

EFFECT OF EXTRUSION ON BIOACTIVE COMPOUNDS FOUND IN  
ARTICHOKE LEAF POWDER AND ON THEIR *IN VITRO*  
BIOACCESSIBILITIES

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Approval of the thesis:

**EFFECT OF EXTRUSION ON BIOACTIVE COMPOUNDS FOUND IN  
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## ABSTRACT

### EFFECT OF EXTRUSION ON BIOACTIVE COMPOUNDS FOUND IN ARTICHOKE LEAF POWDER AND ON THEIR *IN VITRO* BIOACCESSIBILITIES

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The functional properties of extruded products can be enhanced by addition of herbal ingredients. In this study, artichoke leaf powder (ALP) was added to extrudates, at 3%, 6% and 9% concentrations, to increase amount of bioactive nutrients. Aim of the study was to investigate the effect of extrusion on functional components and *in vitro* bioaccessibility of selected phenolic compounds. Extrusion feed flow rate was  $55 \pm 1$  g/min, screw speed was 250 rpm and barrel temperature zones were set at 80°C, 90°C, 130°C and 150°C (die: 128°C). Total phenolic content and antioxidant activities were measured. LC-MS/MS was used for quantification of selected phenolic compounds.

Total phenolic content of extrudates and raw mixtures increased with increasing ALP concentration. Extrusion process caused a significant decrease in total phenolic content for all ALP concentrations. Similarly, antioxidant activities of samples

increased with increasing ALP concentration. Antioxidant activities of 3% and 6% ALP added samples did not change after extrusion. Antioxidant activity of 9% ALP added samples decreased after extrusion process.

Extrusion process adversely affected cynarin and cynaroside contents of samples. However, *in vitro* bioaccessibilities of cynarin and cynaroside compounds increased after extrusion process.

**Keywords:** functional food, phenolic content, antioxidant activity, cynarin, food matrix

## ÖZ

# EXTRÜZYON İŞLEMİNİN ENGİNAR YAPRAĞI TOZUNDA BULUNAN BİYOAKTİF BİLEŞENLERE VE *İN VİTRO* BİYOERİŞİLEBİLİRLİKLERİNE ETKİSİ

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Ekstrüde ürünlerin fonksiyonel özellikleri bitkisel bileşenler kullanılarak artırılabilir. Bu çalışmada, biyoaktif bileşen miktarını artırmak amacıyla, ekstrüde ürünlere %3, %6 ve %9 oranlarında enginar yaprağı tozu (EYT) eklenmiştir. Çalışmanın amacı, ekstrüzyon işleminin; fonksiyonel bileşenlere ve belirli biyoaktif fenolik maddelerin *in vitro* biyoerişilebilirliklerine etkisini araştırmaktır. Ekstrüzyon işleminde kullanılan besleme akış hızı  $55 \pm 1$  g/dk ve vıd hızı 250 rpm'dir. Namlu bölgelerinin sıcaklıkları sırasıyla 80 °C, 90 °C, 130 °C ve 150 °C olarak belirlenmiştir. Kalıp sıcaklığı ise 128 °C'dir. Fenolik maddelerin miktar tayini LC-MS/MS kullanılarak yapılmıştır.

Toplam fenolik miktarı artan EYT miktarı ile artmıştır. Ekstrüzyon işlemi farklı EYT konsantrasyonuna sahip örneklerdeki toplam fenol miktarında kayda değer ölçüde azalmaya neden olmuştur. Benzer şekilde, EYT miktarı arttıkça antioksidan aktivitesinin de arttığı görülmektedir. %3 ve %6 EYT eklenmiş örneklerde antioksidan

aktivitesi ekstrüzyon işleminden sonra deęişmemiştir. %9 EYT içeren örneklerde ise antioksidan aktivitesi ekstrüzyon işlemi sonrasında azalmıştır.

Ekstrüzyon işlemi sinarin ve sinarozid miktarlarının azalmasına sebep olmuştur; ancak ekstrüzyon işlemi bu maddelerin *in vitro* biyoerişilebilirliklerini artırmıştır.

**Anahtar Kelimeler:** fonksiyonel gıda, fenolik madde, antioksidan aktivitesi, sinarin, gıda matrisi

*To my parents...*

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## **CHAPTER 1**

### **INTRODUCTION**

Modern consumers suffer from limited time and busy work schedules. Therefore, they prefer to consume snacks which are both delicious and easily consumed (Wani & Kumar, 2016a). However, snacks have disadvantages that they are traditionally fatty, salty and sweet food products. They are energy-dense and have poor nutritional value (Caruso, Klein, & Kaye, 2014). Thus, consumption of traditional snacks contributes to an unhealthy diet (De Vet, Stok, De Wit, & De Ridder, 2015). However, the snacking habit is not necessarily unhealthy. Actually, it is a good behavior because it can contribute to energy balance if snack choices are made wisely (Barnes, French, Harnack, Mitchell, & Wolfson, 2015). Increasing consumer demand for quick and nutritious food puts an importance on the development of functional ready-to-eat products (Ahmed & Abozed, 2015).

Extruded products are one of the most important product group among snacks (Potter, Stojceska, & Plunkett, 2013). Extrusion is a promising technology to produce snacks having convenient portions with low fat, low salt and good fiber content (Potter et al., 2013; Rodríguez-Miranda et al., 2011). The addition of ingredients having health benefits to regular products is a way of producing healthy snacks (Peřksa et al., 2016). Thus, functional extruded snacks can be produced by fortification.

## **1.1 Functional Foods**

The functional food concept was first proposed by Japan scientists in 1984 as a result of studies of the relationship between nutrition, organoleptic properties of food, fortification and physiological systems (Siró, Kápolna, Kápolna, & Lugasi, 2008). After Japan, in the United States and Europe functional food gained attention with regard to both community health care and marketing perspectives (Eussen et al., 2011; Siró et al., 2008). In 1991 approval criteria including establishment of specific health claims for FOSHU (food for specified health uses) were introduced by the Japanese Ministry of Health (Siró et al., 2008). Today, there are three criteria for a health claim which are taken into consideration by European Food Safety Authority (EFSA) and they are “i) definition and characterization of the food or functional ingredient; ii) definition of the (beneficial physiological) claimed effect; and iii) establishment of a cause and effect relationship between consumption of the food or functional ingredient and the claimed effect” (De Boer, Urlings, & Bast, 2016).

Functional food is defined as an ordinary food which is proven to have health benefits or potential to reduce risk of a chronic disease beyond its nutritional value (Younesi & Ayseli, 2015). In addition, nutritional drawbacks caused by intolerances, strict diets or individual choices can be prevented by functional foods (Bigliardi & Galati, 2013; Siró et al., 2008). Therefore, at the interface between food and pharmaceuticals, together with dietary supplements, functional foods are important actors with regard to both public health care and marketing perspectives (Eussen et al., 2011).

Functional foods, such as fortified cereals and probiotic yoghurt, appear similar to traditional products and consumed as a part of usual diet (Eussen et al., 2011). The perception of health benefits of food and its components will continue to grow (Crowe & Francis, 2013). That is why the popularity of functional foods is

increasing and new products are being constantly launched (Bigliardi & Galati, 2013).

There are four functional food product categories: i) products fortified by additional nutrients ii) products enriched with new nutrients which are not found in that particular food normally, iii) products from which an undesired constituent is eliminated or substituted by a beneficial one and iv) products containing a naturally enhanced component (Bigliardi & Galati, 2013). The most common functional food types are probiotics, prebiotics, functional drinks, functional cereals, bakery products, spreads, functional meat and functional eggs (Siró et al., 2008). The development of functional food processes are complicated since many aspects of products should be considered. Functional food research and development processes brings health, nutrition and technology concepts together; therefore, a specialist, a nutritionist and a food technologist are the main actors in these processes (Bigliardi & Galati, 2013). Thus, development of functional foods is achieved by a collaborative work of academic and industry scientists (Roberfroid, 1999). The success of a functional food product relies on good management of interdisciplinary studies and good analysis of consumer researches (Falguera, Aliguer, & Falguera, 2012).

The composition of plant species plays an important role in studies of biological activity and active substances, and plants are good natural sources of functional ingredients (Betoret et al., 2012; Vieira da Silva, Barreira, & Oliveira, 2016). Therefore, development of novel foods derived from fruit and vegetables is an emerging activity in functional food studies (Betoret et al., 2012).

## **1.2 Antioxidants**

Antioxidants have gained an important role in preservation of foods and modern health care since they prolong shelf life of food products without any undesired change in organoleptic and nutritional properties (Ignat, Volf, & Popa, 2011;

Shahidi & Ambigaipalan, 2015). Antioxidants are roughly defined as the chemical species which have the ability of being oxidized instead of other compounds (Seifried, Anderson, Fisher, & Milner, 2007).

Free radicals are highly reactive chemical substances which have unshared electrons (Carocho & Ferreira, 2013). They are formed as a result of metabolic activities in mitochondria and formation of these chemicals in human body is accelerated by environmental factors (Carocho & Ferreira, 2013).

Reactive oxygen species (ROS), which are free radicals, is one of the major factors causing medical problems related to pathophysiology (Shahidi & Ambigaipalan, 2015). Reactive oxygen species can harm essential molecules in cells such as DNA, proteins and lipids and cause carcinogenesis, cardiovascular disease and other chronic diseases (Dimitrios, 2006; Seifried et al., 2007). If oxygen homeostasis, balance between oxidant and antioxidant species, is not ensured oxidative stress occurs in the cell (Seifried et al., 2007). Oxidative stress caused by overabundant ROS is potentially harmful (Seifried et al., 2007). These hazardous species are kept under control in the cell with the help of antioxidant mechanisms and endogenous enzymes (Dimitrios, 2006; Seifried et al., 2007). Therefore, antioxidants have an important role in preventing the negative effects of ROS and the other free radical species.

### **1.3. Phenolic Compounds**

Secondary metabolites of plants, in other words phytochemicals, are non-nutritional plant-based bioactive molecules found in plants (Acosta-Estrada, Gutiérrez-Urbe, & Serna-Saldívar Centro, 2014; Embuscado, 2015). Phenolic compounds, carotenoids, plant sterols, glucosinolates and other sulfur-containing groups are considered as plant phytochemicals (Embuscado, 2015). Terpenes and terpenoids, alkaloids and phenolics are the three main classes of plant secondary metabolites (Murphy, 2011). These secondary metabolites are responsible for growth activities,

development and responses to environmental conditions (Murphy, 2011). Moreover, they also play an important role in defense system of plants (Kosseva & Webb, 2013).

Phenolics, often called “polyphenols”, constitute one of the most important secondary metabolites group in plants (Balasundram, Sundram, & Samman, 2006; Naczka & Shahidi, 2004). They are important phytochemicals which provide various physiological and morphological benefits to plants (Balasundram et al., 2006; Carochi & Ferreira, 2013). Some functions of polyphenols are structural defense, environmental adaptation and reproduction by attracting pollinators and seed-dispersing animals (Embuscado, 2015). Plants produce phenolic compounds in order to protect themselves against UV light, insects, viruses and bacteria (Embuscado, 2015; Heleno, Martins, Queiroz, & Ferreira, 2015).

Chemically, polyphenols have at least one aromatic ring containing at least one hydrogen substituted by a hydroxyl group (Heleno et al., 2015). Phenylalanine and tyrosine are the common precursors for the large part of natural polyphenols which provide the aromatic ring (Heleno et al., 2015). Phenolic compounds are classified by phenol ring number and other structural components binding these rings to one another (Ignat et al., 2011).

Phenolic antioxidants are separated into two main groups which are synthetic and natural phenolics (Shahidi & Ambigaipalan, 2015). Phenolic acids, flavonoids, stilbenes, coumarins, lignans and tannins are the subgroups of natural phenolic antioxidants (Shahidi & Ambigaipalan, 2015). Terpenes, tocopherols, phospholipids and polyfunctional organic acids are other naturally-occurring antioxidants (Shahidi & Ambigaipalan, 2015).

Among all polyphenolics, flavonoids are the most commonly consumed compounds with dietary purposes (Kosseva & Webb, 2013). Flavonols, flavanols, anthocyanins, isoflavonoids, flavanones and flavones constitute flavonoids group

(Carocho & Ferreira, 2013). Flavonoids inhibit lipoxygenase and cyclooxygenase enzymes, which are involved in oxidative rancidity development in foods (Embuscado, 2015). In addition, free radicals and catalytic metal ions can be inactivated by flavonoids (Embuscado, 2015). Two major phenolic acid groups are hydroxybenzoic acids and hydroxycinnamic acids (Heleno et al., 2015). These molecules are rapidly metabolized bioactive species which have antioxidant antitumor and antimicrobial effects (Heleno et al., 2015).

Phenolic compounds are used to enhance product quality in industrial applications and they have many health benefits. “In food, phenolics may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability of food.” (Naczek & Shahidi, 2006). Moreover, phenolic compounds can be added to foods in order to increase antioxidant content and promote healthiness of products (Acosta-Estrada et al., 2014).

Naturally-occurring phenolic compounds may play a role in preventing serious health problems due to their antioxidant effect (Shahidi & Ambigaipalan, 2015). They have “anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects” (Shahidi & Ambigaipalan, 2015).

#### **1.4 Agri-Industrial By-Products in Functional Food Design**

Agri-industrial by-products draws an increasing interest since they can be exploited in various industries such as food, pharmaceuticals and cosmetics due to their potential economic and environmental benefits (Laufenberg, Kunz, & Nystroem, 2003; Peschel et al., 2006; Schieber, Stintzing, & Carle, 2001). There are several purposes of agri-industrial by-product usage in agriculture and food industries such as utilizing them as feed or fertilizer, using them as shelf-life-increasing additives by preventing lipid peroxidation and oxidative damage in food products, using them in solid state fermentation and thus doing bioconversion and lastly using them as

bioadsorbents for waste water treatment (Laufenberg et al., 2003; Moure et al., 2001; Schieber et al., 2001). In addition to these, value-adding ingredients produced from agri-industrial by-products are used in particularly in the bakery and beverage industries in novel food design studies (Laufenberg et al., 2003).

## **1.5 Artichoke**

The globe artichoke (*Cynara scolymus L.*) is an ancient plant which has been consumed by humans since the 4<sup>th</sup> century B.C. both for feeding and medical purposes (Lattanzio, Kroon, Linsalata, & Cardinali, 2009). This nonarberool (herbaceous) perennial plant belongs to Asteracea family (Lattanzio et al., 2009; Pandino, Lombardo, Mauromicale, & Williamson, 2011; Zuorro, Maffei, & Lavecchia, 2016). The artichoke, which is one of the most important vegetable crops grown in Mediterranean countries, is mainly produced in Italy and Spain but also in France and Greece (Lattanzio et al., 2009; Lombardo et al., 2010). Consequently, commercial production of artichoke makes an important contribution to the agricultural economy in these countries and in Mediterranean areas (Abu-Reidah, Arraez-Roman, Segura-Carretero, & Fernandez-Gutierrez, 2013; Lattanzio et al., 2009).

The artichoke is a popular vegetable used in many food recipes for its delicious bitter taste and health promoting nutritious properties (Pandino et al., 2011; Sánchez-Rabaneda et al., 2003). The palatable part of the plant is the immature inflorescence consumed raw or cooked, boiled, steamed or fried (Pandino et al., 2011; Ruiz-Cano et al., 2014). The edible immature inflorescence part, receptacle together with the inner bracts, is covered by outer bracts (fleshy green leaves) which can be consumed if they are prepared by cooking properly (Pandino et al., 2011).

The edible part of artichoke, which is also called the capitula is harvested when it is still in early development stages (Lattanzio et al., 2009; Lombardo et al., 2010). It has a weight between 150g and 600g which changes according to cultivar and

harvest time (Pandino et al., 2011). The weight ratio of receptacle (“heart”) to the whole head is 10-18% while that of tender inner part including receptacle and the inner bracts is 35-55% depending on the cultivar and harvest time (Abu-Reidah et al., 2013; Pandino et al., 2011). In total, the ratio of the edible part to the total plant biomass is less than 15-20% (Lattanzio et al., 2009).

The edible fractions, especially heart, of artichoke are consumed both raw and processed, canned or frozen, worldwide and the plant gains an additional economic value (Abu-Reidah et al., 2013; Lattanzio et al., 2009). In addition to mature heads, immature baby artichokes are also sold in markets (Lutz, Henríquez, & Escobar, 2011). Capsules, powder extracts, liquid extracts (produced from leaves) and juices (produced using the heads) constitute another group of commercial artichoke products in several European countries and USA (Fritsche, Beindorff, Dachtler, Zhang, & Lammers, 2002). The production of leaf extracts is a way to utilize artichoke leaves. However there is still a management problem of artichoke wastes (Sánchez-Rabaneda et al., 2003). It was reported that almost 60 % of heads coming from harvested crops which is 80 – 85 % of the total biomass consisting of external flowers, bracts and stems is discarded during manufacturing operations (Conidi, Rodriguez-Lopez, Garcia-Castello, & Cassano, 2015; Llorach, Espín, Tomás-Barberán, & Ferreres, 2002; Zuorro et al., 2016). This agri-industrial waste cannot be exploited and remains as an environmental problem (Conidi et al., 2015; Llorach et al., 2002).

The waste (by-products) of artichoke industry is rich in inulin, 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 waste product (especially the outer green leaves, and floral stems) as a sources 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).

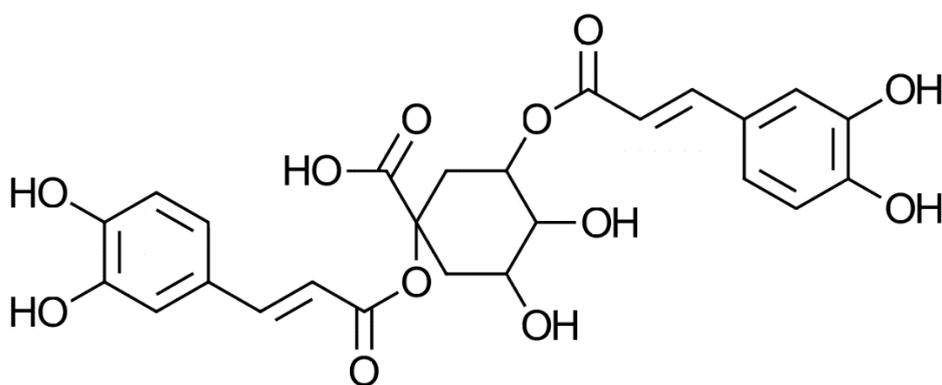
Artichoke and artichoke leaf extracts have long been used in folk medicine for their choleric, diuretic and hypocholesterolemic activities (Fritsche et al., 2002; Lombardo et al., 2010). Moreover, in herbal medicine, consumption of artichoke leaf tea is very common in solving problems related to digestion and liver due to its antidyspeptic and hepatoprotective properties (Pistón et al., 2014). The artichoke is considered to solve hyperlipidemia, dropsy, rheumatism and cholesterol metabolism problems and to reduce coronary heart disease risk and atherogenic risk (Gebhardt, 1997; Lattanzio et al., 2009). Having a lipid-lowering effect and high antioxidant capacity, the artichoke has a favorable cardiovascular profile (Lattanzio et al., 2009). The artichoke is also believed to have a slimming effect besides its health-promoting, therapeutic and nutritious profile and artichoke-based products are becoming more and more popular among consumers (Lattanzio et al., 2009; Lutz et al., 2011; Pistón et al., 2014).

The main chemical composition of globe artichoke consists of a large amount of inulin, fiber, minerals and bioactive phenolic compounds (Lattanzio et al., 2009). These components provides a substantial nutritious profile and gives a rich medicinal substances profile to the plant (Lattanzio et al., 2009). Most of the biological effects and mentioned health promoting properties are related to the high amounts of inulin and phenolic compounds, mainly caffeoylquinic acids and flavonoids such as luteolin (Conidi et al., 2015; Jun et al., 2007; Lattanzio et al., 2009).

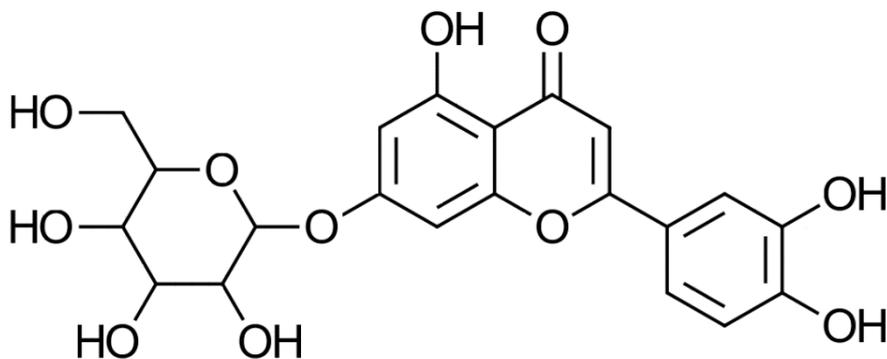
The artichoke was proven to have antioxidative and hepatoprotective potential due to its specific composition of phenolic substances including caffeoylquinic acid and luteolin derived components (Abu-Reidah et al., 2013; Gebhardt & Fausel, 1997). Many studies have reported that health promoting and antioxidative properties of artichoke rely on its phenolic fraction composed of especially mono- and dicaffeoylquinic acids and flavonoids (Fратиanni, Tucci, Palma, Pepe, & Nazzaro, 2007). Moreover, the main individual phenolic compounds in artichoke were

proven to have a strong antioxidative effect such as protecting low density lipoproteins (LDLs) from oxidative damages (Garbetta et al., 2014; Lattanzio et al., 2009).

The main compounds in artichoke are caffeoylquinic acid isomers. Among these compounds cynarin (1,3-di-O-caffeoylquinic acid) was found to be the most effective compound to prevent cholesterol biosynthesis and LDL oxidation (Lattanzio et al., 2009; Pistón et al., 2014). Thus, it may have a role in decreasing cardiovascular disease risk. In the literature, there is a confusion about the IUPAC name of cynarin; this confusion is eliminated by using post-IUPAC nomenclature system and 1,3-di-O-caffeoylquinic acid is accepted for “cynarin”(Lattanzio et al., 2009). Cynaroside (luteolin-7-O-glucoside) is another abundant phenolic compound found in artichoke. Chemical structures of cynarin and cynaroside were given in Figures 1 and 2.



**Figure 1** Chemical structure of cynarin.



**Figure 2** Chemical structure of cynaroside.

Various studies have been carried in order to determine the phenolic composition and profile of artichoke plant (Lombardo, Pandino, Ierna, & Mauromicale, 2012; Pandino, Lombardo, & Mauromicale, 2013; Sihem et al., 2015; M. Wang et al., 2003). However, it is hard to draw a conclusion about which part has the highest amount of bioactive components and which compound is the most abundant one in different parts. The reason is that the phenolic profile of artichoke is affected by several factors which are cultivar, variety, maturity stage of plant, agronomical parameters and storage conditions after harvest (Ruiz-Cano et al., 2014; Sihem et al., 2015; M. Wang et al., 2003). Therefore, as these parameters differs among analyzed artichokes, suggested phenolic substance profile also becomes different.

Inulin is another important constituent of artichoke. It is a hydrophilic storage carbohydrate found in artichoke in high levels (Lattanzio et al., 2009). Especially, fleshy green leaves and artichoke root system are rich sources of inulin (Lattanzio et al., 2009). It is a non-digestible oligosaccharide which has prebiotic effect and it is thought to decrease colon cancer risk preventing lesions and tumors in colon (Lattanzio et al., 2009). Moreover, artichoke inulin is suitable for food processing

practices due to its high degree of polymerization and it can be used as fat and carbohydrate replacer in order to produce nutritionally enhanced products which are also acceptable in terms of flavor and other organoleptic properties (Lattanzio et al., 2009).

## **1.6 Extrusion Technology**

The literal meaning of “extrusion”, which is a word originated from the Latin word “*extrudere*” is “pushing out” (Berk, 2009). In engineering terminology “extrusion” defines the action of pushing material out from a narrow gap (Berk, 2009). In extrusion cooking, the raw material is forced to flow through a hole to produce various types of foods using specified process parameters (Alam, Kaur, Khaira, & Gupta, 2015). Extrusion is a remarkable technology which has been used in food processing since the mid-1930s (Brennan, Brennan, Derbyshire, & Tiwari, 2011). In the early times of food extrusion, applications were limited to processes of mixing and shaping of pasta and cereal pellets (Alam et al., 2015). Today, extrusion is a versatile operation which is applicable to different food processes and as a product segment, extruded foods is growing rapidly with various new and creative products (Alam et al., 2015). As new applications of extrusion technology have been developed, the interest in effects of extrusion on physicochemical, nutritional and functional properties of products has increased (Singh, Gamlath, & Wakeling, 2007).

Extrusion is a continuous, stepwise, thermomechanical and high-temperature-short-time process which depends on high pressure and shear force application for cooking and shaping purposes (Huang & Ma, 2016; Obradović, Babić, Šubarić, Ačkar, & Jozinović, 2014; Ti, Zhang, Zhang, et al., 2015; Wani & Kumar, 2016b). Generally, the main ingredients of extruded products are plant-based materials containing high amounts of starch and protein which gives the final products their specific characteristics (Obradović et al., 2014).

In extrusion, shearing, mixing, cooking and expansion of the raw material occurs at the same time and the intended structure is reached (Hirth, Leiter, Beck, & Schuchmann, 2014). The required energy for these mechanical and thermal processes are supplied to integrated extrusion system by rotating screws and heated barrels (Emin, Teumer, Schmitt, Rädle, & Schuchmann, 2015). At the end of the process, all these applied forces and heat cause several physicochemical and molecular transformations and chemical reactions in the raw material (Alam et al., 2015; Emin et al., 2015). Gelatinization and melting are two examples of these complex changes occurring mainly in starch-based products (Hirth et al., 2014).

In food manufacturing, the major types of extruders are piston, roller-type and screw extruders among which the most common type is screw extruders (Alam et al., 2015). A screw extruder is simply a metal tube or barrel in which there is a screw system carrying the raw material and leading to friction forces (Alam et al., 2015). Heat is supplied by conversion of mechanical energy, and additionally, barrels can be heated separately by steam (Alam et al., 2015) or they can be heated by electrical power.

For higher nutritional quality attributes, such as higher digestibility of nutrients and higher retention of bioactive compounds mild extrusion conditions (high moisture content, low temperature and screw speed) are preferred (Singh et al., 2007). Low temperature is a component of mild extrusion process and twin-screw extruders are preferred when low temperatures are desired in processes such as soluble dietary fiber preparation from legumes (Obradović et al., 2014; Singh et al., 2007). Low residence time and high moisture content are the other desired conditions for production of higher nutritional quality products (Singh et al., 2007). Temperature values higher than 200 °C and moisture values lower than 15% are not good for maintaining the nutritional quality (Singh et al., 2007). Short residence time decreases the occurrence frequency of undesired reactions (Hirth et al., 2014).

Extruded food manufacturing is a multi-billion dollar market which serves products for both human and animal consumption (Alam et al., 2015; Morales et al., 2015; Paraman, Sharif, Supriyadi, & Rizvi, 2015). Ready-to-eat cereals, snack foods, pasta, baby foods, texturized vegetable proteins, meat products, confectionery, chewing gum, dried soups and dry beverage mixes are some of the extruded products (Alam et al., 2015; Morales et al., 2015). The processing of cereals and cereal-like materials by extrusion is common and it has an advantage of developing high nutritional quality products for the consumers (Kowalski, Medina-Meza, Thapa, Murphy, & Ganjyal, 2016; Morales et al., 2015). In addition, products such as gluten-free snacks and meat analogs or substitutes can be produced for specific consumer segments by this technology (Emin et al., 2015; Morales et al., 2015).

One positive aspect of extrusion in terms of highly nutritive food production is increasing protein and carbohydrate content (Singh et al., 2007; Wani & Kumar, 2016b). Secondly, extrusion may improve the digestion properties of protein and starch with decreased nutrient destruction (Singh et al., 2007; Wani & Kumar, 2016b). Moreover, dietary fiber content can be increased or modified and solubility of the fibers can be altered during extrusion (Alam et al., 2015). There are protein enrichment applications by addition of legume proteins or whey protein to extrudates (Obradović et al., 2014). Moreover, high fiber products can be developed by cereal, legume, fruit and vegetable fibers addition (Obradović et al., 2014). Extrusion is an effective way of elimination of anti-nutritional or toxic substances such as aflatoxins, tannins and phytates and inhibition of undesired enzymatic changes and lipid oxidation (Alam et al., 2015; Wani & Kumar, 2016b).

Extrusion is increasingly practiced in product development studies since it is suitable for value addition by using additional ingredients in order to increase nutritional and textural quality and improve functional properties of the product (Alam et al., 2015; Obradović et al., 2014; Wani & Kumar, 2016b). Incorporation of fruit and vegetable based ingredients as mineral, fiber and phytochemical sources

into extruded material can improve the nutritional quality and increase consumer interest of cereal-based extruded products (Alam et al., 2015; Hirth et al., 2014; Paraman et al., 2015). By-products of fruit and vegetable and their waste are promising ingredients for improvement of extruded products in terms of nutritional quality. Although there are a number of studies on incorporation of vegetable by-product usage in extrusion, there is still limited information on extrusion processing of these products (Alam et al., 2015). Vegetable and fruit powder addition may provide an increase content of nutritive compounds in extruded products. In order to get the highest nutritional quality, process parameters (temperature, moisture and screws speed) should be optimized (Obradović et al., 2014).

Bioactive compounds in extruded products are generally the phytochemicals coming from cereal, legume, fruit and vegetables (Obradović et al., 2014). Cereals, especially whole grains, have a high antioxidative activity due to their high phytochemical content (Brennan et al., 2011; Obradović et al., 2014). On the other hand, fruit and vegetable by-products are increasingly used for improving the bioactive matter content of extruded products (Brennan et al., 2011). The effect of additional ingredients may lead to changes in physical properties of extruded products; however, these effects cannot be generalized since they can change according to the cereal type and added material (Obradović et al., 2014). In terms of bioactive compounds, extrusion also has several effects such as alteration of phenolic material structures and antioxidant activity (Ti, Zhang, Li, Wei, & Zhang, 2015). In order to maintain the antioxidant activity of products and minimize the destruction of bioactive substances, extrusion process parameters can be optimized (Leyva-Corral et al., 2016).

### **1.7 Bioaccessibility and Bioavailability**

Bioaccessibility is generally defined as the solubilized amount of a food compound or nutrient which becomes available for subsequent absorption in the gut after ingestion (Hedrén, Diaz, & Svanberg, 2002; Helal, Tagliazucchi, Verzelloni, &

Conte, 2014; Tagliazucchi, Helal, Verzelloni, & Conte, 2012). Unless a food constituent is released from food matrix and gains the ability of passing through the intestinal barrier it is not bioaccessible (Hedrén et al., 2002; Rubió et al., 2014; Saura-Calixto, Serrano, & Goni, 2007).

Bioavailability is defined as the absorbed and metabolized fraction of an ingested compound (Rubio et al., 2014). The main steps for bioavailability are excretion of the substance from the matrix, absorption through the intestine and reaching the tissue (Porrini & Riso, 2008). Thus, there is a strong relationship between bioavailability and bioaccessibility (Tagliazucchi et al., 2012) that a substance needs to be bioaccessible in order to be bioavailable. Bioavailability also can be defined as the proportion of a nutrient which gets into the circulatory system and reaches the target tissue where it exerts its physiological effect (Porrini & Riso, 2008).

Bioaccessible polyphenolic compounds are potentially bioavailable and only bioavailable compounds can exert their positive effect (Celep, Charehsaz, Akyüz, Acar, & Yesilada, 2015; Li, Deng, Liu, Loewen, & Tsao, 2014). Polyphenols become bioaccessible when they are released from the food matrix and/or modified either by the enzyme activities in small intestine or by bacterial activities in large intestine to exert their function (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2014; Saura-Calixto et al., 2007). Moreover, biological benefits of polyphenolic compounds depend on their stability under gastro-intestinal conditions (Garbetta et al., 2014).

Polyphenolic substance are found in food matrix mostly bound to the carbohydrates, organic acids or one phenolic compound is linked to another (Bohn, 2014). Interactions in food matrix and its composition strongly affects the stability, bioaccessibility, absorption and consequently bioavailability of polyphenols (Ortega, Macià, Romero, Reguant, & Motilva, 2011).

Complete absorption of a nutrient is not ensured and may be related to the interactions of other food constituents (Parada & Aguilera, 2007). “Food matrix” refers to the concept that a food system is a large continuous medium (a cellular origin or a processed microstructure) containing nutrients having smaller molecular weights (Parada & Aguilera, 2007). In this system nutrients interact with the other substances and structural elements. They can be found either as individual molecules or they can form complexes with other molecules such as carbohydrates or proteins (Parada & Aguilera, 2007).

Physiological dose, particle size reduction, heating processes, presence of lipids and lowering the amounts of protein and indigestible carbohydrates have positive effects on bioaccessibility of bioactive compounds (Bohn, 2014). When a polyphenolic compound is a part of food matrix with other nutrients its bioaccessibility and bioavailability can be modified (Ortega et al., 2011). Suppression of food matrix negatively affects the total amount of nutrients; however, it has a positive effect on bioavailability of nutrients (Parada & Aguilera, 2007). Food processing technologies such as heating, freezing, canning or cooking cause food matrix suppression and they can improve bioaccessibility of nutrients. Mechanical, enzymatic, chemical and colloidal activities during digestion process also may improve bioaccessibility (Parada & Aguilera, 2007).

*In vitro* digestion models are useful for studying the effect of digestion process and food matrix effects on bioaccessibility and bioavailability of polyphenols (Helal et al., 2014). They are rapid and cheaper alternatives of *in vivo* studies (Hur, Lim, Decker, & McClements, 2011). An *in vitro* digestion model is a simulation of gastro-intestinal process in which gastric solutions and enzymes are used under simulated conditions of gastrointestinal tract.

## **1.8 Analytical Considerations**

It was critical in the study design that any analytical measurement technique which was selected, should provide a high degree of confidence in the correct identification of bioactive components and also have the capability to quantitate with good precision. Both unprocessed artichoke, as well as the extruded product containing artichoke leaf powder, are complex matrices containing a large number of components. Separating these components at low levels from co-occurring plant constituents, confirming identification and accurately quantifying therefore presents a considerable challenge. The separating power of high performance liquid chromatography (LC) combined with high specificity of mass spectrometric detection (LC-MS) offers a powerful analytical tool. Tandem mass spectrometry (MS/MS) adds a further dimension to the degree of specificity that can be achieved and therefore LC-MS/MS was selected as the optimum measurement tool for this study.

## **1.9 Objectives of the Study**

The objective of the study was to investigate the effect of extrusion on functional components found in artichoke leaf powder and *in vitro* bioaccessibility of selected phenolic compounds after extrusion. The effect of extrusion on total phenolic content and antioxidant activity was studied. The effect of extrusion on cynarin and cynaroside contents, and *in vitro* bioaccessibility of cynarin and cynaroside compounds were investigated.

## CHAPTER 2

### MATERIALS AND METHODS

#### 2.1 Materials

Wheat flour was supplied by Söke Değirmencilik Sanayi ve Ticaret A.Ş. (Aydın, Turkey). Artichoke leaves were purchased from local groceries (Ankara, Turkey). Selected green fleshy outer leaves were washed and allowed to dry at room temperature not exposed to sunlight directly. Dried leaves were ground (Fritsch Industriestrasse 8, D-55743 Idar-Oberstein, Tüv-Cert) and sieved (1 mm, Fritsch). Moisture contents of both flour and artichoke leaf powder was determined by halogen moisture analyzer at 160 °C (MIX-50, AND, Japan).

Samples were prepared having 3%, 6%, and 9% artichoke leaf powder concentrations on dry basis. Final moisture content was adjusted to 20% during mixing (Kitchen aid, Ariston, USA). Measured moisture contents of prepared samples were  $19.73 \pm 0.19$  %. Prepared samples were kept at +4 °C overnight and before extrusion they were allowed to equilibrate at room temperature for 2 hours.

All reagents used in analyses except LC-MS/MS analyses were of analytical grade. Chemicals used in LC-MS/MS analyses were HPLC grade.

Cynarin was purchased from Sigma (Saint Louis, MO, USA), cynaroside was purchased from Extrasynthese (Genay, France) and chlorogenic acid was purchased

from Dr. Ehrenstrofer GmbH (Ausburg, Germany) and used as analytical standards in LC-MS/MS analyses.

## **2.2. Methods**

### **2.2.1. Extrusion**

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

Four heating zones of the extruder was controlled by electrical heating and water cooling. 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 feed into extruder.

The feed flow rate was  $55 \pm 1$  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  $\pm 2$  °C from the set temperatures.

After extrusion, moisture contents of extrudates were  $11.48 \pm 0.45$  %. Extruded samples to be used in analyses were kept in closed bags at -20 °C in the dark until the analysis.

**Table 1** Screw configurations of the extruder

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8 *D* Twin lead feed screws

7x30° Forward kneading elements

4 *D* Twin lead feed screws

4x60° Forward kneading elements

4x30° Reverse kneading elements

2*D* Twin lead feed screws

6x60° Forward kneading elements

4x30° Reverse kneading elements

1 *D* Single lead feed screws

7x90° Kneading elements

2 *D* Single lead feed screws

Die

---

Screw diameter (*D*) = 25 mm.

One kneading element = 0.25 *D*.

### **2.2.2 Extraction of Phenolic Compounds Used in Total Phenolic Content and Antioxidant Analysis**

Extraction was carried out using a modified procedure suggested by Anton et al. (2009). 1g of finely ground extruded product or 1g of raw mixture was mixed with acetone-water (80:20 v:v). Extraction took place by continuously stirring the mixture at 200 rpm/min (JeioTech-Multichannel Stirrer, MS-52 M) for 2 hours in the dark at room temperature. Samples were centrifuged at 3000 g speed for 12 minutes (2-16PK, Sigma Laborzentrifugen, Germany). Centrifuged samples were filtered through 0.45 µm PTFE syringe filter.

### **2.2.3 Total Phenolic Content**

Total phenolic contents of samples were determined by using Folin Ciocalteu method as described by Anton et al. (2009) with modifications. 3 ml of 10-fold diluted Folin Ciocalteu reagent (Merck, Germany) was added to 0.4 ml of extract. After waiting for a time period of 5 minutes, 3 ml of Na<sub>2</sub>CO<sub>3</sub> solution (60 g/L) was added to the mixture. Then the final mixture was allowed to react at room temperature in the dark for 90 minutes. After the incubation period, the absorbance values were measured at 725 nm by using spectrophotometer (Shimadzu, UV-Visible Spectrophotometer, UV-1700, Japan). A calibration curve was drawn with gallic acid (3,4,5-Trihydroxybenzoic acid, Sigma-Aldrich, Germany) with a concentration range of 5 – 50 mg/L which covered the samples measured (Appendix C, Figure 15). Results were reported as mg gallic acid equivalent (GAE) / g dry weight.

### **2.2.4 Antioxidant Activity**

Antioxidant activities of samples were measured by using DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich, Germany), which is a stable radical having the characteristic of color change in the presence of antioxidants, as described in Anton

et al. (2009) with some modifications. 0.8 ml of extract was mixed with DPPH solution (63  $\mu$ M). They were allowed to react at room temperature in dark for 40 minutes. Then, absorbance values were measured at 517 nm by using spectrophotometer (Shimadzu, UV-Visible Spectrophotometer, UV-1700, Japan). A calibration curve was drawn with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) with a concentration range of 0.01 – 0.1 mmol/L which covered the samples measured (Appendix C, Figure 16). Results were reported as mmol trolox equivalent (TE) / g dry weight.

### **2.2.5 *In Vitro* Digestion Analysis**

*In vitro* digestion analyses were done at Food Engineering Department of Ege University, Izmir, Turkey. Digestion process was carried out at three steps as described by Minekus et. al. (2014) with some modifications. Mouth, stomach and small intestine liquids were prepared according to Table 2.

pH values of mouth, stomach and small intestine liquids were adjusted to 7, 3 and 7 respectively.

*Digestion in the mouth:* For each sample to be digested 1g of sample was weighed in falcon tube. 5 ml of distilled water was added to the sample. 4 ml of mouth liquid (Table 2) mixed with sample and water mixture. Then 25  $\mu$ l of CaCl<sub>2</sub> (0.3 M) was added. pH was adjusted to 7 and volume was completed to a value of 10 ml. Final mixture was incubated at 37° C for 2 minutes.

*Digestion in the stomach:* In this step 8 ml of stomach liquid (Table 2) was added to the tube. 5  $\mu$ l of CaCl<sub>2</sub> (0.3 M) was added and pH was adjusted to 3. After that 1 ml of pepsin solution (2000 U/ml) was added and volume was completed to a final value of 20 ml. Then, incubation was done for 2 hours in order stomach digestion to be completed.

**Table 2** Composition of mouth, stomach and small intestine liquids

Compound	Stock Concentration (g L <sup>-1</sup> )	SSF* (pH 7)		SGF* (pH 3)		SIF* (pH 7)	
		Stock Volume (ml)	Concentration in SSF (mmol L <sup>-1</sup> )	Stock Volume (ml)	Concentration in SGF (mmol L <sup>-1</sup> )	Stock Volume (ml)	Concentration in SIF (mmol L <sup>-1</sup> )
KCl	37.3	0.5	15.1	6.9	6.9	6.8	6.8
KH <sub>2</sub> PO <sub>4</sub>	68	0.5	3.7	0.9	0.9	0.8	0.8
NaHCO <sub>3</sub>	84	1	13.6	12.5	25	42.5	85
NaCl	117	2	-	11.8	47.2	9.6	38.4
MgCl <sub>2</sub> (H <sub>2</sub> O) <sub>6</sub>	30.5	0.15	0.15	0.4	0.1	1.1	0.33
(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	48	0.5	0.06	0.5	0.5	-	-

\* SSF, SGF and SIF represent mouth, stomach and small intestine liquids respectively.

*Digestion in the small intestine:* 11 ml of small intestine liquid (Table 2) was added to the tube. 40  $\mu$ l of CaCl<sub>2</sub> (0.3 M) solution was mixed with sample. 2.5 ml of ballast water (160 mM) was added. pH was adjusted to 7 and 5 ml of pancreatin solution (100 U/ml) was added. Volume was completed to a final value of 40 ml. Incubation was done at 37 °C for 2 hours.

After the last incubation period 5 ml of digested sample was taken from each tube and 45  $\mu$ l of Pefabloc® stock solution (500 mM) was added and digestion was stopped. Samples were centrifuged at 10000xg for 15 min. Supernatant (bioaccessible part) was frozen and kept at -20 °C.

*In vitro* bioaccessibilities of cynarin and cynaroside were calculated with the formulas below after LC-MS/MS analyses:

$$\% \text{ Bioaccessibility of cynarin} = 100 \left( \frac{\text{Cynarin amount after digestion}}{\text{Cynarin amount before digestion}} \right) \quad (1)$$

$$\% \text{ Bioaccessibility of cynaroside} = 100 \left( \frac{\text{Cynaroside amount after digestion}}{\text{Cynaroside amount before digestion}} \right) \quad (2)$$

### **2.2.7 Extraction of Phenolic Acids and Flavonoids for LC-MS/MS Analysis**

Extraction of phenolic acids and flavonoids from raw mixtures and extruded samples was based on a method introduced by Takahashi et. al. (2014) with some modifications. 5 g of finely ground extruded product or raw mixture was mixed with methanol. Extraction took place ultrasonic bath (Jeiotech, Lab Companion, US Portable Cleaners) at room temperature for 1 hour in the dark at room temperature. Samples were centrifuged at 15000 g for 10 min (2-16PK, Sigma Laborzentrifugen, Germany). After centrifugation, supernatant was separated, and 15 ml methanol was added to the residue. Then, residue-methanol mixture was sonicated again and centrifuged at 15000 g for 10 min. Supernatants from first and second extraction steps were mixed. Then, combined mixture was centrifuged again at 15000 g for 10 min. Final supernatant was separated and it was completed to a final volume of 50 ml. Centrifuged extracts were first filtered through 0.45 µm PTFE syringe filter, and then, a second filtration through 0.22 µm PTFE syringe filter was done. Each sample was diluted by 1/5 ratio with mobile phase B (CH<sub>3</sub>CN with 1% formic acid). Obtained sample was immediately analyzed via described LC-MS/MS system.

Extraction of phenolic acids and flavonoids from digested samples was done as follows: 0.5 ml of digested liquid sample was taken into two separate Eppendorf tubes. Both tubes were centrifuged at 13000 rpm (22673g) for 90 seconds (Eppendorf, Minispin Plus personal microcentrifuge). 0.4 ml of supernatants were transferred to different tubes, and 0.4 ml of mobile phase B (CH<sub>3</sub>CN with 1% formic acid) was added to each tube. Tubes were centrifuged again at 13000 rpm (22673g) for 90 seconds. After centrifugation, 0.2 ml supernatant from each tube was taken and diluted by adding 0.8 ml of mobile phase B. Diluted samples were centrifuged at 13000 rpm (22673g) for 90 seconds and supernatants were combined. Final supernatant was filtered through 0.45 µm PTFE syringe filter first and then it was filtered through 0.22 µm PTFE syringe filter. Obtained sample was immediately analyzed via described LC-MS/MS system.

### 2.2.8 LC-MS/MS Analysis

A mass spectrometer coupled to an UHPLC gradient pumpset system (SPH1240<sup>TM</sup>, Spark Holland) through an ESI interface with an autosampler (Alias<sup>TM</sup>, Spark Holland) was used in order to determine the amount of cynarin (1,3-di-O-caffeoylquinic acid), cynaroside (luteolin-7-O-glucoside) and chlorogenic acid (3-O-caffeoylquinic acid). The method was adapted based on the method described by Sanchez-Rabaneda et al. (2003). The column was a 2.0 mm x 50 mm C<sub>18</sub> Synergi 4 μm Fusion-RP 80 Å (Phenomenex). It was kept at room temperature. Oven temperature was +4 °C. Mobile phases were (A): H<sub>2</sub>O with 1% formic acid and (B): CH<sub>3</sub>CN with 1% formic acid. The gradient profile of solvent A was as follows (min/%A): 0.0/0, 1.0/15, 2.0/20, 2.2/10, 2.5/10, 3.3/0 at a constant flow rate of 200 μl/min.

In order to obtain MS/MS data a 3200 Q TRAP mass spectrometer (AB Sciex) was used. Turbo Ionspray source in negative ion mode, multiple reaction monitoring (MRM) mode was used in analyses. Ionspray voltage was -4500 V. Ion source temperature was 550 °C. N<sub>2</sub> was used as nebulizer gas, curtain gas and collision gas. Cynarin was monitored at the transition 515.115 → 191.000, cynaroside was monitored at the transition 447.055 → 285.000 and chlorogenic acid was monitored at the transition 352.966 → 191.100 for quantitative determination. For qualitative analysis cynarin was monitored at the transition 515.115 → 353.100 and cynaroside was monitored at the transition 447.055 → 133.000. Analyst 1.6 software system was used for instrument control and data acquisition.

Limit of quantification (LOQ) was determined by signal to noise ratio (S/N) method. LOQ was assumed as the minimum concentration giving an S/N value of 10. Calibration curves for the standards were prepared before the analyses of each data set separately (with a standard concentration range of 5 - 2000 ng/mL for “before *in vitro* digestion” samples and with a standard concentration range of 0 -

40 ng/mL for “after *in vitro* digestion” samples). Sample calibration curves were given in Appendix C.

### **2.2.9 Statistical Analysis**

In order to determine whether there is a significant difference between the samples ( $p \leq 0.05$ ) analysis of variance (ANOVA) was used. When there was a significant difference Tukey’s Multiple Comparison Test was applied to determine means which significantly differ from others ( $p \leq 0.05$ ). In statistical analysis Minitab 16 Statistical Software (Minitab Inc., State College, Pennsylvania) was used.

## **CHAPTER 3**

### **RESULTS AND DISCUSSION**

#### **3.1 Effect of Extrusion on Total Phenolic Content**

The results given in Table 3 indicated that ALP addition has a significant effect on total phenolic content; as ALP concentration increased total phenolic content showed an increasing trend. Even though extrusion process decreased total phenolic content (Table 3), extrudates retained 64.6% - 76.5% of their original phenolic content in the study.

The effect of extrusion process on total phenolic content depends on various conditions including extrusion temperature, screw speed, moisture content, and formulation of ingredients. It is possible to achieve optimum processing conditions to retain the highest amount of polyphenols. Although majority of the studies in the literature showed that extrusion adversely affected total phenolic content, in some cases increases in total phenolic content after extrusion were reported.

**Table 3** Total phenolic content of raw mixtures and extrudates for different artichoke leaf powder concentrations

<b>Total Phenolic Content (mg GAE / 10g dry weight)</b>		
<b>ALP Concentration</b>	<b>Raw Mixture</b>	<b>Extrudate</b>
<b>Control</b>	3.39 ± 0.23 <sup>Ad</sup>	2.19 ± 0.18 <sup>Bd</sup>
<b>3%</b>	4.89 ± 0.48 <sup>Ac</sup>	3.59 ± 0.18 <sup>Bc</sup>
<b>6%</b>	6.55 ± 0.60 <sup>Ab</sup>	5.08 ± 0.20 <sup>Bb</sup>
<b>9%</b>	8.18 ± 0.77 <sup>Aa</sup>	6.26 ± 0.59 <sup>Ba</sup>

Results are mean ± SD (n=8). Significantly different values in the same row are followed by different capital letters (A, B) (p≤0.05). Significantly different values of the same column followed by different letters in lowercase (a, b, c, d) (p≤0.05).

The reduction in total phenolic content after extrusion is generally thought to be due to high temperature and shear forces. Moisture content was also reported to have an effect on total phenolic content (Leyva-Corral et al., 2016). Phenolic compounds are not heat-resistant and they are destroyed at high temperatures. High extrusion temperatures may lead to degradation of phenolic compounds or cause structural changes (Obradović et al., 2014). Therefore, phenolic compounds may become chemically less reactive or less extractable due to certain degree of polymerization. (Obradović et al., 2014).

Kowalski et al. (2016) conducted a study by extruding quinoa (*Chenopodium quinoa* Willd.) var. Cherry Vanilla. They investigated the effect of the parameters such as moisture, last zone temperature and screw speed on total phenolic content. Moisture, last zone temperature and screw speed values were 15% - 20% - 25%,

120 °C – 140 °C – 160 °C and 300 – 400 – 500 rpm respectively in separately conducted trials. Except one combination of these variables (15% moisture content, 160 °C last zone temperature and 500 rpm screw speed) total phenolic content decreased significantly after extrusion. Results showed that extrusion decreased the total phenolic content; however an optimum combination of process parameters could be achieved to maximize the total phenolic content of extruded products.

Total phenolic content of extruded products produced from a mixture of oat flour, potato starch and apple pomace significantly decreased at different moisture content and temperature values (Leyva-Corral et al., 2016). The decrease in phenolic content were explained by the combination of the effects related to feed moisture content with high shear forces and temperatures used during extrusion (Leyva-Corral et al., 2016).

Sharma et al. (2016) conducted a study on production of pineapple fruit leather by using a single screw extruder. Lower temperatures, between 60°C and 100°C, and screw speed values, between 50 rpm and 150 rpm, provided relatively higher total phenolic content in final product. They reported that increasing temperature and screw speed adversely affected the total phenolic content. They suggested that at higher temperatures decarboxylation occurring in phenolic acids could cause a decrease in total phenolic content. Increasing screw speed also caused an increase in temperature and the adverse effect of process on phenolic compounds increased.

In a study of Wani & Kumar (2016) extrudates produced from fenugreek seed, fenugreek leave powder, oats, dried green pea, rice and corn flour showed a lower total phenolic content than the raw mixture. They selected temperature, feed moisture content and screw speed as 100 °C, 12% and 200 rpm respectively. Similar to the other mentioned studies destruction of the phenolic compounds were explained by the heat treatment.

Morales et al. (2015) found that extrudates produced from lentil flour enriched with fiber had higher total phenolic content than the raw mixture. In the study, different formulations and different sources of fiber were used to enrich lentil flour to be used in extrusion process. Samples containing only lentil flour with no additional fiber did not show a significant difference before and after extrusion. However, fiber added extrudates had a higher total phenolic content than the raw mixtures. Extrusion showed a positive effect on total phenolic content. According to Morales et al. (2015) the reason may be that hydrolysis of polyphenols bound to fiber and/or proteins occurred and the extraction rate of the polyphenols increased. This resulted in a higher total phenolic content in all samples after extrusion.

In a study of milled fractions of black rice (rice bran, brown rice and polished rice) Ti et al. (2015), the total phenolic contents of extrudates produced from rice bran, brown rice and polished rice were investigated. It was reported that the total phenolic content of rice bran increased after extrusion; however, the total phenolic contents of both brown rice and polished rice samples were significantly lower than the raw samples. It was suggested that this was mainly because of the different phenolic compositions and different matrices of rice bran, brown rice and polished rice.

### **3.2 Effect of Extrusion on Antioxidant Activity**

The antioxidant activities of both raw mixtures and extruded samples are listed in Table 4. The antioxidant activity of the samples increased significantly as ALP concentration increased. According to data, except for the 9% ALP containing samples extrusion did not significantly affect antioxidant activity.

**Table 4** Antioxidant activity of raw mixtures and extrudates for different artichoke leaf powder concentrations

<b>Antioxidant Activity (<math>\mu\text{mol TE} / 10 \text{ g dry weight}</math>)</b>		
<b>ALP Concentration</b>	<b>Raw Mixture</b>	<b>Extrudate</b>
<b>Control</b>	$7.20 \pm 3.14^{\text{Ad}}$	$6.92 \pm 2.80^{\text{Ad}}$
<b>3%</b>	$14.47 \pm 2.38^{\text{Ac}}$	$12.18 \pm 2.37^{\text{Ac}}$
<b>6%</b>	$21.36 \pm 1.94^{\text{Ab}}$	$19.16 \pm 2.02^{\text{Ab}}$
<b>9%</b>	$29.54 \pm 2.35^{\text{Aa}}$	$24.38 \pm 2.50^{\text{Ba}}$

Results are mean  $\pm$  SD (n=4). Significantly different values in the same row are followed by different capital letters (A, B) ( $p \leq 0.05$ ). Significantly different values of the same column followed by different letters in lowercase (a, b, c, d) ( $p \leq 0.05$ ).

Phenolic compounds are antioxidants, but reduction in phenolic content does not always lead to a decrease in antioxidant activity. Antioxidant activity of extruded products does not only depend on the quantity of bioactive substances. The composition of bioactive substances also has a substantial effect on antioxidant activity (Sharma et al., 2016). It is well known that there are phenolic compounds which do not act as antioxidants in antioxidant activity assays or a part of antioxidant activity might be masked by flavonoids and proteins (Altan, McCarthy, & Maskan, 2009). There are studies showing correlation or no correlation between total phenolic content and antioxidant activity. For example, in the study of Altan et al. (2009), it was reported that there was not a correlation between antioxidant activity and total phenol content of extrudates. However, Gui & Ryu (2014) reported that total phenolic content and antioxidant activity of products increased after extrusion and showed a strong correlation. They stated that increase in total

phenolic content led to an increase in antioxidant activity. They also reported that the increase in antioxidant activity of extruded white ginseng powder was higher than that of red ginseng powder. It showed that content of the samples had an effect on alteration of antioxidant properties of extruded materials. Similarly, Chalermchaiwat et al. (2015) reported that both total phenolic content and antioxidant activity of brown rice increased after extrusion. They concluded that increase in total phenolic content caused increase in antioxidant activity.

Leyva-Corral et al. (2016) stated that extrusion process decreased the total and individual phenolic content of extruded materials composed of oat flour, potatoes starch and apple pomace. Although phenolic content was affected negatively, they observed a slight increase in antioxidant activity. This slight increase in antioxidant activity was associated with the formation of new antioxidative compounds due to Maillard reactions. It was also reported that among all products the extrudates having the highest total phenol content showed the highest antioxidant activity.

According to a study of Ti et al. (2015) antioxidant activity of rice bran increased after extrusion while antioxidant activity of polished and brown rice samples decreased. They suggested that antioxidant activity of rice bran increased due to the release of bound bioactive compounds and formation of Maillard reaction products after extrusion. Decreasing antioxidant activity values of polished and brown rice were explained with the decrease in phytochemicals such as phenolics and anthocyanins. The samples were differently affected by the extrusion treatment because they had different phenolic compound profiles and different food matrices which could affect extractability of phenolic compounds.

In a study of Wani & Kumar (2016) a mixture of oat flour, dried green pea flour, fenugreek leaf powder and fenugreek seed powder were added to rice and corn flour for extrusion treatment. After extrusion process, antioxidant activity was found slightly higher than calculated value by considering the percentage contributions of raw materials. They suggested that pigments formed during Maillard reaction were

responsible for this increase. They also reported that heat treatment had an increasing effect on antioxidant activity.

In a study of Sharma et al. (2012) the effect of extrusion on antioxidant activity of barley extrudates was investigated. Die temperature values of co-rotating twin screw extruder system was between 150 °C – 180 °C and screw speed was 400 rpm. Under these conditions, extrusion led to a significant increase in antioxidant activity compared with raw mixtures. They suggested that antioxidant activity increased after extrusion since thermal processing caused formation of antioxidants and Maillard reaction pigments having antioxidant properties.

Extrusion may increase or decrease antioxidant activity depending on the amount of pigment formation due to Maillard browning reaction and that of degraded phenolic compounds due to thermal treatment. In our study, except 9% ALP added samples, antioxidant activity did not decrease after extrusion (Table 4). This may be because of that amount of antioxidative compound production due to Maillard browning was lower than degraded antioxidative polyphenol amount in 9% ALP added extrudates. For 3% and 6% ALP added samples amount of Maillard browning reaction products were almost equal to amount of destructed phenolic compounds, and we did not observe significant differences between raw mixtures and extrudates.

### **3.3 Effect of Extrusion on Cynarin and Cynaroside Content**

According to the results given in Table 5 and Table 6, extrusion decreased the amounts of cynarin and cynaroside significantly for all ALP concentration levels due to their heat sensitivity. Sample chromatograms were given in Appendix B.

Leyva-Corral et al. (2016) have conducted a study on effect of extrusion process on polyphenolic compounds. They used oat flour, potato starch and apple pomace mixture for producing cereal-based snacks and reported the effect of extrusion on

individual phenolic compounds chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, rutin and phlorizin. All these compounds are chemical relatives of cynarin and cynaroside coming from either hydroxycinnamic acids group or they are flavonoids. It was reported that extrusion process led to a reduction in quantity of all these phytochemicals which is consistent with our study (Leyva-Corral et al., 2016).

Sharma et al. (2016) reported that extrusion process decreased total flavonoid content during pineapple fruit leather production. According to Sharma et al. (2016), during extrusion the synergistic effects of friction and high temperature may lead to degradation of chemical structure of flavonoids after extrusion.

**Table 5** Cynarin contents of samples before and after *in vitro* digestion and their % *in vitro* bioaccessibilities.

	Cynarin Content Before Digestion (µg / g dry weight)		Cynarin Content After Digestion (µg / g dry weight)		% <i>in vitro</i> Bioaccessibility	
ALP Concentration	Raw Mixture*	Extrudate*	Raw Mixture**	Extrudate**	Raw Mixture**	Extrudate**
Control	<LOQ	<LOQ	<LOQ	<LOQ	-	-
3%	14.25 ± 1.59 <sup>Ac</sup>	3.74 ± 0.34 <sup>Bc</sup>	0.62 ± 0.21 <sup>Bb</sup>	1.17 ± 0.08 <sup>Ab</sup>	4.53 ± 1.30 <sup>Ba</sup>	32.85 ± 2.68 <sup>Aa</sup>
6%	28.86 ± 2.75 <sup>Ab</sup>	6.09 ± 0.70 <sup>Bb</sup>	0.93 ± 0.18 <sup>Bab</sup>	2.07 ± 0.49 <sup>Ab</sup>	3.11 ± 0.57 <sup>Ba</sup>	36.09 ± 9.28 <sup>Aa</sup>
9%	44.56 ± 4.37 <sup>Aa</sup>	9.61 ± 0.97 <sup>Ba</sup>	1.34 ± 0.40 <sup>Ba</sup>	4.36 ± 0.83 <sup>Aa</sup>	3.15 ± 0.93 <sup>Ba</sup>	47.22 ± 10.35 <sup>Aa</sup>

Results are mean ± SD (n=4)\*. Results are mean ± SD (n=3)\*\*. Significantly different values of the same row are followed by different capital letters (A, B) (p≤0.05). Significantly different values of the same column followed by different letters in lowercase (a, b, c.) (p≤0.05).

**Table 6** Cynaroside contents of samples before and after *in vitro* digestion and their % *in vitro* bioaccessibilities.

ALP Concentration	Cynaroside Content Before Digestion (µg / g dry weight)		Cynaroside Content After Digestion (µg / g dry weight)		% <i>in vitro</i> Bioaccessibility	
	Raw Mixture*	Extrudate*	Raw Mixture**	Extrudate**	Raw Mixture**	Extrudate**
Control	<LOQ	<LOQ	<LOQ	<LOQ	-	-
3%	4.23 ± 0.56 <sup>Ac</sup>	2.40 ± 0.42 <sup>Ba</sup>	<LOQ	<LOQ	-	-
6%	7.08 ± 0.63 <sup>Ab</sup>	2.78 ± 0.50 <sup>Ba</sup>	0.49 ± 0.11 <sup>Aa</sup>	1.36 ± 0.54 <sup>Aa</sup>	7.11 ± 1.64 <sup>Ba</sup>	44.68 ± 12.45 <sup>Aa</sup>
9%	8.65 ± 0.84 <sup>Aa</sup>	3.41 ± 0.62 <sup>Ba</sup>	0.54 ± 0.15 <sup>Ba</sup>	1.43 ± 0.21 <sup>Aa</sup>	6.63 ± 2.21 <sup>Ba</sup>	45.25 ± 9.29 <sup>Aa</sup>

Results are mean ± SD (n=4)\*. Results are mean ± SD (n=3)\*\*. Significantly different values of the same row are followed by different capital letters (A, B) (p≤0.05). Significantly different values of the same column followed by different letters in lowercase (a, b, c) (p≤0.05).

In another study of Sharma et al. (2012), it was reported that total flavonoid content of barley also decreased after extrusion cooking. They suggested that since flavonoids are heat sensitive compounds, they were damaged during high temperature extrusion. They observed reduction of flavonoids at two different barrel zone temperature combinations which are 50, 100, 125, 150 °C and 50, 100, 140, 180 °C.

In our study, in addition to cynarin and cynaroside contents, chlorogenic acid amount in the samples was also investigated. Chlorogenic acid data also showed a decreasing trend after extrusion. However, since the confirmation ion could not be detected in LC-MS/MS analysis for chlorogenic acid, the results were only reported in the appendix (Table 7).

### **3.4 Effect of Extrusion on *In Vitro* Bioaccessibilities of Cynarin and Cynaroside**

As shown in Table 5, even though extrusion decreased cynarin content, *in vitro* bioaccessibility of extruded samples were higher than raw mixtures for all ALP concentrations. Changing ALP concentration did not affect *in vitro* bioaccessibility significantly for raw or extruded samples (Table 5). Similar results were obtained for cynaroside. Extruded samples had higher *in vitro* cynaroside bioaccessibility compared to raw samples (Table 6). There was not significant difference between 6% and 9% ALP added samples in terms of *in vitro* bioaccessibility either for raw or extruded samples (Table 6).

There were not specific studies on effect of extrusion on bioaccessibility of artichoke polyphenols. However, the reasons for relatively higher bioaccessibility of phenolic compounds in extruded products could be explained by the studies on food processing and *in vitro* polyphenol digestion.

Parada & Aguilera (2007) stated that food microstructure, especially interactions between phenolics and other constituents, has an important role in bioaccessibility of bioactive nutrients. Presence of molecules that have either positive or negative effect on bioaccessibility and formation of stable compounds manipulate release of molecules from the food matrix. Food processing leads to formation of new microstructures which may cause interactions between phenolic compounds and other components and structures in food matrix (Dueik & Bouchon, 2016).

Bioaccessibility of polyphenols can be affected positively or negatively by high temperature treatment and extrusion (Wang, He, & Chen, 2014). High temperature treatments may lead to degradation of phenolic compounds or they may increase bioaccessibility since they make liberation of polyphenolic compounds easier. Overall effect of process on bioactive compounds depends on which effect is predominant. Wang et al. (2014) suggested that processing technologies may increase bioaccessibility of bound phenolic compounds in cereals by decreasing particle size, damaging the cereal matrix or leading to degradation of fibers. In our study, artichoke leaf powder is the main source for cynarin and cynaroside in extruded products. Gaafar & Salama (2013) reported that artichoke by-products have both free and bound polyphenolic compounds. Artichoke has a high fiber content and the reason of increase in bioaccessibility can be the easier release of cynarin and cynaroside from the matrix due to degradation of fibers after extrusion.

In a study of D'Antuono et al. (2015), *in vitro* digestion of six different isomers of cynarin extracted from artichoke heads was investigated. According to the results isomers including 1,3-di-O-caffeoylquinic acid, 1,4-di-O-caffeoylquinic acid, 4,5 di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, 1,5-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid were found to have different bioaccessibilities. Schütz et al. (2004) reported that isomerization of cynarin could occur during juice production from artichoke. It was also reported that heating processes such as pasteurization and blanching led to isomerization of mono- and di-caffeoylquinic

acids in fresh artichoke and its pomace (Schütz et al., 2004). Therefore, isomerization of cynarin could occur during extrusion since extrusion is a high temperature process similar to pasteurization or blanching. Formation of more bioaccessible isomers could be another reason of higher bioaccessibility of cynarin after extrusion.

In a study of Hole et al. (2013), effect of extrusion on *in-vivo* digestion of phenolic acids in oat and barley was investigated. They reported that in growing pigs bioaccessibility of phenolic acids in extruded oat and barley samples was higher than that of unprocessed samples. They also reported that extrusion is a process which improves bioaccessibility of both free and bound phenolic acids.



## CHAPTER 4

### CONCLUSION AND RECOMMENDATIONS

The extrusion process caused a significant decrease in total phenolic contents of raw mixtures containing 3%, 6% and 9% ALP. In both extrudates and raw mixtures ALP addition significantly increased total phenolic content.

Extrusion process did not significantly affect antioxidant activity of 3% and 6% ALP added raw mixtures while antioxidant activity of 9% ALP added raw mixtures decreased after extrusion process. Increasing ALP concentration increased antioxidant activity of extrudates.

Cynarin and cynaroside contents of raw material significantly decreased after the extrusion process. Cynarin amount increased in raw mixtures and extrudates with increasing ALP concentration. Cynaroside content of raw mixtures significantly increased with increasing ALP concentration; however, for different ALP concentrations cynaroside amounts of extrudates were not significantly different. *In vitro* bioaccessibility percentages of cynarin and cynaroside were higher for extruded products.

In conclusion, the ALP can be added as a functional ingredient to the extrudates. Although extrusion process affected phenolic compounds negatively, *in vitro* bioaccessibility of bioactive molecules were higher after extrusion process. Findings

of the study would be useful in design of food and food processing for functional food industry.

*In vitro* digestibility of nutrients in functional products is important because nutrients can exert their beneficial health effect only when they are bioaccessible. In the literature, there are very few studies on the effects of extrusion on bioaccessibility of phenolic compounds. This study showed that extrusion have a positive effect on *in vitro* bioaccessibility of phenolic compounds. However, *in vivo* studies are required to support findings of *in vitro* studies. In addition, further research on different individual polyphenols will be beneficial in understanding and explaining the relationship between extrusion and digestibility of phenolic compounds.

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## APPENDICES

### APPENDIX A

#### EFFECT OF EXTRUSION ON CHLOROGENIC ACID CONTENT

**Table 7** Chlorogenic acid content of raw mixtures and extrudates at different ALP concentrations

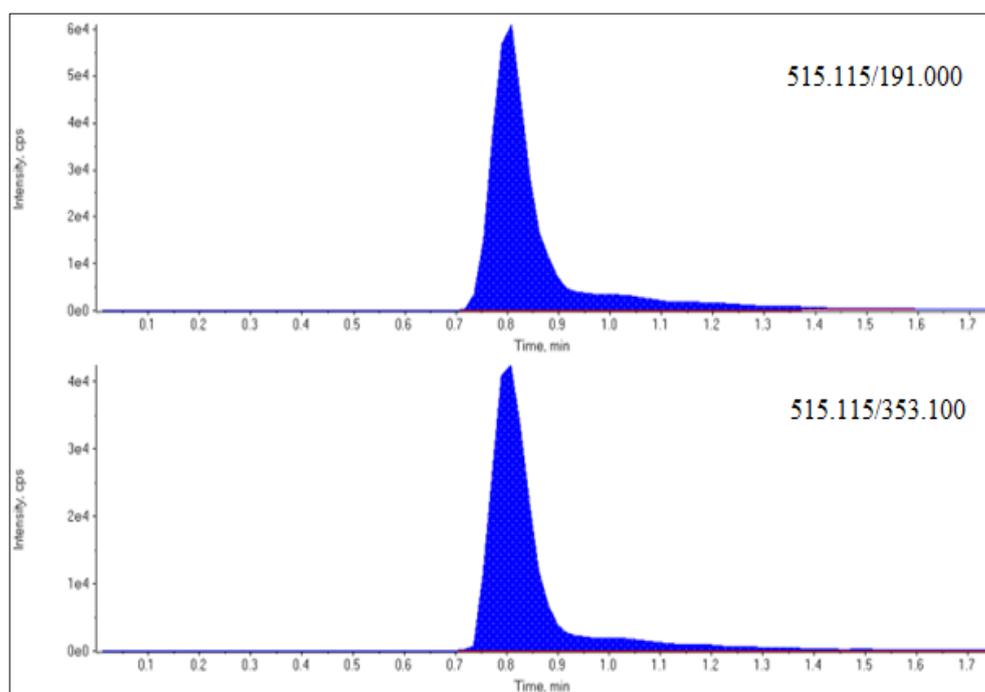
Chlorogenic acid ( $\mu\text{g}$ / g dry weight )		
ALP Concentration	Raw Mixture	Extrudate
Control	<LOQ	<LOQ
3%	$15.98 \pm 2.38^{\text{Ac}}$	$4.83 \pm 0.86^{\text{Bc}}$
6%	$32.73 \pm 5.19^{\text{Ab}}$	$8.12 \pm 0.34^{\text{Bb}}$
9%	$44.46 \pm 7.46^{\text{Aa}}$	$11.64 \pm 0.71^{\text{Ba}}$

Results are mean  $\pm$  SD (n= 4). Significantly different values in the same row are followed by different capital letters (A, B) ( $p \leq 0.05$ ). Significantly different values of the same column followed by different letters in lowercase (a, b, c, d) ( $p \leq 0.05$ ).

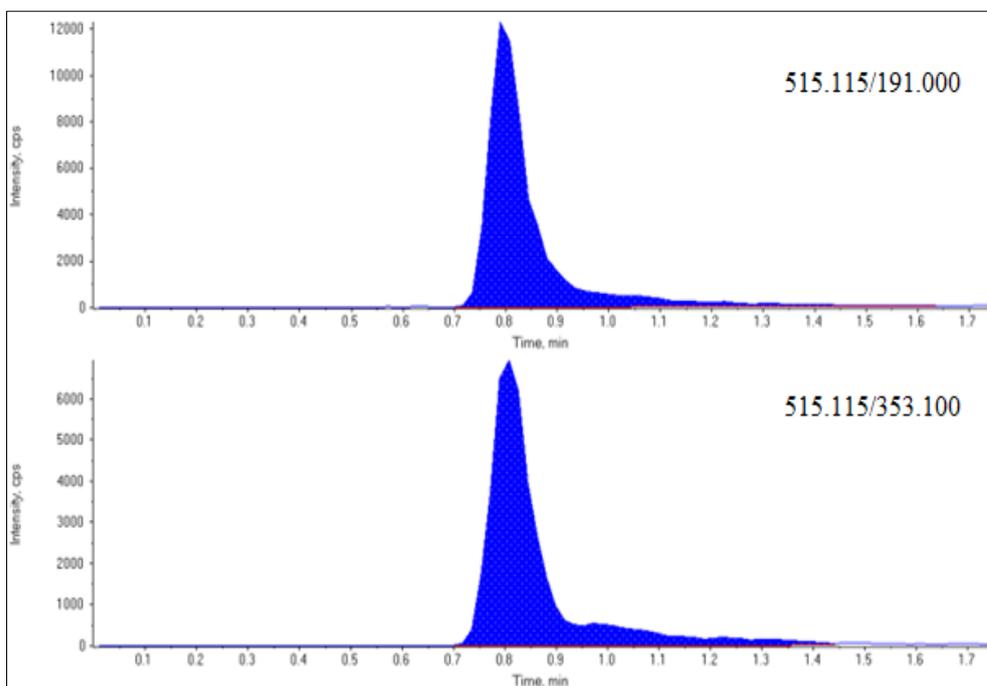


## APPENDIX B

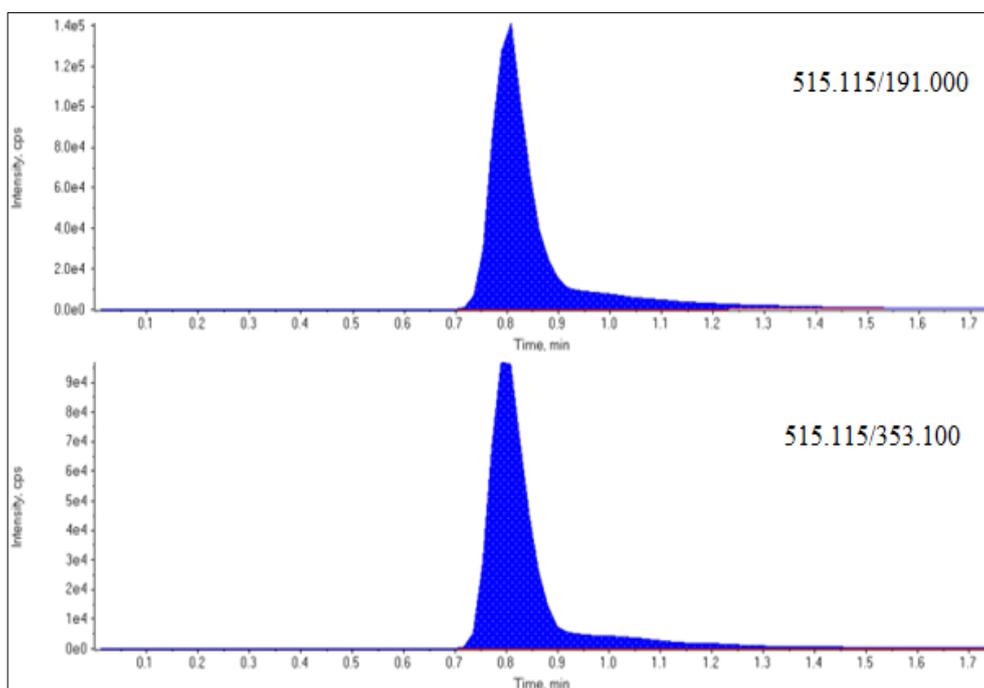
### LC-MS/MS CHROMATOGRAMS



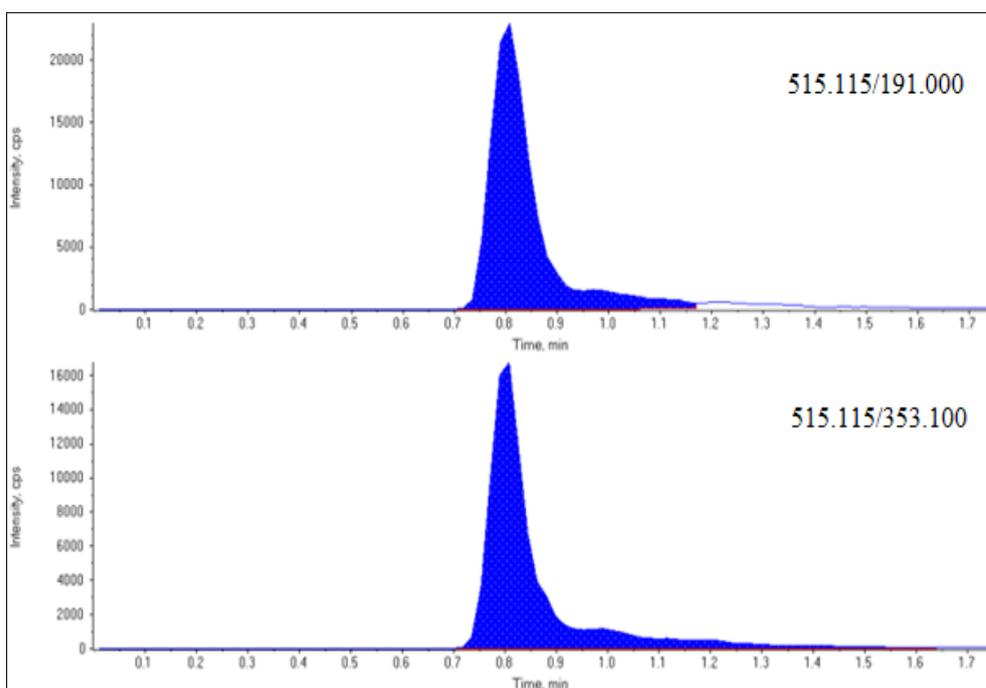
**Figure 3** Sample ion chromatograms of 3% ALP added raw mixture extract for cynarin.



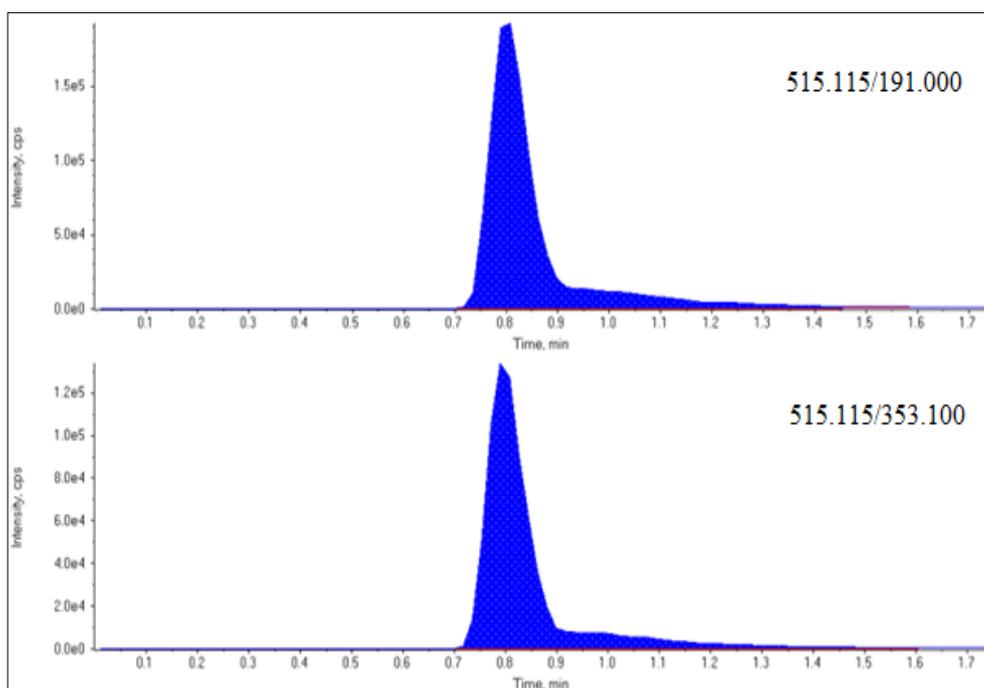
**Figure 4** Sample ion chromatograms of 3% ALP added extrudate extract for cynarin.



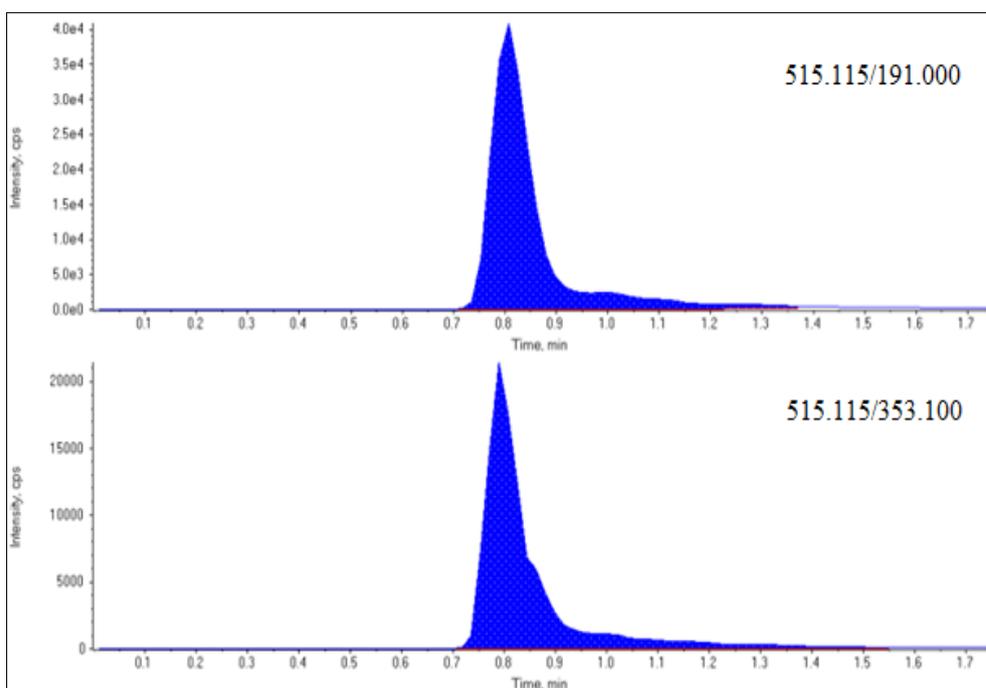
**Figure 5** Sample ion chromatograms of 6% ALP added raw mixture extract for cynarin.



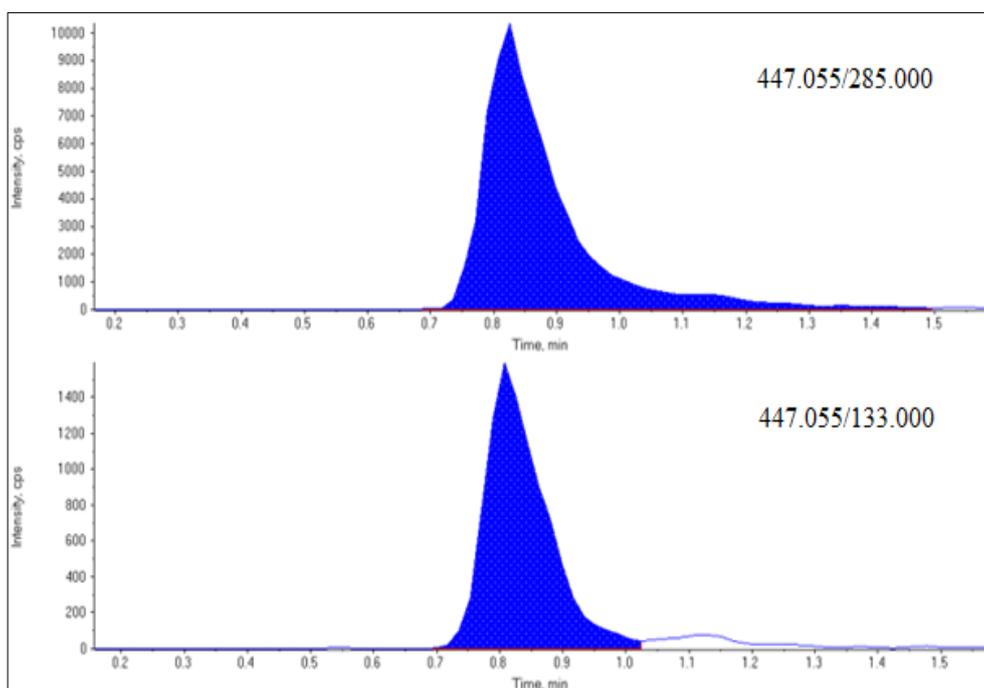
**Figure 6** Sample ion chromatograms of 6% ALP added extrudate extract for cynarin.



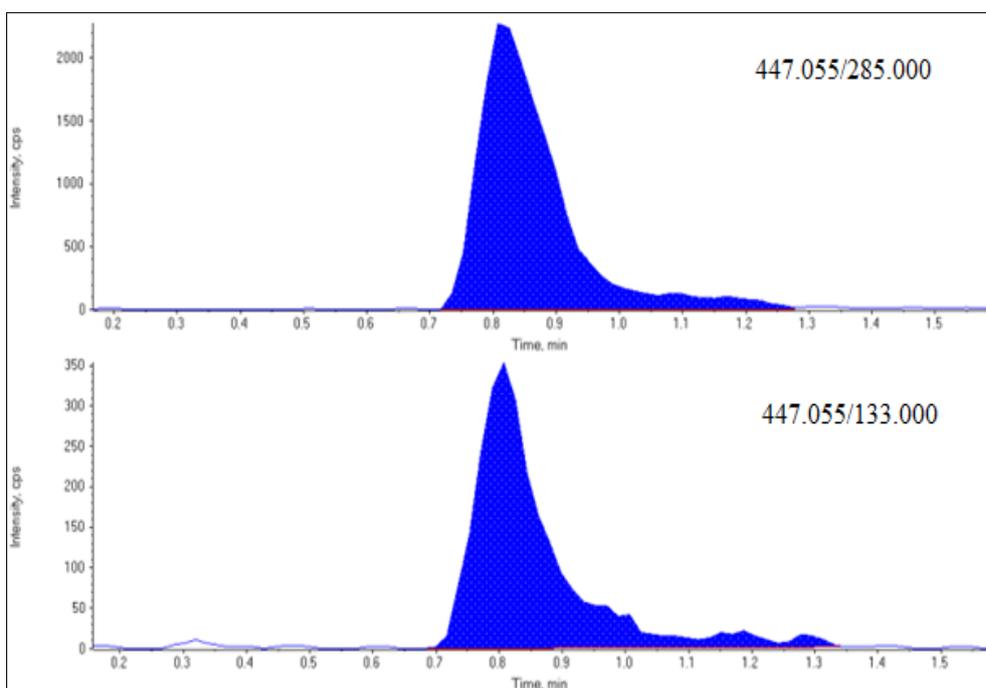
**Figure 7** Sample ion chromatograms of 9% ALP added raw mixture extract for cynarin.



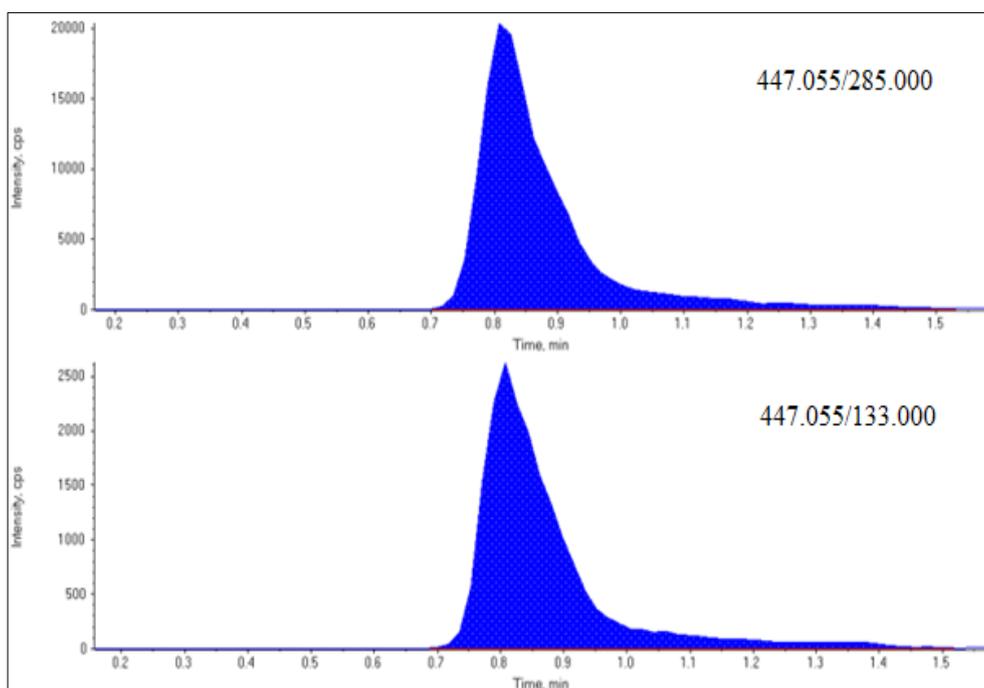
**Figure 8** Sample ion chromatograms of 9% ALP added extrudate extract for cynarin.



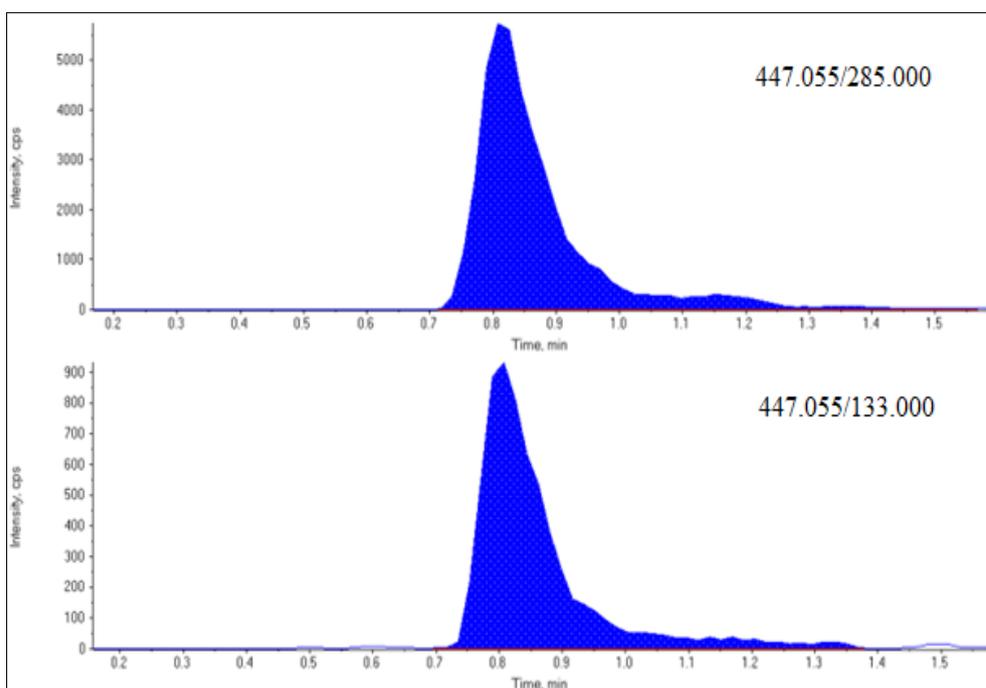
**Figure 9** Sample ion chromatograms of 3% ALP added raw mixture extract for cynaroside.



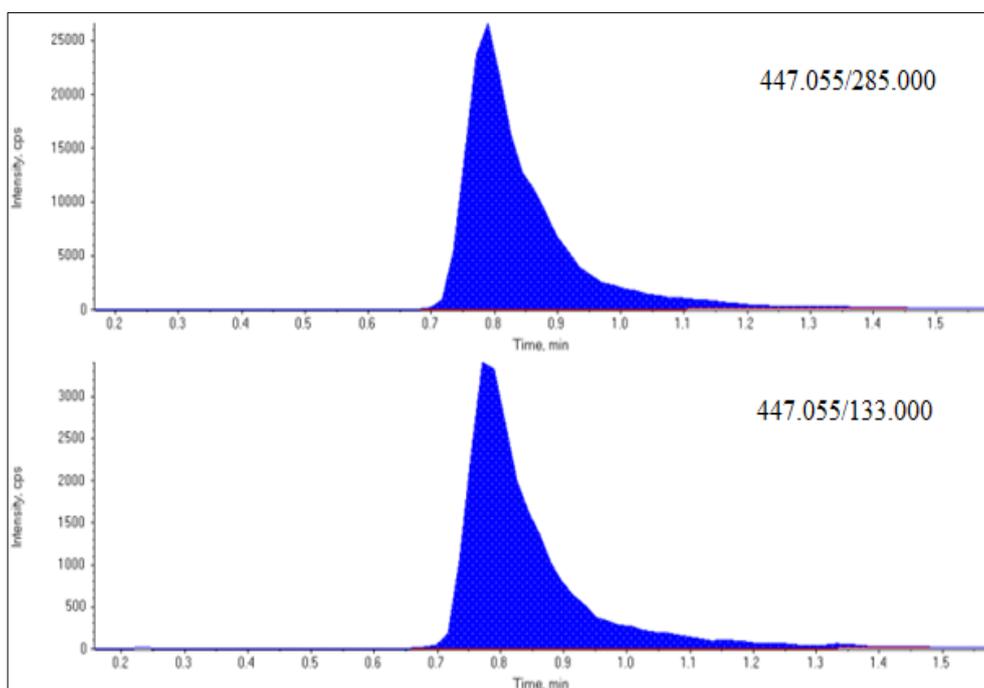
**Figure 10** Sample ion chromatograms of 3% ALP added extrudate extract for cynaroside.



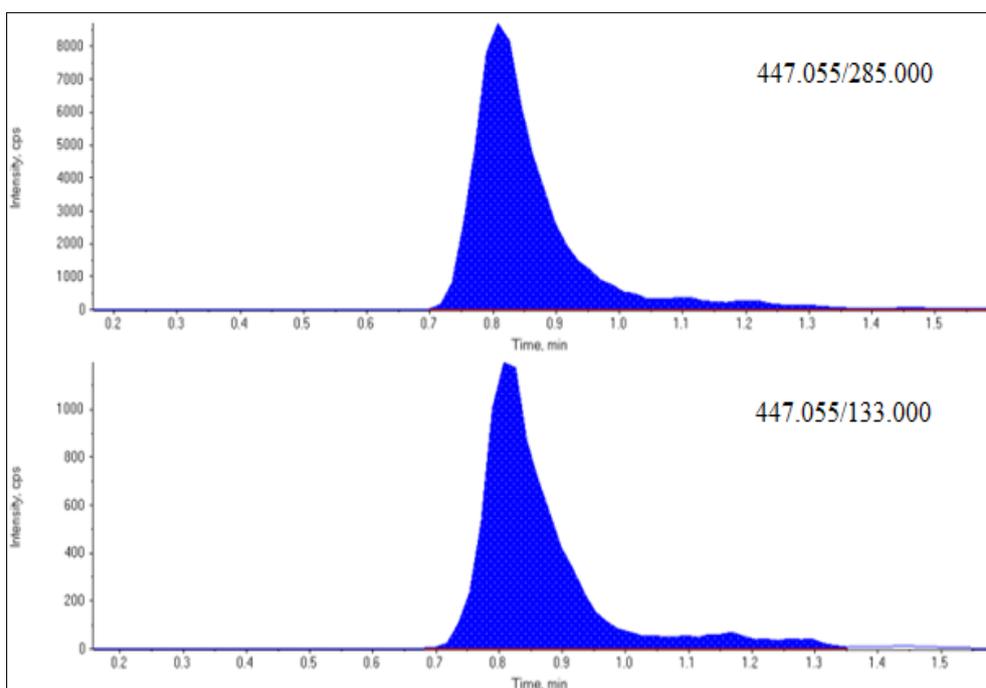
**Figure 11** Sample ion chromatograms of 6% ALP added raw mixture extract for cynaroside.



**Figure 12** Sample ion chromatograms of 6% ALP added extrudate extract for cynaroside.



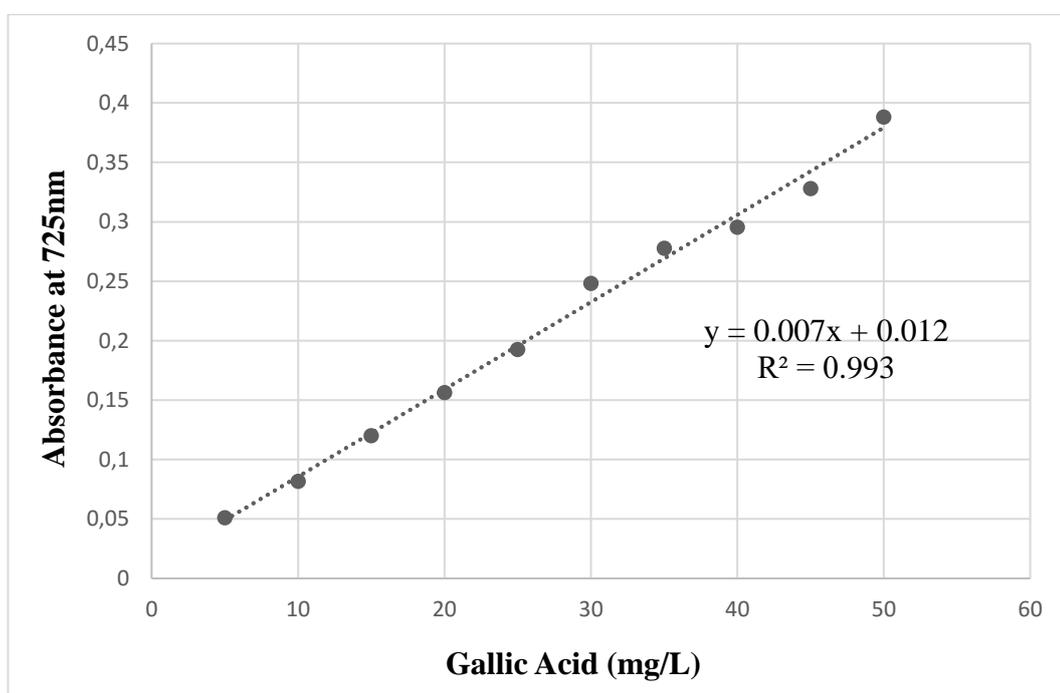
**Figure 13** Sample ion chromatograms of 9% ALP added raw mixture extract for cynaroside.



**Figure 14** Sample ion chromatograms of 9% ALP added extrudate extract for cynaroside.

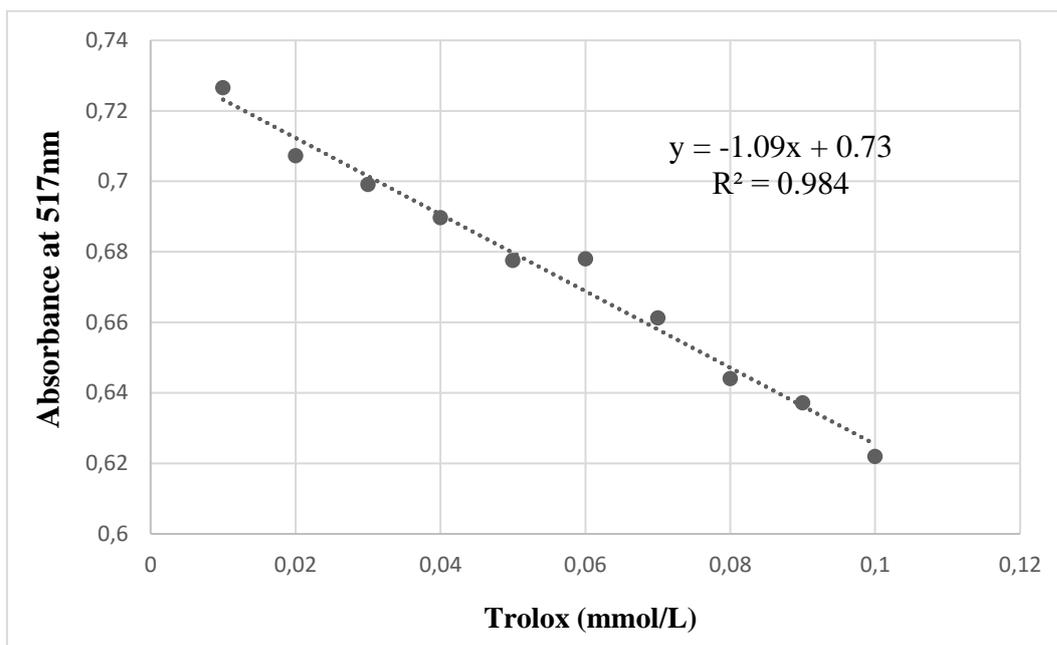
## APPENDIX C

### CALIBRATION CURVES



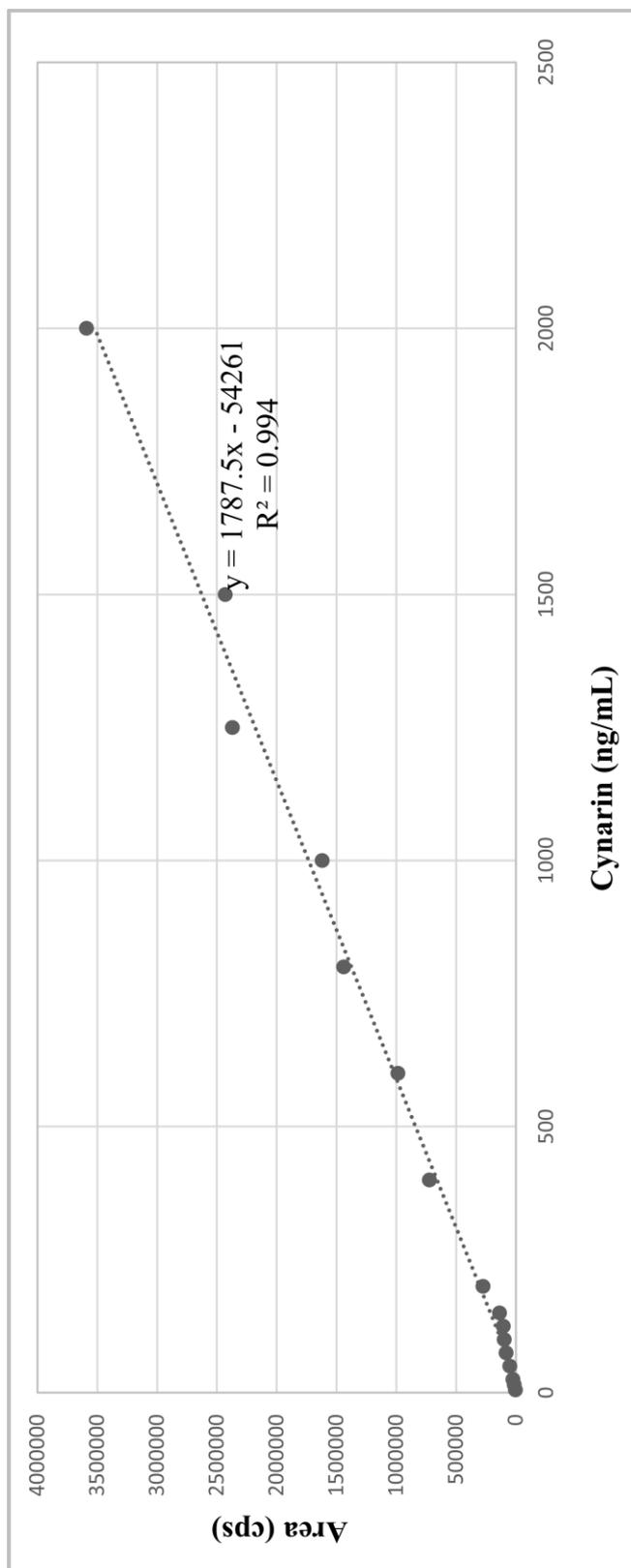
**Figure 15** Calibration curve used for GAE calculation in total phenol content analysis prepared by gallic acid in ethanol.

$$\text{abs ( at 725 nm )} = 0.007 \left( \frac{\text{mg Gallic Acid}}{\text{L}} \right) + 0.012 \quad R^2 = 0.993$$

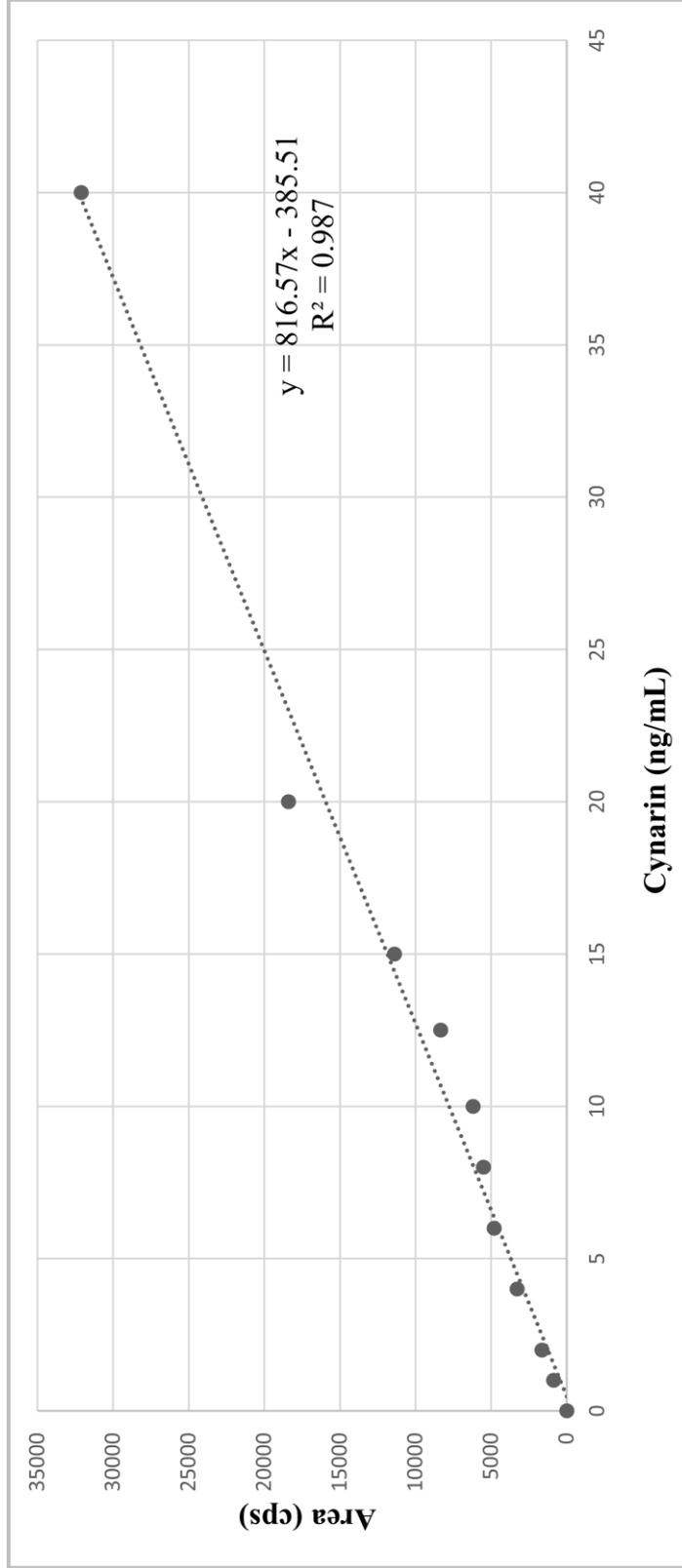


**Figure 16** Calibration curve used for TE value calculation in antioxidant activity analysis prepared by trolox in methanol.

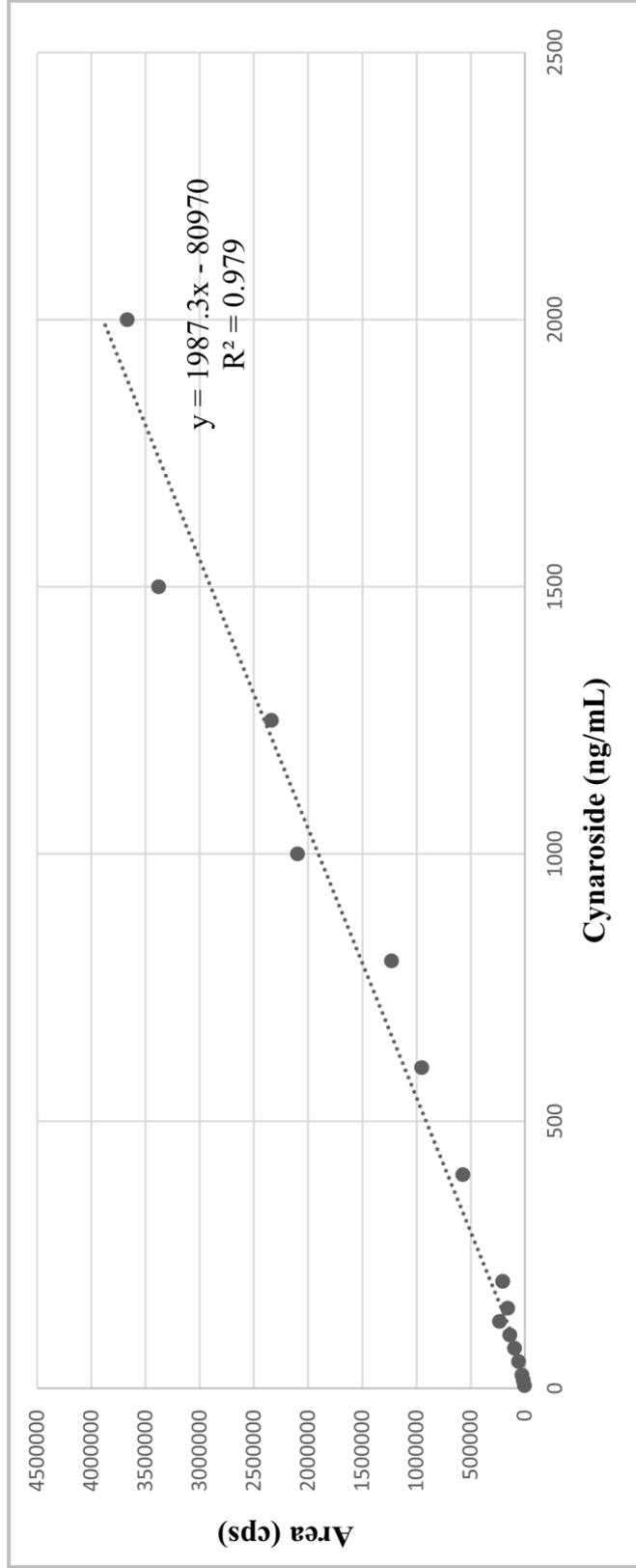
$$\text{abs ( at 517 nm )} = -1.09 \left( \frac{\text{mmol Trolox}}{\text{L}} \right) + 0.73 \quad R^2 = 0.984$$



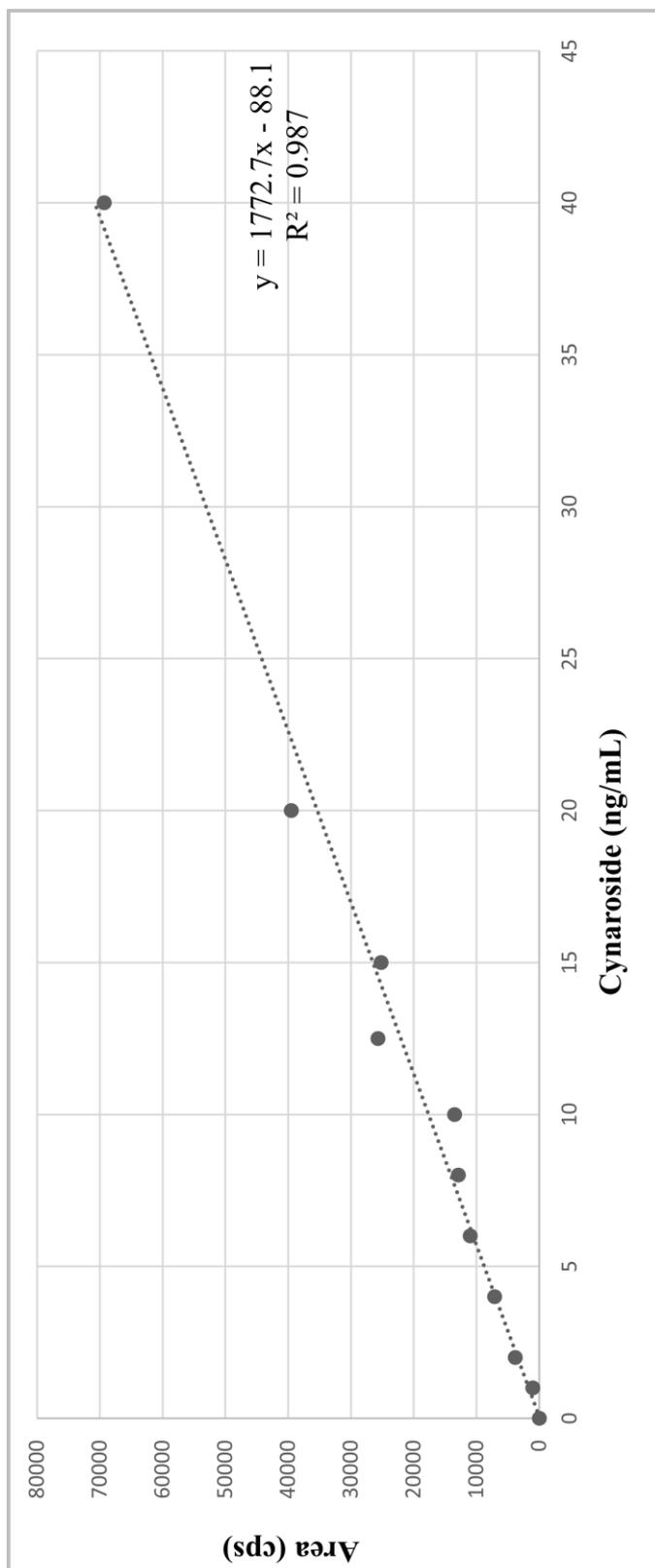
**Figure 17** Sample calibration curve used for quantification of cynarin in raw mixtures and extruded samples before *in vitro* digestion.



**Figure 18** Sample calibration curve used for quantification of cynarin in samples after *in vitro* digestion.



**Figure 19** Sample calibration curve used for quantification of cynaroside in raw mixtures and extruded samples before *in vitro* digestion.



**Figure 20** Sample calibration curve used for quantification of cynaroside in samples after *in vitro* digestion.