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Food processing and digestion: The effect of extrusion process on bioactive compounds in extrudates with artichoke leaf powder and resulting *in vitro* cynarin and cynaroside bioaccessibility

Ozge Guven[a], Ilkay Sensoy[a,*], Hamide Senyuva[b], Sibel Karakaya[c]

[a] Middle East Technical University, Food Engineering Department, Ankara, Turkey
[b] FoodLife Int. Ltd, ODTU Teknokent, Middle East Technical University, Ankara, Turkey
[c] Ege University, Food Engineering Department, Turkey

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ABSTRACT

Although herbal ingredients could be used to increase bioactive compounds in food products, it is more crucial to investigate the effect of processing on their bioaccessibility and their content. In this study, artichoke leaf powder (ALP) was added to wheat flour (WF) at ratios of 0:100, 3:97, 6:94 and 9:91 g ALP:g WF on dry basis. The effect of extrusion process on the bioactive compounds themselves and *in vitro* bioaccessibilities of cynarin and cynaroside, which are found in ALP, were investigated. Total phenolic content (TPC) and antioxidant activity (AA) of extrudates and raw mixtures were also measured. The extrusion process reduced TPC from the range of 3.4–8.2 to 2.2–6.3 g gallic acid equivalent/kg. Antioxidant activities of the samples with ratios of 3:97 and 6:94 remained the same whereas it decreased from 30 to 24 mmol trolox equivalent/kg for the sample with the ratio of 9:91. The extrusion process also caused a decrease in cynarin and cynaroside contents. The process increased *in vitro* bioaccessibility of cynarin by 30–44% for all ALP concentrations and that of cynaroside by 38% for both ratios, 6:94 and 9:91. Obtained results could be useful in product and processing design to improve the bioaccessibility of functional compounds.

1. Introduction

The artichoke (*Cynara scolymus* L.) has antioxidative and hepatoprotective potential due to its composition of phenolic substances. Health promoting and antioxidative properties of artichoke rely on its phenolic compound profile composed of especially mono- and di-caffeoylquinic acids and flavonoids (Fratianni, Tucci, De Palma, Pepe, & Nazzaro, 2007). Among these compounds cynarin (1,3-di-*O*-caffeoylquinic acid), which is a phenolic acid, is the most effective one in preventing cholesterol biosynthesis and low density lipoprotein oxidation (Lattanzio, Kroon, Linsalata, & Cardinali, 2009; Piston et al., 2014). Cynaroside (luteolin-7-*O*-glucoside), which is an abundant flavonoid-glycoside in artichoke, is another artichoke polyphenol.

The edible fractions, especially heart, of artichoke are consumed both raw and processed, canned or frozen worldwide (Abu-Reidah, Arraez-Roman, Segura-Carretero, & Fernandez-Gutierrez, 2013; Lattanzio et al., 2009). Most of the total artichoke biomass (80–85%) is harvested and 60% of the harvested crops, consisting of external flowers, bracts and stems, is discarded during manufacturing operations (Conidi, Rodriguez-Lopez, Garcia-Castello, & Cassano, 2015; Llorach, Espin, Tomas-Barberan, & Ferreres, 2002; Zuorro, Maffei, & Lavecchia, 2016). Although there are several applications for artichoke waste utilization such as production of leaf extracts, this unexploited agri-industrial waste still remains as an environmental problem (Conidi et al., 2015; Llorach et al., 2002; Sanchez-Rabaneda et al., 2003). Artichoke waste is a rich source of fibers, bioactive phenolic substances and antioxidants with its low proportion of fat (Lattanzio et al., 2009; Ruiz-Cano et al., 2014). The use of this valuable product, especially the outer green leaves and floral stems, as a source of bioactive compounds or utilizing them in functional product development will be an economically feasible solution to the waste problem (Ruiz-Cano et al., 2014; Sihem et al., 2015).

Extrusion is a continuous, stepwise and high-temperature-short-time thermomechanical operation where high pressure and shear force help cooking and shaping (Huang & Ma, 2016; Obradovic, Babic, Subaric, Ackar, & Jozinovic, 2014; Ti et al., 2015b; Wani & Kumar, 2016). Incorporation of fruit and vegetable based ingredients as mineral, fiber and phytochemical sources into extruded products can improve the nutritional quality and increase consumer interest of cereal extrudates (Alam, Kaur, Khaira, & Gupta, 2015; Hirth, Leiter, Beck, &

* Corresponding author.
E-mail address: isensoy@metu.edu.tr (I. Sensoy).

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Schuchmann, 2014; Paraman, Sharif, Supriyadi, & Rizvi, 2015). There are a number of studies on incorporation of vegetable by-product usage in extrusion, yet the information on effect of extrusion on functional components is limited.

The term bioavailability is defined as the absorbed and metabolized fraction of an ingested compound (Rubio et al., 2014). Bioaccessibility refers to the solubilized amount of a food compound or nutrient which becomes available for subsequent absorption in the gut after ingestion (Hedren, Diaz, & Svanberg, 2002; Helal, Tagliazucchi, Verzelloni, & Conte, 2014; Tagliazucchi, Helal, Verzelloni, & Conte, 2012). In order for a food constituent to be bioaccessible, it has to be released from a food matrix and gain the ability of passing through the intestinal barrier (Hedren et al., 2002; Rubio et al., 2014; Saura-Calixto, Serrano, & Goni, 2007).

Food matrix, structure and composition strongly affect the stability, release and absorption properties of polyphenols (Ortega, Macia, Romero, Reguant, & Motilva, 2011). Processing technologies can affect releasing properties of polyphenols by causing alterations in the food matrix.

In the literature, there are limited studies on the effects of extrusion on *in vitro* bioaccessibility of phenolic compounds. Therefore, the objective of this study was to investigate how extrusion process affects cynarin (Fig. 1), which is a hydroxycinnamic acid, and cynaroside (Fig. 2), which is a flavone, contents and to investigate the effect of extrusion on *in vitro* bioaccessibilities of these two phenolic compounds in artichoke. The effect of the extrusion process on the total phenolic content (TPC) and antioxidant activity (AA) were also investigated.

2. Materials and methods

2.1. Raw materials and preparation of feed for extrusion

Wheat flour (WF) was supplied from Soke Milling Industry and Trade Inc. (Aydın, Turkey) and artichoke (*Cynara scolymus* L.) leaves were supplied from local groceries (Ankara, Turkey). Artichoke leaf powder (ALP) was prepared as follows: fleshy outer green leaves were washed and dried at room temperature up to 2 weeks. Dried leaves were pulverized in a pulverisette 16 mill (Fritsch, Idar-Oberstein, Germany) and sieved with 1 mm size sieve (Fritsch, Idar-Oberstein, Germany). Obtained ALP, which had a moisture ratio of 84 ± 14 g/kg FW (fresh weight) was stored at room temperature in dark bags, which were stored in closed jars up to a year. Moisture contents of both raw and processed materials were determined by MIX-50 halogen moisture analyzer at 160 °C (AND, Tokyo, Japan). Feed samples were prepared with ratios of 0:100, 3:97, 6:94 and 9:91 g ALP:g WF on dry basis. Final moisture contents of the feed samples were adjusted to 197 ± 2 g/kg by distilled water addition during mixing (KitchenAid, Greenville, OH, USA). Prepared samples were kept at +4 °C overnight. They were allowed to equilibrate at room temperature for 2 h before the extrusion process.

2.2. Extrusion

The extruder was a laboratory scale co-rotating twin screw extruder (Feza Machine Co. Ltd., Istanbul, Turkey) with computer control and data acquisition system. Screw configurations of the extruder were given in Table 1. Die diameter was 3 mm and barrel length to diameter ratio (L:D) was 25:1 cm:cm. Four heating zones were controlled by electrical heating and water cooling systems. Barrel zone temperatures, rotor speed and flow rate were monitored by means of computerized data acquisition system. A twin screw volumetric feeder integrated with the extruder system was used to feed the prepared raw mixture into extruder.

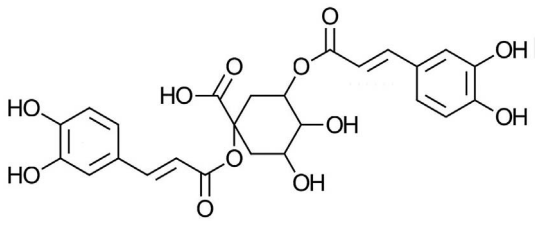

Fig. 1. Chemical structure of cynarin.

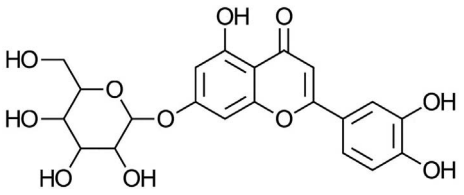

Fig. 2. Chemical structure of cynaroside.

Table 1
Screw configurations of the extruder.

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

Screw diameter (*D*) = 25 mm.
One kneading element = 0.25 D.

The feed flow rate was 55 ± 1 g/min for all samples. Screw speed was 250 rpm. Barrel temperature zones were set at 80 °C, 90 °C, 130 °C and 150 °C (die: 128 °C). Samples were taken only when actual measured barrel zone temperatures and die temperatures varied maximum ± 2 °C from the set temperatures.

Moisture ratios of extrudates were 115 ± 5 g/kg FW. Extruded samples to be used in analyses were kept in closed bags at −20 °C in the dark.

2.3. Chemical reagents

All reagents used in analyses apart from liquid chromatography with mass spectrometry (LC-MS/MS) analyses were of analytical grade (Merck, Darmstadt, Germany and Sigma, St. Louis, MO, USA). Chemicals used in LC-MS/MS analyses were high pressure liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany). Cynarin was purchased from Sigma (St. Louis, MO, USA) and cynaroside was purchased from Extrasynthese (Lyon, France).

2.4. In vitro digestion analysis

Digestion was carried out at three steps according to the procedure described by Minekus et al. (2014) with some modifications. Simulated salivary (SSF), gastric (SGF) and intestinal fluids (SIF) were prepared according to Table 2 (Minekus et al., 2014).

2.4.1. Oral digestion

Firstly, 5 mL of distilled water was added to 1 g of sample in a falcon tube. After that, 4 mL of SSF was mixed with sample and water mixture. Then 25 μL of $CaCl_2$ (0.3 mol/L) was added. pH was adjusted to 7