar *et al.*[33]'s study, the energy and torque of the extrusion had a negative impact on the lycopene content of the tomato added corn grit extrudates. Even though the lycopene amount decreased, the bioaccessibility of lycopene increased. The feed of extrusion had bioaccessibility between 16-56 % where the extruded products gave a result between 19-105 % .[33] Dehghan-Shoar *et al.*[33] stated that the destructive effect of heat and mechanical energy of extrusion caused the fibers and other substances within the tomato to decompose, making easier for lycopene to be released from the cell. Shi and Le Maguer[6] said that the promoted bioavailability due to damages to cell wall was a result of the broken bonds between lycopene and its surrounding matrix.

4. Conclusion

Total phenolic content and the antioxidant activity of the feed material was significantly decreased with the extrusion process. When the treatment temperature was increased during extrusion, the obtained result showed that the extrudate samples which had 160°C last zone treatment temperature had significantly higher total phenolic content and antioxidant activity compared with the samples which had 130°C last zone treatment temperature.

The extrudates had lower lycopene content compared to feed. *In vitro* bioaccessibility of extrudates with last zone temperature of 130°C was not different from feed while *in vitro*

bioaccessibility of lycopene of extrudates with last zone treatment temperature of 160°C was significantly higher than the feed and extrudates with last zone treatment temperature of 130°C. These results could serve as a starting point for the food processers where they want to minimize the loss and maximize the bioavailability of functional components in the extrudates.

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Table 1 Screw configurations of the extruder.

8 *D* Twin lead feed screws
7 x 30° Forward kneading elements
4 *D* Twin lead feed screws
4 x 60° Forward kneading elements
4 x 30° Reverse kneading elements
2 *D* Twin lead feed screws
6 x 60° Forward kneading elements
4 x 30° Reverse kneading elements
1 *D* Single lead feed screws
7 x 90° Kneading elements
2 *D* Single lead feed screws
Die

Screw diameter (*D*) = 25 mm.

One kneading element = 0.25 D.

Table 2 Composition of simulated salivary, gastric and duodenal fluids (Minekus *et al.*[25]).

			Simulated salivary fluid		Simulated gastric fluid		Simulated duodenal fluid	
			pH 7		pH 3		pH 7	
Constituent	**Stock Conc. g L^{-1}**	**Stock Conc. mol/L^{-1}**	**Volume of Stock mL**	**Concentration mmol/L^{-1}**	**Volume of Stock mL**	**Concentration mmol/L^{-1}**	**Volume of Stock mL**	**Concentration mmol/L^{-1}**
KCl	37.3	0.6	15.1	15.1	6.9	6.9	6.8	6.8
KH_2PO_4	68	0.5	3.7	3.7	0.9	0.9	0.8	0.8
$NaHCO_3$	84	1	6.8	13.6	12.5	25	42.5	85
NaCl	117	2	-	-	11.8	47.2	9.6	38.4
$MgCl_2(H_2O)_6$	30.5	0.15	0.5	0.15	0.4	0.1	1.1	0.33
$(NH_4)_2CO_3$	48	0.5	0.06	0.06	0.5	0.5	-	-
For pH adjustment								
	mol L^{-1}		**mL**	**mmol L^{-1}**	**mL**	**mmol L^{-1}**	**mL**	**mmol L^{-1}**
NaOH	1		-	-	-	-	-	-
HCl	6		0.09	1.1	1.3	15.6	0.7	8.4

Table 3 Total phenolic content of feed and extrudate samples where last zone temperatures were 130°C and 160°C.

Sample	GAE mg/ g dry weight
Feed	15.37 ± 0.09^{a}
130°C	6.14 ± 0.07^{c}
160°C	7.49 ± 0.11^{b}

Results are means ± SD (n= 3); values of the same column, followed by the different letter (a,b,c) are statistically different ($p \leq 0.05$).

Table 4 The antioxidant activities of feed and extrudate samples where last zone temperatures were 130°C and 160°C.

Sample	TE µmol /g dry weight
Feed	16.81 ± 0.29^{a}
130°C	12.55 ± 0.21^{c}
160°C	13.19 ± 0.28^{b}

Results are means ± SD (n= 3); values of the same column, followed by the different letter (a,b,c) are statistically different ($p \leq 0.05$).

Table 5 Lycopene content of the samples before and after *in vitro* digestion.

	Lycopene (µg / g dry sample)			
	Before *in vitro* digestion	**Dialyzed portion**	**Not dialyzed portion**	***In vitro* bioaccessibility (%)**
Feed	12.04 ± 2.05^{a}	0.085 ± 0.004^{a}	3.36 ± 0.10^{a}	29.15 ± 4.93^{b}
Extrudate (130 °C)	2.84 ± 0.44^{c}	0.068 ± 0.006^{b}	0.77 ± 0.17^{c}	29.49 ± 3.19^{b}
Extrudate (160 °C)	4.48 ± 0.17^{b}	0.071 ± 0.019^{ab}	2.34 ± 0.26^{b}	53.86 ± 4.62^{a}

Results are means ± SD (n = 3); values of the same column followed by the different letter (a,b,c) are statistically different ($p \leq 0.05$).