EFFECT OF EXTRUSION ON FUNCTIONAL COMPONENTS AND *IN VITRO* BIOACCESSIBILTY OF β-CRYPTOXANTHIN AND ZEAXANTHIN

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ABSTRACT

EFFECT OF EXTRUSION ON FUNCTIONAL COMPONENTS AND *IN VITRO* BIOACCESSIBILTY OF β-CRYPTOXANTHIN AND ZEAXANTHIN

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Red pepper pulp was added as a functional ingredient to the extrudates. Effects of extrusion on the functional properties of the red pepper pulp added corn grit extrudates were investigated. Antioxidant activity, total phenolic content, and the amounts of xanthophyll carotenoids in the product, such as β-cryptoxanthin and zeaxanthin, were determined before and after the extrusion process. *In vitro*

bioaccessibility of these carotenoids from the extrudates and the feed were also investigated. Starch gelatinization (with DSC), pectin, protein, moisture and ash contents were determined for the feed and the product.

Two different temperature profile were applied for the barrel zones during extrusion: 80°C, 90°C, 100°C and 130°C (die: 121°C) and 80°C, 105°C, 130°C and 160°C (die: 142°C). Screw speed (225 rpm) and feed flow rate (36 g/min) were kept constant. Feed moisture content was 25 ± 0.5%.

Total phenolic content of the samples were found to decrease about 41.72% and 47.68% whereas antioxidant activity of the samples were found to decrease about 28.92% and 22.89% after 130°C and 160°C last zone temperature extrusion process, respectively. No gelatinization peak was observed on DSC thermograms after extrusion whereas a peak was observed before extrusion. High performance liquid chromatography (HPLC) analysis showed a decrease in β-cryptoxanthin content about 17.53% and 28.87% whereas a reduction was observed in zeaxanthin content about 49.83% and 40.07% after 130°C and 160°C last zone temperature extrusion process, respectively. There was not statistically significant difference between *in vitro* bioaccessibility of β-cryptoxanthin and zeaxanthin of feed and extrudates.

The results suggest that red pepper pulp can be added as a functional ingredient to develop new functional extruded food products.

Keywords: Extrusion, red pepper, *in vitro* bioaccessibility, β-cryptoxanthin, zeaxanthin

ÖZ

EKSTRÜZYONUN FONKSİYONEL BİLEŞENLER VE β-KRİPTOZANTİN VE ZEAKSANTİNİN *IN VITRO* BİYOERİŞİLEBİLİRLİĞİ ÜZERİNE ETKİSİ

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Kırmızı biber posası ekstrüde ürünlere fonksiyonel bileşen olarak eklenmiştir. Ekstrüzyonun mısır irmiğine kırmızı biber posası eklenmiş ekstrüde ürünlerin fonksiyonel bileşenleri üzerine etkisi incelenmiştir. Üründeki antioksidan aktivite, toplam fenol miktarı ve ksantofil karotenoidler olan β-kriptozantin ve zeaksantin miktarları ekstrüzyon işlemi öncesi ve sonrası araştırılmıştır. Besleme ve ekstrüde

ürünlerde bu karotenoidlerin *in vitro* biyoerişilebilirliği de incelenmiştir. Besleme ve ürünlerin nişasta jelatinizasyonu (DSC ile), pektin, protein, nem ve kül miktarları ölçülmüştür.

Ekstrüzyon işlemi sırasında silindir bölgelerinde iki farklı sıcaklık seti uygulanmıştır: 80°C, 90°C, 100°C ve 130°C (kalıp: 121°C) ve 80°C, 105°C, 130°C ve 160°C (kalıp: 142°C). Vida hızı (225 dev/dk) ve besleme hızı (36 gr/dk) olarak sabit tutulmuştur. Beslemenin nem miktarı %25 ± 0.5 dir.

130°C ve 160°C son bölüm sıcaklığında ekstrüzyon sonrası, örneklerin toplam fenol miktarı sırasıyla %41.72 ve %47.68 azaldığı tespit edilirken örneklerin antioksidan aktivitelerinin %28.92 ve %22.89 azaldığı gözlemlenmiştir. Ekstrüzyon işleminden sonra DSC termogramlarında jelatinizasyon piki gözlenmezken ekstrüzyon işlemi öncesi pik görülmüştür. Yüksek performanslı sıvı kromatografisi (HPLC) analizi 130°C ve 160°C son bölüm sıcaklığında ekstrüzyon sonrası β-kriptozantin miktarında sırasıyla %17.53 ve %28.87 azalma gösterirken zeaksantin miktarında %49.83 ve %40.07 azalma göstermiştir. Besleme ve ekstrüde ürünlerin β-kriptozantin ve zeaksantin *in vitro* biyoerişilebilirliği arasında istatistiksel olarak anlamlı bir fark yoktur.

Sonuçlara göre fonksiyonel özelliklere sahip yeni ekstrüde gıda ürünleri üretebilmek için kırmızı biber posası fonksiyonel katkı maddesi olarak eklenebilir.

Anahtar Kelimeler: Ekstrüzyon, kırmızı biber, *in vitro* biyoerişebilirlik, β-kriptozantin, zeaksantin

Dedicated to my family

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INTRODUCTION

1.1 Snack Foods

Scientists have been focused on the functional foods that are demanded by consumers for the past few years. Some of the ready to eat (RTE) breakfast cereals and snack foods are extruded foods. They have become a part of the daily food intake of many people all around the world. Snacks are ready to eat and cheap foods. Because of that consumption of snack foods in all age groups, including adolescents and children has increased during the last twenty five years. Snacks have become the considerable part of total dietary intake of the adolescents (Sebastian et al., 2008). In Turkey, regular meal consumption is low among the Turkish adolescents and 61% of the Turkish adolescents are skipping regular meals and consume snacks instead (Akman et al., 2010).

Snack foods are the first commercial extruded products and today, food manufacturers produce many foods and consumption of the extruded foods has been increasing. New market and new consuming trend is snacks. However, consumers think that snack foods are not healthy (Guy, 2001). Consumers have been concerned about not only the nutritional quality, but also functional properties of these products. Snack foods are consumed as light meals. The main snack foods are popcorn, chips, crisps, baked or fried snacks and starch-based snacks (Guy, 2001). Corn snack is popular among the extruded foods; however, snack has high glycemic index and it has not enough nutritional value (Conti-Silva et al., 2012; Brennan et al., 2013).

Some scientists studied to develop the nutritional value of the extrudates (Altan et al., 2008a,b, 2009; Anton et al., 2009; Limsangouan et al., 2010).

Rice flour or starch is mainly used for producing extruded snack products; thus, these foods have low protein content, biological value and essential amino acids concentration. Because of this, researchers have been studying on developing extruded foods with cereals and legumes which are higher in protein and lysine content especially corn, red kidney beans and soy to make nutritive foods having essential amino acids for human (Baskaran and Bhattacharaya, 2004).

Researches about digestibility of starches in snack foods demonstrated that snacks have rapidly digestible starch (Goni et al., 1997; Singh et al., 2007). There is a tendency to promote the use of grain incorporated with fibers causing raised slowly digestible starch and resistant starch in snack foods, and compounds beneficial to health; for example, antioxidants. The new generation of snacks should be nutritive and be beneficial to the consumer's health.

Snacks generally include cereals, vegetable proteins and starches. The ingredients give texture, structure, mouth feel, and bulk desired for extruded snack foods (Launay and Lisch, 1983; Tahnoven et al., 1998). Extruded products are accepted by consumers because of the attractive appearance, value, convenience and texture (Anton and Luciano, 2007).

Recently, investigating the relationship between health and nutrition has become a top priority of researchers. Scientists studied on the antioxidant compounds of vegetables and fruits because of the beneficial effects for health. In these foods, there are important vitamins (A, C and E), polyphenols, dietary fibers, and other antioxidants (Marin et al., 2004). Fruit and vegetables have fibers, minerals and vitamins (A, B, C and K) that provide benefits for human health. Moreover, they contain polyphenols (flavonols and anthocyanins), lutein, zeaxanthin and lycopene which may have positive effects on some chronic diseases (Socaciu, 2007). Cereals play a significant role in human nutrition. Cereals and beans contain beneficial

constituents for health, such as antioxidants and anti-disease factors (Ragaee et al., 2006).

According to Guo (2009), conventional foods have some characteristics which are giving pleasure, providing energy to body and contributing to the health. In addition, functional foods have properties of conventional foods and are able to decrease the risk of diseases. Characteristic of the functional foods are enhancing health benefits and preventing diseases (Jackson and Paliyath, 2011). Functional foods contain some ingredients or external healthful substances which are added to them and have an impact on human health (Roberfroid, 2000).

Recently, adding dietary fiber into snacks has become popular since it has a role of nutrition and health. Scientists have studied on the incorporation of nutrient rich by-products of vegetables and fruits for fiber supplementation and paste, fruit juice, powders, peels, pomace, trimmings and seeds to enhance the functionality of the extruded food (Camire et al., 2007; Stojceska et al., 2008; Yagci and Gogus, 2008; Altan et al., 2008a; Altan et al., 2009; Yagci and Gogus, 2009; Stojceska et al., 2009; Stojceska et al., 2010; Dehghan-Shoar et al., 2010; Limsangouan et al., 2010; Karkle et al., 2012). However, there are little published researches on the influences of extrusion on carotenoids and phytochemicals. The effects of processing on the functional components should be determined to investigate the health promoting benefits (Guy, 2001). The extruded products are good candidates for adding functional value to foods. Incorporation of fruits rich in anthocyanin to cereals during extrusion has been investigated to obtain nutritional extruded foods and develop marketability since natural food colorants are preferred by consumers (Gerdes, 2004).

Snack extruded food is dense in energy; however, it has poor nutritional profile. Therefore, it is good to add functional ingredients to these products. Red peppers with peel can be important functional ingredients due to the presence of specific bioactive compounds such as antioxidants, phenolics, flavonoids, and carotenoids.

1.2 Sweet Red Peppers

Sweet red peppers (*Capsicum annuum* L.) belong to the genus *Capsicum* of the family Solanaceae. They are used as a pungent spice or condiment (red, chilli or cayenne peppers) and as a non-pungent vegetable (sweet or bell peppers, paprika, pimentos). The genus *Capsicum* contains near to 20 species. The large-fruited, non-pungent bell peppers, paprikas and pimentos, together with the small-fruited, extremely pungent bird peppers, as well as most of the Mexican chillies are all included in *Capsicum annuum*. (Macrae et al., 1993).

An inner cavity of the all *Capsicum* fruits containing the seeds is surrounded by an outer wall (pericarp) varied with thickness and moisture content. Water loss is retarded with a highly, impermeable outer cuticle. They are fruits having characteristic color, tangy taste and crunchy texture (Minguez-Mosquera et al., 2008). The color of peppers originates from carotenoid pigments which are produced during fruit ripening. About 30 different pigments have been detected in pepper fruits (Matus et al., 1991). The pigments giving attractive red color of red peppers are oxygenated carotenoids including capsanthin, capsorubin and crypto-capsin which are shown to be free radical scavengers and they are exclusive to *Capsicum* genus (Matsufuji et al., 1998).

Peppers have rich antioxidant content, natural colors, ascorbic acid content, and other antioxidant materials. Genus *Capsicum* has a rich phenolic content (Lee et al., 1995; Howard et al., 2000). Sweet peppers include a rich polyphenol pattern including flavonols, hydroxycinnmates and flavones (Marin et al., 2004). Sweet pepper has been assessed as main sources of vitamin C, non provitamin A carotenoid pigments including lycopene and zeaxanthin, and provitamin A carotenoids. The carotenoids and phenolics contribute to the antioxidant characteristics of sweet peppers (Topuz and Ozdemir, 2007). Ascorbic acid content of fresh sweet peppers is in the range 76-243 mg in 100 g fresh weight basis (Howard et al., 1994). Red pepper is a good source of tocopherols, and more particularly of α-tocopherol (vitamin E) (Koch et al., 2002). Vitamin E has a protective effect against a number of disorders, such as

atherosclerosis, ischaemic heart disease and different tumours (Azzi et al., 2002). Red pepper contains high amount of β-carotene and β-cryptoxanthin (provitamin A) (Minguez-Mosquera and Hornero-Mendez, 1993).

According to Marin et al. (2004), 90.7 mg ascorbic acid, 2.3 mg dehydroascorbic acid and 93.0 mg vitamin C in 100 g fresh weight were found in sweet red pepper (*Capsicum annuum* L.). Moreover, phenolics content of sweet peppers were found as 0.44 mg total hydroxycinnamics and 2.54 mg total flavonoids in 100 g fresh weight. They also stated that the phenolic compounds were mainly located in the peel of the sweet peppers. Furthermore, carotenoid pigments content of sweet red bell peppers were found as approximately 2.29 mg violaxanthin, 1.49 mg β-cryptoxanthin, 4.29 mg β-carotene, 3.00 mg capsorubin, 0.91 mg capsanthin 5-6, 19.89 mg capsanthin, 1.06 mg antheraxanthin, 6.30 mg cis-capsanthin, 2.35 mg cucurbitaxanthin A, 3.66 mg zeaxanthin, 0.35 mg cis-zeaxanthin and 45.59 mg total pigments in 100 g fresh weight.

Red pepper contains non-provitamin A carotenoid, which is zeaxanthin. The predominant pigments of red pepper are the uncommon or species-specific carotenoids that are capsorubin and capsanthin (Rodriguez-Amaya, 2001). Various major carotenoids of red bell peppers which are capsanthin, β-carotene, violaxanthin, cryptoxanthin, capsorubin and cryptocapsin were found 35%, 10%, 10%, 6%, 6% and 4% of the total carotenoids, respectively (Curl, 1962). The peel of the most vegetables and fruits has higher carotenoid content than their pulp (Rodriguez-Amaya and Kimura, 2004).

O'Connell et al. (2007) investigated *in vitro* bioaccessibilities of xanthophyll carotenoids and carotene from some vegetables and fruits with HPLC. They found that the % bioaccessibility of carotenoids of red peppers as about 97%, 29%, 21%, and 77% for lutein, β-cryptoxanthin, β-carotene and zeaxanthin, respectively.

O'Sullivan et al. (2010) found that red chili peppers obtained from Turkey contain 5241.6 ± 538.0 μg β-carotene, 3338.4 ± 221.6 μg β-cryptoxanthin, 1297.9 ± 153.7

μg lutein and 664.8 ± 113.8 μg zeaxanthin in 100g. Bioaccessibility (%) of these carotenoids were found 11.6 ± 1.6 for β-carotene, 30.3 ± 3.0 for β-cryptoxanthin, 67.0 ± 5.0 for lutein and 86.1 ± 13.1 for zeaxanthin.

Increased intake of bell peppers has been associated with reduced risks of prostate cancer (Ambrosini et al., 2008). Peppers have radical scavenging activity (Materska and Perucka, 2005; Sun et al., 2007) and prevent the oxidation of docosahexaenoic acid and cholesterol during heating (Sun et al., 2007). Vitamin C in pepper fruits chelates heavy metal ions (Namiki, 1990), suppresses peroxidation, and reacts with free radicals and singlet oxygen, reducing the risk of arteriosclerosis, some cancers and cardiovascular diseases (Navarro et al., 2006).

Compositions of the red pepper are listed in Table A. 1. in Appendix A.

1.3 Extrusion

Today, extrusion is an important food processing technology since it has been first introduced in the mid-1930's to produce ready to eat snacks and breakfast cereals. Extruders were used in snack food industry for producing corn curls from corn grits in the 1930's (Frame, 1999). This technology was developed for conveying and shaping dough and pastes. Cereal, protein, pet food and feed are processed by using extrusion technology. Extrusion process units have been improved and gained popularity for the last decades. The reasons of popularity of extrusion cooking are versatility of products, lower processing cost, productivity or high throughput and product quality. Another reason is that extrusion is an environmentally-friendly technology owing to processing at low-moisture, not producing important process effluents, reducing environmental pollution and water treatment costs (Guy, 2001).

Extrusion is a very versatile process that combines the mechanical and the thermal treatment to produce products with the desirable structure and texture. In the food and feed industry, extrusion cooking method has lower processing cost than other

cooking methods and it has continuous processing capability (Altan et al., 2009). Extrusion process is economically viable, flexible and versatile; thus, extruder can be used for different applications with minor adjustments of the proses parameters. In the food industry, extrusion cooking is a process that is used to convert protein-based dough into meat replacements and starch-based dough into snacks and breakfast cereals. Energy input is mostly separated into mechanical energy supplied by the main motor and thermal energy provided by heating system (Kokini et al., 1991).

In an extrusion process, food materials are forced to move under shear, mixing and heating through the die that shapes the materials of the food. Extrusion process includes heat and mass transfer and momentum; thus, it is a complex process of food. Extruder operates in a dynamic steady-state equilibrium which means inputs are balanced with outputs. To give desired properties of extrudate, inputs having multiple variables must be set at the correct levels to give the dependent physical conditions and chemical process changes (Guy, 2001).

Extrusion-cooking processes are often called HTST (High Temperature/Short Time) because the feed temperatures of 200°C can be reached during the process while the residence time is very short approximately 5 to 10 s where pressure can be up to 20 MPa. The feed is mixed, compressed, melted and plasticized during process (Moscicki and van Zuilichem, 2011). Extrusion processing at high temperature generally in the range between 100°C and 180°C for a short time provides good products quality by retaining heat sensitive food ingredients. In the extruder, pasta and half-product pellet dough are processed at low temperatures whereas flatbreads and extruded snacks are processed at high temperatures. Pressure is used to control shaping, to increase shearing forces in some screw types and to keep water in the vapor state (Guy, 2001).

Stable and consistent feeding into the extruder is a very important process factor. Inconsistent flow rates of feed results for producing products having large size distribution, variety of texture and poor shape (Frame, 1999).

Screw speed affects the residence time distribution, degree of barrel fill and shear stress on the extruded material. The moisture content of the feed is between 14-20%, screw speed of higher than 250 rpm are normal for the ready to eat cereals, snacks and pet foods (Frame, 1999). Increasing of screw rotation speed results increase of shear rate and decrease of residence time (Kokini et al., 1991). The die pressure and barrel torque change with screw speed. The minimum screw speed is in the range of 70-100 rpm; however, the cost of the extrusion process becomes high for manufacturers and volumetric capacity is highly limited below this level (Frame, 1999).

Recently, extrusion cooking has been investigated broadly for producing different foods such as snack foods, pasta products, ready to eat breakfast cereals, baby foods, dry beverage mixes, texturized vegetable protein, dried soups, and pet foods since extrusion improves digestibility and bioavailability of nutrients (Gu et al., 2008; Singh et al., 2010). Moreover, extrusion processing of cereals and pulses blends studied by many researchers to produce of high fibre and protein, and low fat extruded products (Lazou et al., 2010; Jisha et al., 2010; Frias et al., 2011; Adamidou et al., 2011).

Protein denaturation, starch gelatinization, and inactivation of microbes, enzymes and anti-nutritional factors are occurred by the thermomechanical action during extrusion in a shear environment (Bhattacharya and Prakash, 1994).

During extrusion process of the food, starches are gelatinized, proteins are denatured, growth inhibitors are inactivated, fat-splitting enzymes are destroyed and the food is pasteurized. Therefore, the extruded product has a very good shelf life (Kokini et al., 1991). Snacks produced by the extrusion cooking process have a shelf life for up to 9 months. Extruded snack products of cereals are produced from a foamed matrix, mostly including gelatinized starch at $a_w < 0.4$ (Lillford, 2008).

Extrusion process assures the safety of food or feed by processing food at 130°C or higher temperatures and produces foods having long shelf life. Therefore, extrusion

technology can be an important solution for nutrition problem in less-developed countries (Guy, 2001).

1.3.1 Advantages of the Extrusion Technology

Extrusion technology has some benefits over the traditional food processing methods such as jacketed batch cookers and continuous rotary steam cookers. These advantages are processing many different foods by changing a small ingredient or process parameters of the extruder, providing various shapes, textures, colors and appearances by small changes of the process parameters or hardware, and lowering energy cost. Moreover, cooked flavors are not generated by this technology so that it is a HTST process (Guy, 2001).

Extruder effectively converts electrical energy into heat; thus, extrusion has a low gelatinization cost. Furthermore, the required space and staff per kilogram of product are lower than other cooking methods (Lorenz and Jansen, 1980).

Extrusion offers some advantages to produce diversity of products with different textural benefits which are mouthfeel, expansion and crispiness. Moreover, it is a versatile process and has important properties of high productivity, energy efficiency, low operating cost, and short cooking time (Guy, 2001).

Extruder is comprised of one or two screws carrying the ingredients which are mixed before through the barrel (Guy, 2001). Extruder cooks the feed thermomechanically at high shear stress, temperature and pressure which is formed in the screws and barrel. Then, texture and shape of the cooked melt were formed in the die (Arhaliass et al., 2003).

1.3.2 Types of the Extruder

Extruders are classified according to the amount of generated mechanical energy. Low shear extruder is produced for lowering mechanical energy as much as possible to prevent cooking. This extruder is used for producing some types of snack foods and breakfast cereals, and pasta. High shear extruder is produced to generate high mechanical energy converted to heat for cooking. It is used for making puffed snack foods, breakfast cereals and pet foods (Frame, 1999).

In the 1940's, single screw extruder was designed to produce snacks from cereal grits or flours. Extruder having higher capacity is required for increasing demand for precooked cereals required; therefore, extruder having five ton capacity per hour was designed in the 1960's. In addition to this, numerous news applications were developed such as snacks, infant feeding and pet foods. In the 1970's, foods including two or more components were produced. Twin screw extruders were begun to be used for food processing at the end of the 1970's (Mercier and Feillet, 1975; Harper, 1979; Linko et al., 1981).

There are four most commonly used types of the extruder and choosing the right extruder is very important for successful extrusion. These types are single screw wet extruders, single screw dry extruders, single screw interrupted flight extruders and twin screw extruders (Guy, 2001).

1.3.2.1 Single Screw Extruder

Single screw extruder has been in use since 1940 in food and feed industry (Guy, 2001). Single screw extruders with large electrical drive motors were introduced in the 1940's to make snacks from cereals (Kokini et al., 1991).

Developments of the single screw extruders help to increase efficiency and versatility. Common products produced by the single screw extruder are ready to eat

breakfast cereal, direct expanded corn snack, production of full fat soy, texturized vegetable protein, rice bran stabilization, breading, pet foods (Guy, 2001).

These extruders depend on drag flow to carry feed through the barrel and produce pressure at the die. They do not resemble the positive displacement pump and are drag flow machines. Maize grits have a high coefficient of friction resulting production of drag flow in single screw extruder; therefore, they are extruded at high die pressure. Extruded snacks and breakfast cereals are produced by using this technology (Frame, 1999).

Single screw barrel consists of three processing zones which are feeding zone that has a deep channel, kneading zone and final cooking zone. Water and steam can be injected at the kneading zone in order to make dough, and increase heat transfer in the barrel and viscosity of feed. Material shows rubbery texture and enters final cooking zone that has screw flights which are shallow and short pitch. This zone compresses and pumps the plasticized material to the die. Temperature and pressure rise quickly and reach maximum. The final product that is the extrudate expands owing to vaporization of the moisture since it leaves from the die into a lower pressure. Knife placed at the exit cuts extrudate into desired lengths and shapes (Guy, 2001).

Mixing ability of the single screw extruder is poor; thus, generally feed is premixed and preconditioned with water prior to extrusion process. Preconditioning enhances extrusion process taking advantage of the longer equilibrium time and higher moisture level, prolongs the life of wearing components of the extruder and improves product quality (Guy, 2001).

1.3.2.2 Single Screw Wet Extruder

The most commonly used extruder in the food and feed industries is the segmented screw or barrel single screw extruder. During processing, steam or water is injected

into the extruder barrel means wet. They have cooling and heating jackets. Also, they have higher process capacity of foods than other types of extruders (Guy, 2001).

Single screw segmented wet extruder is easily operated and can be used by less trained operators. Single screw extruder has a cost which is half of the cost of the twin screw extruder. Also, maintenance cost of the single screw is less than twin screw maintenance cost. When compared to dry extruder, wet extruder has higher capital investment and has lower cost of operation. Moreover, dry extruder which needs large drive motor per unit throughput has lower capacity than wet extruder. Furthermore, wet extruder that has more process control produce better shaped products than dry extruder (Guy, 2001).

1.3.2.3 Single Screw Dry Extruder

Extruder that does not need jacket heating, external source of heat and steam injection is called dry extruder. Feed is heated by mechanical friction in this extruder. Feed having moisture content between 10% and 40% can be processed by dry extruders. Dry extruder has the ability of water injection during extrusion process. At the die of the dry extruder, moisture is lost as steam flash-off. Exit temperature of the product and initial moisture level of the feed determine the expansion property of the product (Guy, 2001).

Dry extruders consist of choke plates on the shaft or screw segments and steam-locks to increase heat and shear. These restrictions are arranged with increasing diameter of the screw to the die to produce more shear and pressure to restrict feed material pass through and to flow material back when feed moves through the extruder barrel. If the temperature, pressure and shear increase, the material plasticizes. The higher shear is occurred in dry extruders to create heat when compared to wet extruders (Guy, 2001).

Dry extruders are used for processing food, food recycling, feed and by-products of the feed. However, they are mainly used to prepare oilseeds for screw pressing. Moreover, they are used in processing of cereals, breakfast cereals, snack foods, starches, texturized vegetable protein, aquaculture feed, pet food, animal feeds and animal by-products (Guy, 2001).

Dry extruder requires lower capital investment. It can be fitted all types and sizes of installations. Dry extruder need higher horse power for operating than other types of extruders. Furthermore, it is not flexible as twin screw and wet extruders. Initial moisture level of the feed is critically important and high fat content foods may not be cooked well. Highly viscous materials are hard to process with dry extruders (Guy, 2001).

1.3.2.4 Single Screw Interrupted Flight Extruder

Single screw interrupted flight extruders are also known as expanders. Interrupted flight extruders were developed in the United States in the latter 1950's to process pet foods and cereal products. Interrupted flight extruders are developed from a screw press; therefore, it has different mechanical properties from other types of extruders. Screw press is a more massive and has a high cost but it generates high pressure and has a barrel part which allows oil to separate from solids by flowing away. Generally interrupted flight extruders do not have steam-heated and water-cooled jacket, and heat created by direct steam injection and mechanical shear of the ingredients (Guy, 2001).

Applications of interrupted flight extruders mainly are oilseeds preparation for solvent extraction. They are also used for producing pet foods, floating aquatic feeds, feeds for animals, full fat soybeans, rice bran stabilization, and snack foods in the feed and food industries (Guy, 2001).

Interrupted flight extruders are cheaper than single and twin screw extruders. These types of extruders are the simplest extruder and are operated easily by less trained operators. They are very rugged and have easily replaceable wear sections. Same quality products can be produced for long runs (Guy, 2001).

1.3.2.5 Twin Screw Extruder

An extruder with two equal length screws placed in the barrel is named twin screw extruder. Twin screw extruder was developed for plastic industry initially. In the food industry, they had the popularity between the year's mid-1980's and mid-1990's. Demand of innovative food products by consumers caused adopting twin-screw extruder for producing different extruded foods (Guy, 2001).

Twin screw extruder has three processing zones which are feeding, kneading and final processing zone like single screw extruder (Guy, 2001).

There are two kinds of twin screw extruders which are classified based on the screw rotation direction. They are classified into counter rotating twin screw extruders and co-rotating twin screw extruders. These are also subdivided into intermeshing and non-intermeshing types (Guy, 2001).

1.3.2.5.1 Counter Rotating Twin Screw Extruder

The screws of counter rotating twin screw extruder rotate in opposite directions. In food industry, applications of counter rotating twin screw extruder are limited with low viscosity systems requiring positive displacement pumping. In this extruder high pressure can be generated; thus, expanded cereal products are not produced economically. Additionally, wear becomes very important since high pressure produces large forces deflecting the screws onto the barrel walls (Frame, 1999).

1.3.2.5.2 Co-Rotating Twin Screw Extruder

The screws of co-rotating twin screw extruder are placed side by side and rotate in same direction. Self-wiping type of this extruder is mostly used in the food industry to produce the different kinds of the extruded products (Guy, 2001). It is the most popular device for design food product among food manufacturers (Frame, 1999). This type of extruder is chosen for its high capacity in the food industry (Kokini et al., 1991).

The material is moved from one screw to other screw in co-rotating extruders (Kokini et al., 1991). These extruders are common in the design of fully intermeshing and self-wiping. It is a drag flow machine similar to the single screw extruder. It has a flow mechanism formed from a positive displacement and drag flow (Frame, 1999).

Co-rotating twin screw extruder operates at a higher screw speed as compared to a counter rotating type (Kokini et al., 1991). In addition, co-rotating twin screw extruder presents better conveying and shorter residence time distributions when compared to the single screw extruder. The food ingredients are moved from one screw to other screw. This type of extruder exhibits the most flexibility during producing a variety of foods (Frame, 1999).

1.3.2.5.3 Intermeshing and Non-Intermeshing Type Extruder

Co-rotating and counter rotating twin screw extruders are subdivided into intermeshing and non-intermeshing according to the screws intermesh degree. Non-intermeshing twin screw extruder depends on friction for extrusion. In these types of extruders, pumping and mixing are not positive and do not contribute a positive displacement flow. The screws of intermeshing twin screw extruder partially overlap each other; thus, provide efficient mixing, self-wiping action and positive pumping. Counter rotating extruders have lower mixing capability compared to co-rotating

extruders. Intermeshing twin screw extruder provides a positive displacement flow for pumping the feed through the barrel to the die by rotating the screws (Guy, 2001).

Counter rotating intermeshing twin screw extruder is a positive displacement pump because it forms closed C-shaped chambers for minimizing the mixing and the backflow owing to pressure. In co-rotating extruder, drag flow and positive displacement flow are occurred due to pushing action of the screw in the intermeshing region (Kokini et al., 1991).

1.3.3 Twin Screw vs. Single Screw Extruder

Twin screw extruders are chosen among producers because of their high capability of processing viscous and hard-to-break materials, lower energy consumption and producing more diverse and specialized food products. Only disadvantages of the twin screw extruder are the more complicated design and the cost (Moscicki and van Zuilichem, 2011). The cost of twin screw extruder is at least double price of the single screw extruder. Moreover, maintenance cost of twin screw extruder is higher than maintenance cost of single screw extruder (Guy, 2001). Nowadays, twin screw co-rotating extruders are used more commonly due to their high productivity, good mixing and high screw speed (Moscicki and van Zuilichem, 2011).

Twin screw extruders can be processed highly oily, wet and viscous materials. Fat content of food for operating with single screw extruder is between 12% and 17% whereas food containing internal fat greater than 18% up to 22% can be used with twin screw extruder. In single screw extruder, above 17% fat content reduces friction due to lubrication and prevent transformation of mechanical energy into heat. Twin screw extruder maintains the mechanical energy when processing food containing high fat content. Twin screw extruder runs well under both narrow and wide ranges of moisture content than single screw extruder (Frame, 1999).

Twin screw extruder can handle direct feeding with not only very finely ground ingredients but also many coarse ingredients. Furthermore, it can handle three dimensional and very delicate foods. Twin screw extruders are used to produce candies and sticky caramels which cannot be made by single screw extruders (Guy, 2001). Twin screw extruders can process sticky and difficultly conveying food ingredients thanks to the conveying capability (Frame, 1999).

Single screw extruders are much simpler than twin screw extruders. However, twin screw extruders are more flexible to control the food and process parameters than single screw extruders. Twin screw extruders have the advantages of easy cleaning, faster product changeover and less dependence on rheological properties compared to single screw types. Twin screw extruder is used instead of single screw extruder due to the need of products with small sizes and different shapes (Guy, 2001).

At the end of the twin screw extrusion process, water and steam can clean the extruder barrel and screws from inside because they have two shafts and two screws that swipe each other. Moreover, twin screw extruder is more easily used by inexperienced operators (Guy, 2001).

1.4 Structure of the Extrudate

The structure of the extrudate is occurred by forming a melt fluid from biopolymers and blowing water vapor bubbles into fluid to shape foam. The cell walls of gas bubbles are formed by fluid melts of biopolymers that allow these bubbles to expand because the superheated water is vaporized rapidly at lower pressure. After expansion, temperature decreases rapidly due to evaporation, viscosity increases because of losing moisture and cellular structure rigidifies. After viscosity is rapidly increased, glassy state is occurred (Guy, 2001).

High shear and high temperature extrusion process develops a plastic starch melt. The melt expands when it leaves through the die because of dropping pressure

suddenly and vaporizing of water at high temperatures (Dehghan-Shoar et al., 2011). The physical properties of the extruded products show the suitability of ingredients and effectiveness of the process (Patil et al., 2005).

Inside extrudates, a sponge-like, expanded, porous structure is occurred because of that a lot of tiny steam bubbles are produced after leaving the die due to rapid release of pressure (Suknark et al., 1997). Decreasing porosity at higher temperatures can occur by weakening of structure and raised dextrinization. This was seen when temperature was higher than 150°C (Mendonça et al., 2000).

Nucleation sites allow growth of bubbles in the product. When the extrudate exists from die, it swells because of the elasticity of the melt. After that, bubbles grow in the die or outside the die according to the temperature of the process and type of the feed used. Some bubbles collapse if the wall of bubbles is very thin because it cannot sustain the water vapor pressure. The degree of collapse is related to moisture content and rheological properties of the melt (Kokini et al., 1991).

1.5 Raw Materials of the Extrusion Process

1.5.1 Cereals

In extrusion process different types of the cereals can be used for producing extruded products. These are wheat, maize (corn), rice, oats, barley, rye, triticale and sorghum.

The main composition of cereals is starch. Starch gives structure and texture of extruded food products. In addition, cereals have protein with varying amounts of fiber and fat, and small amount of sugar, vitamins and minerals. Cereals used commonly in snack foods are wheat, oats, rice and corn but other cereals can be used due to flavor acceptance (Frame, 1999). Extruded products produced from cereals contain rich vitamins content (Tiwari and Cummins, 2009).

Cereals are important nutrition and have health benefits for human. They contain antioxidants and anti-disease factors (Ragaee et al., 2006).

1.5.2 Maize (Corn)

Maize (*Zea mays* L.) is known as corn and is found in varieties according to grain morphology and color. There are white, yellow and red maize in nature. Maize grains have two kinds of endosperm (Frame, 1999).

The composition of the maize approximately is consist of the amount of 71-81% starch, 6-10% proteins, 0.8-2% lipid, 12-15% moisture, 0.5-0.7% fibre and 0.5-0.6% ash (Frame, 1999).

The main protein is called zein protein in the maize. This protein swells in water and reacts during extrusion cooking (Frame, 1999).

Corn is the most common cereal used to produce expanded snack products and is used for low cost and has the properties of expanding well in all extruders. Degerminated corn expands better when compared with a whole corn because the amount of oil is lower in degerminated corn. Corn is processed by extrusion in a variety forms from grits to fine flour. Twin screw extruder is more flexible because it can operate with from fine flour to coarse granules (Frame, 1999).

The major carotenoids in corn are zeaxanthin and lutein; on the other hand, β-cryptoxanthin and β-carotene are found in smaller amounts (Rodriguez-Amaya and Kimura, 2004).

The ingredients of different corn products are shown in Table 1. Compositions of the corn are listed in Table A. 2. in Appendix A.

Table 1. Proximate analyses of different corn products adapted from Frame (1999).

Feed	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Crude fibre (%)	Starch (%)
Corn flower, whole	11	7	4	1.5	3	73
Corn meal, degerminated	11-14	6-8	<1	0.3	0.4	76-80
Corn flour, degerminated	11-13	5-7	2.5 max.	0.6 max.	0.5 max.	76-80

1.5.3 Water

Water acts as a plasticizer for the polymers such as starch and protein. Furthermore, water reduces starch and protein interactions and causes decrease in energy input in moisture content 10-25% on a wet weight basis (Frame, 1999).

Moisture level is a critical parameter for extrusion process that affects protein denaturation and starch gelatinization. Generally formulas have moisture content between 20% and 28%. Steam or water used in the preconditioner or extruder barrel in order to soften raw food materials and reduce their abrasiveness (Frame, 1999).

During extrusion, water hydrates and solves the starch and protein polymers. Feed containing more than 10% water level has sufficient water in order to begin to move and slide across each other for polymers. At this water level, physical state of extrudates converts from glassy state to viscous elastic fluid and the mass is heated quickly. If the water content is raised the viscosity decreases, the fluidity of the mass raises and lower mechanical energy is needed by the screw conveying. The heat input is provided mostly by viscous dissipation and friction when moisture level in a starch complex is approximately 25% whereas barrel heating is required to attain temperature higher than 120°C at higher moisture content. If there is excess water in

starch, free water is available; therefore, crystalline structure of starch granules melts and then swells (Guy, 2001).

1.5.4 Starch

The starch granules mainly containing 97-98% dry matter are amylose and amylopectin. Starch polymer is found as glucose units in nature (Guy, 2001). Amylose is a linear polymer of α-D-glucose linked by α-1,4 bonds (Delcour and Hoseney, 2010). Amylose in maize can be high as 70% of the total starch and the highest level is found in the amylomaize. Amylopectin is large branched chain molecules of linear polymers containing 500000 glucose units (Guy, 2001). Amylopectin is a branched polymer where α-D-glucose linked mainly by α-1,4 bonds and 4-5% of the glycosidic α-1,6 bonds (Delcour and Hoseney, 2010). In cereals, general starch composition is a ratio of 3 amylopectin to 1 amylose (Guy, 2001). Starch contains several minor components such as lipid in the level between 0.5-1.0%, phosphorus and nitrogen (Delcour and Hoseney, 2010).

In breakfast cereal, snack food and biscuit markets, the main extruded products are formed from starch. Starch polymers which are available in cereals such as maize, barley, wheat, oats, rice and sorghum are very good at glassy state function and well-expanded structures (Guy, 2001). Starches act as fillers in the extrudates. During expansion, they can raise the nucleation of bubbles within the extrudates. As a result of this they create a finer texture (Frame, 1999).

Most natural starches, having a very large average polymer size, are not proper for optimum expansion. Amylopectin that is most wide polymer gives poor flow characteristics in a bubble cell wall and lower expansion due to large molecular weight whereas amylomaize containing more smaller molecular weight polymer amylose allow more flow in a gas cell wall and high expansion. During extrusion, using high level of mechanical shear can decrease the mean molecular weight. Extrusion process may be affected by the physical size and shape of starch granules.

In the extruder barrel, small starch granule has shorter distance for the heat to increase temperature up to critical melting point; thus, it becomes soft in a very short time (Guy, 2001).

Starches are melted and dispersed into the continuous phase in most extruded products; however, some of the granules remaining in the aggregated form act as dispersed phase at low shear processes. Amlyomaize starches have much more strongly bonded structure. They melt and become soft at higher temperatures than normal starches (Guy, 2001). Amlyomaize starches have higher gelatinization temperatures than common cereals and remain ungelatinized in the melt fluid (Frame, 1999). Starch of maize has a gelatinization temperature around 62-72°C (Delcour and Hoseney, 2010). High amylopectin starches have importantly higher degree of gelatinization when compared with high amylose starches (Kokini et al., 1992).

Starch must be gelatinized to be digested by enzymes of human digestive system, because ungelatinized starch cannot be easily digested by humans. Weaning should eat very digestible foods; however, adults can eat less digestible foods. Starch may be pre-digested during extrusion. Amylopectin branches are broken in the extrusion barrel easily. Molecular weight of the amylopectin and amylose molecules decreases during process. After eating meals, human blood sugar and insulin levels increase rapidly due to rapidly-digested starch. These increases can cause Type II diabetes and insulin insensitivity (Guy, 2001).

Recent studies have demonstrated that consuming excessive gelatinized starch promotes to diabetes, increased blood triglycerides and obesity (Jenkins et al., 1980). Absorbed and slowly digested carbohydrates are important for dietary of diabetes.

Limsangouan et al. (2010) found that resistance starch content of corn grit is the second highest one among the other raw cereals and legumes which are rice starch, red kidney bean flour, broad bean flour, soy flour, black soy flour and green pea flour.

Resistant starch has been known as a functional fiber (prebiotic), and plays a significant role in the digestive physiology of the colon that it bypasses digestion and provides fermentable carbohydrates for colonic bacteria. Furthermore, resistant starch provides a better appearance, texture and feeling in the mouth than conventional fibers (Martinez et al., 1998).

1.5.5 Protein

Proteins consist of chains of amino acids. Albumins, globulins, gliadins and glutenins are found in cereals. Proteins are used to form structure in extrudates. During extrusion cooking protein denaturation exposing enzyme-access sites has been occurred that improves protein digestibility. Denaturation causes loss of activity of enzymes and enzyme inhibitors that are proteins. During extrusion process, low moisture content and high temperature favor Maillard reaction that is the reaction of amino acids and reducing sugar. During shear starch and sucrose, reducing sugars are produced and react with amino acids. Therefore, nutritional value of the protein decreases. Proteins are used to produce extruded product having meat-like properties for replacement of meat in dried foods, pet foods and ready meals (Guy, 2001).

Proteins act as fillers in the extruder. Proteins hydrate and become soft viscoelastic dough during melt transition of the extrusion process. Proteins become soft into small pieces of globular shapes by the shearing forces in the extruder (Frame, 1999).

1.5.6 Fiber

Dietary fibers are the edible parts of the carbohydrates or plants and include oligosaccharides, lignin and polysaccharides. Fibrous material including comprised of hemi-cellulose, lignin and cellulose tend to stay stable and firm. Therefore, they are not decreased in size during extrusion process (Guy, 2001). Consumption of fiber is related to helpful physiological effects such as glucose attenuation, modulation of

blood cholesterol and laxation. Soluble dietary fiber reduces the risk of heart disease. Incorporation of 20-40 g fiber is recommended in the daily diet (Brennan, 2005).

Dietary fiber of vegetables demonstrated higher total dietary fiber content and better soluble or insoluble dietary fiber ratios than cereals (Grigelmo-Miguel and Martin-Belloso, 1999).

As a raw material, fibrous materials act as fillers in the extruder. Outside layer of a grain like bran have fibrous materials which are presented as a large firm particles made up of rigid cellulose cells. During extrusion process, these fibrous materials cannot break down and protect their shape and size. These are significantly important for the shape, texture and expansion of the extruded product (Frame, 1999).

Larrea et al. (2005) stated that extrusion process increased soluble fiber and decreased insoluble fiber. Yanniotis et al. (2007) investigated the influence of pectin with wheat fiber on the structural and physical characteristics of extruded cornstarch. According to their research, fibers increased number of the cells and decreased size of them and pectin reduced expansion ratio and increased porosity of the extrudates. The hardness of extrudates was increased due to fibers effect on cell thickness. Ng et al. (1999) observed that solubility of hemicelluloses and pectic polymers is increased by extrusion cooking due to raise in swelling of the cell wall component.

1.5.7 Oil and Fat

Oils and fats act as lubricants among the screws and particulate matter; therefore, they are important for extrusion process. Foods including less than 10% lipids are extruded because high lipid content decreases slip in the barrel of the extruder that makes extrusion hard. Fats appear as solids and contain crystalline material at ambient temperature; however, in the extruder they become liquids at higher than 40°C temperature (Guy, 2001).

Oils and fats have two main purposes for extrusion processing. The first one is that they have a strong lubricant effect in polymer mix. The second one is modifying the eating qualities of extruded foods (Frame, 1999).

Oils and fat may reduce the applied shear level. Oils and fats provide lubrication interacting particles in feed. Oils cause the reduction of the friction among components in the feed and surfaces of the screw (Guy, 2001).

1.5.8 Other Raw Materials

Sugar, salts, nucleating substances, coloring substances, and flavoring substances can be added during preparation of feed before the extrusion (Guy, 2001). As lubricants, emulsifiers can be added to the formula of feed (Frame, 1999).

1.6 Physical and Chemical Changes During Extrusion

Physical and chemical changes are occurred in the food ingredients through macromixing or micromixing during twin screw extrusion process. Mixing is an important factor for transfer of energy playing a main role in transformation of the material. The parameters effecting quality of the product are material and barrel temperature and pressure which must be controlled during process (Guy, 2001). Extruders provide thermal and shear energy to the food; therefore, significant physical and chemical changes occur during process. During extrusion, complex physicochemical transformations including protein denaturation, gelatinization of starch and hydrogen bond rupture occur (Miller, 1993).

The components except starch prevent formation of air bubbles and limit gelatinization of starch needed for expansion of extrudates and arise less expanded and hard products (Ainsworth et al., 2007; Yanniotis et al., 2007; Brennan et al., 2008). Therefore, extrusion processing at high shear and temperature is required for

decreasing the melt viscosity and producing more expanded extrudates incorporated with tomato (Altan et al., 2008a; Yagci and Gogus, 2008). Fibre hinders formation of gas cell and increases wall thickness of gas cell; thus, harder products were produced (Ainsworth et al., 2007; Altan et al., 2008a; Dehghan-Shoar et al., 2010).

The raw materials undergo some order-disorder transitions during extrusion process and extruder die gives a shape to the food material. These transformations are gelatinization of starch, formation of complex between lipid and amylose, denaturation of protein, and degradation reactions of pigments and vitamins. Therefore, during extrusion process feed becomes viscoelastic dough with the help of the, moisture, heat and shear (Lai and Kokini, 1991; Ilo and Berghofer, 1999). Degradation of crystalline structure of starch and macromolecules are occurred due to high temperature, high pressure and high shear (Guha and Ali, 2002). HTST extrusion process initiates starch gelatinization with the help of the mechanical pressure that damages the fiber barrier and makes easier the accessibility to enzymatic activity. Starch gelatinization gives unique textural and structural features to the food. During extrusion cooking starch gelatinization takes place, after cooling retrogradation occurs. It may promote to resistant starch formation, increasing insoluble fibre (Moraru and Kokini, 2003).

Beneficial effects of extrusion contain increase soluble dietary fibre, degradation of antinutritional factors, protein denaturation and texturization, gelatinization of starch, and decrease lipid oxidation. However, Maillard reactions can decrease the nutritional value of the protein, according to the types and composition of the feed material, and process parameters. In addition, heat labile vitamins may be lost to varying extents (Patil et al., 2005; Singh et al., 2007). High barrel temperature and low feed moisture level cause decrease of the ascorbic acid content after extrusion cooking (Killeit, 1994). It was reported that an important reduction in Vitamin E level of the buckwheat approximately 63% after extrusion (Zielinski et al., 2006).

Highly complex physical and chemical changes take place during extrusion processing of cereals that affect the quality properties of the food. Physical changes

are mechanical mixing, shearing, and disintegration of discrete entities such as starch granules, protein bodies with their lipid sheaths. Moreover, some chemical or molecular changes are occurred during extrusion such as hydration and swelling of 23the starch granules, and loss of crystallinity by gelatinization of the starch. Starch undergoes some changes during extrusion. These are the disruption of the crystalline regions in the granule by a loss of granule integrity and amylose-lipid complexes are formed by cereal starches. Also, polysaccharides are degraded during extrusion process (Kokini et al., 1991). An amylose-lipid complex is formed because of the cereal starches including amylose and natural fatty acids during twin-screw extrusion cooking (Mercier et al., 1980).

Extruded snack foods with different physico-chemical properties are produced from corn, rice and wheat because of the differences of the chemical composition of the grain. Corn and rice have lower protein and higher starch content than wheat. Thus, extruded corn and rice products are softer, and more expanded (Guy, 2001).

Proteins include more chemical groups than polysaccharides; therefore, they are more reactive. Lipid-protein, lipid-carbohydrate interactions and protein-protein interactions occur during extrusion process. Lipid-protein interactions are important for structure stabilization at lower lipid contents and improving its solubility. Extrusion process may increase saturation limit because more sites are exposed after the disruption of aggregates maintained by intermolecular hydrophobic interactions or unfolding of native proteins (Kokini et al., 1991). Grain proteins are denatured during extrusion and this leads to open lose structures after that tannin-protein complexes are formed retaining antioxidant activity (Riedl and Hagerman, 2001).

Interactions between proteins, lipids and carbohydrates are formed during formation and cooking of the dough in the extrusion process. The effect of high energy mixing with high temperature gives enough energy for the mechanisms of interaction between lipids, proteins, and carbohydrates (Kokini et al., 1991).

Oxidation, hydrogenation, isomerization or polymerization that takes place during extrusion can affect the nutritional value of lipids. The amount of hydrogenation and cis-trans isomerization of fatty acids is not nutritionally significant (Camire et al., 1990).

Lipid oxidation results for lowering nutritional value and sensory quality in foods. Extrusion process has a short residence time; thus, lipid oxidation may not be occurred. However, during storage of the extrudates it can be occurred. Moreover, extrusion process can inactivate lipolytic and other enzymes which favor oxidation and oxidation resistant starch-lipid complexes are produced in the extruder barrel. Forming air bubbles in extrudates leads to increase surface area and favors oxidation. Opaque containers packaged under vacuum or nitrogen provide longer shelf life for extrudates (Guy, 2001).

The color of corn based foods can change during the extrusion process, because of the expansion of product and disintegration of pigments and causing color reduction (Berset, 1989) and color formation owing to chemical reactions including caramelisation of carbohydrates, Maillard reaction, and oxidative decomposition products of proteins and lipids (Dworschak and Carpenter, 1980).

1.7 Phenolic Compounds and Antioxidants

Phenolic compounds are the members of flavonoid family and protect against oxidation and disease. Higher barrel temperature and moisture protect free phenolics (Guy, 2001). Natural phenolic compounds in foods act as chelators of metal catalysts, singlet oxygen quenchers and free radical terminators. Antioxidant activity of extrudates is depended on the level and composition of bioactive compounds (Brennan et al., 2011).

Antioxidants are known as important nutraceuticals and provide many health benefits (Valko et al., 2007). Natural antioxidants raise the resistance to oxidative stress and may have an important impact on health (Dimitrios, 2006).

Phenolic acids and flavonoids which are phenolic compounds significantly exist in fruits and vegetables. They have become popular owing to many studies stated that consumption of foods having high polyphenol content is associated with a decreased risk of some cancers, cardiovascular diseases and stroke (Prior and Cao, 2000; Kaur and Kapoor, 2001). Flavonoids have antioxidant activity properties that associated with a decreased risk of some cancers (Czeczot, 2000). Flavonoids have anti-allergic and anti-inflammatory (Seelinger et al., 2008), anti-viral activities (Liu et al., 2008).

1.8 Carotenoids

Color is an intrinsic property of food and quality change results change of color of food. Color has an attraction for the consumers. One of the natural colorants is carotenoids. Light absorbances of most of the carotenoids are in the range of 400 to 500 nm (Socaciu, 2007). Carotenoids naturally are fat-soluble pigments. Above 600 carotenoids except trans and cis isomers of them were identified from natural sources (Rodriguez-Amaya and Kimura, 2004). It is found that only 60 carotenoids exist in the human diet and approximately 20 carotenoids found in human blood and tissues. Humans and animals cannot synthesize carotenoids; thus, plants are the primary sources for human diet (Socaciu, 2007).

The five most prominent carotenoids which are β-carotene, lycopene, α-carotene, zeaxanthin, cryptoxanthin and lutein are found in human blood and tissues. The principal carotenoids present in human tissue and plasma are xanthophyll carotenoids especially β-cryptoxanthin, lutein and zeaxanthin (Delgado-Vargas et al., 2000; Rodriguez-Amaya and Kimura, 2004).

Various fruits and vegetables are consumed daily by human and supply intake of carotenoids. Average intake of carotenoids in the United States is about 6.5 mg/day. The average total carotenoid intake is around 14 mg/day in 7 countries in Europe (Socaciu, 2007).

Today, scientists give importance to carotenoids of vegetables and fruits because of their provitamin A activity and antioxidant activity properties (Olives Barba et al., 2006).

1.8.1 Health Benefits of Carotenoids

A number of studies have demonstrated that a high consumption of carotenoid rich foods such as vegetables and fruits reduces the risk of cardiovascular (Arab and Steck, 2000) and other chronic diseases, certain cancers (Giovannucci, 1999; Nishino et al., 1999; Giovannucci et al., 2002; Cooper, 2004; Kelemen et al., 2006; Rao and Rao, 2007) age-related eye diseases, macular degeneration (Snodderly, 1995; Mayne, 1996; Krinsky and Johnson, 2005), cataracts, atherogenesis, bone calcification, neuronal damages (Cantuti-Castelvetri et al., 2000), and osteoporosis (Rao et al., 2007). Indeed, epidemiological studies have demonstrated that intake of carotenoids has a relationship inversely with risk of laryngeal, lung, stomach and colon cancer (Block et al., 1992; Steinmetz and Potter, 1993; Ziegler et al., 1996; Steinmetz and Potter, 1996). Carotenoids have ability to change growth patterns and inhibit growth in tumor cell lines (Krinsky, 1994). The carotenoids and their antioxidant activity properties provide to the healthful effects of vegetable and fruit consumption (Sies and Stahl, 1995; Caris-Veyrat, 2008). Carotenoids which are major source of vitamin A trap lipid peroxyl radicals and quench singlet oxygen species (Stahl and Sies, 1997) deactivate free radicals (Rodriguez-Amaya, 2001). Carotenoids and capsaicinoids show anticancer properties (Aggarwal et al., 2008; Hwang et al., 2009) and antioxidant activities (Matsufuji et al., 1998; Anandakumar et al., 2008).

Bioavailability and bioaccessibility of carotenoids from vegetables and fruits have been gained interest due to many potential benefits on health associated with intake of carotenoids (Stahl and Sies, 2005). Many researchers focus on the potential health advantages of carotenoids and *in vitro* digestion method have been used to provide information of the bioaccessibility of them.

1.8.2 Health Benefits of β-cryptoxanthin, Zeaxanthin and Lutein

Carotenoids especially β-cryptoxanthin, α-carotene and β-carotene are precursors of vitamin A (Howard et al., 1994; Scott and Rodriquez-Amaya, 2000; Hedren et al., 2002). Deficiency of vitamin A is an important problem in developing countries. Vitamin A is necessary for vision, cell differentiation, normal cell growth and immunological functions (Gerster, 1997). Blindness caused by the deficiency of vitamin A is a main problem in many less-developed countries (Guy, 2001). Deficiency of vitamin A is the main cause of childhood blindness. It affects approximately 250000 children a year in the world and is a leading cause of death. Vitamin A supplementation alone results that infant mortality is deceased by 60% (Sommer, 1993; Fawsi et al., 1993). Vitamin A deficiency known as xerophthalmia, blindness and pre-mature death are common in children. Deficiency of vitamin A is a main nutritional problem in many underdeveloped countries throughout the world. It is important in maintaining growth and reproductive efficiency, maintenance of epithelial tissues and prevention of keratinization, and has an important action in immune response (Scott and Rodriquez-Amaya, 2000)

β-cryptoxanthin has the ability of quenching singlet oxygen (Di Mascio et al., 1989). Zeaxanthin and lutein possess preventive effects against peroxidation of lipid (Stahl et al., 1998).

Zeaxanthin and lutein exist in the retina and protect cells from blue light damage (Snodderly, 1995; Krinsky et al., 2003). Dietary intake of zeaxanthin and lutein has a protective affect against blindness among the elderly and age-related macular

degeneration (AMD) (Seddon et al., 1994; Landrum et al., 1996; Mozaffarieh et al., 2003; Perez-Galvez et al., 2003; Beatty et al., 2004; Ribaya-Mercado and Blumberg, 2004) and formation of cataract (Hankinson et al., 1992; Chasan-Taber et al., 1999; Brown et al., 1999; Olmedilla et al., 2003; Calvo, 2005). AMD which is an eye disease causes severe and irreversible loss of vision in developed countries (Fine et al., 2000). Zeaxanthin and lutein protects photoreceptor cells from light-generated oxygen radicals; therefore, they have significant roles in preventing AMD (Krinsky, 2002; Krinsky and Johnson, 2005).

Lutein and zeaxanthin may protect from retina and lens against UV-induced oxidative damage, absorbing damaging blue light, development of AMD and cataracts (Krinsky and Johnson, 2005).

1.8.3 Structure of the Carotenoids

There are hydrocarbon carotenoids and oxidized carotenoids. Hydrocarbon carotenoids are known as carotenes and formed by only carbon and hydrogen. Oxidized carotenoids are also called xanthophylls or oxycarotenoids which have some O-substituent groups such as hydroxy, epoxy and keto groups (Oliver and Palou, 2000). The most common carotenes are β-carotene and lycopene. Xanthophylls have common oxygen substituents; for example, the β-cryptoxanthin has hydroxy group, canthaxanthin has keto group, violaxanthin has epoxy group, and β-citraurin has aldehyde group. The carotenoids are grouped as acyclic such as lycopene, monocyclic such as γ-carotene, and dicyclic such as α-carotene and β-carotene. Naturally carotenoids present mainly in more stable trans form; nevertheless, minor amounts of cis isomers are also exist (Rodriguez-Amaya and Kimura, 2004). In nature, the xanthophylls carotenoids such as β-cryptoxanthin, zeaxanthin and lutein are found in free, monoester and diester forms (Socaciu, 2007).

Foods carotenoids are usually C_{40} tetraterpenoids which are formed by eight C_5 isoprenoid units and they are symmetrical molecules. All carotenoids are lycopene

($C_{40}H_{56}$) derivatives formed by reactions. The conjugated double bonds playing a role as the light absorbing chromophore; therefore, carotenoids have colors and provide the visible absorption spectrum for their identification and quantification (Rodriguez-Amaya and Kimura, 2004).

1.8.4 β-cryptoxanthin, Zeaxanthin and Lutein

β-cryptoxanthin is found in some vegetables and fruits; for example, red pepper, orange, persimmon, mango, papaya and peach (Su et al., 2002). β-cryptoxanthin and β-carotene exist in lower contents in corn (Rodriguez-Amaya and Kimura, 2004). Müller (1997) found β-cryptoxanthin in some vegetables and fruits including red paprika, potato, kohlrabi, "Josta" berry, raspberry, red currant, grape, nectarine, apricot, cherry (morello), peach, cherry (sweet), apple "Jak. Fischer", apple "Elstar", grapefruit, papaya, orange and lemon. β-cryptoxanthin can trap singlet oxygen species and shows provitamin A activity (Su et al., 2002). β-cryptoxanthin shows approximately half of the vitamin A activity of β-carotene which is the most potent and widespread provitamin A (Rodriguez-Amaya and Kimura, 2004).

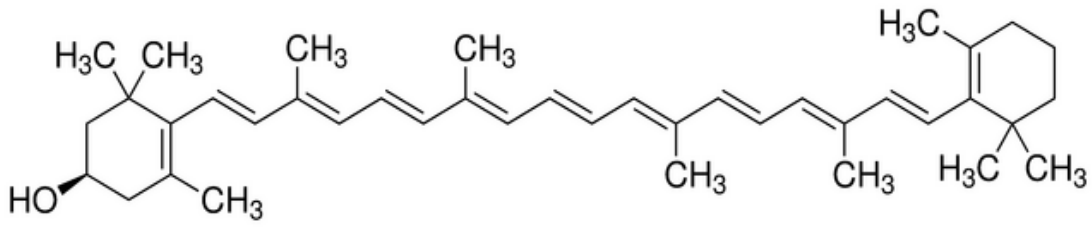

Figure 1. The structure of the β-cryptoxanthin ($C_{40}H_{56}O$, mol wt 552.87).

Lutein and zeaxanthin belong to xanthophylls containing one or more hydroxyl group (Krinsky and Johnson, 2005). Lutein is a major xanthophyll in yellow and leafy green vegetables. Zeaxanthin is found in red peppers, orange, persimmons,

green leafy vegetables and wolfberry. Lutein and zeaxanthin are found in corn, kale, peas, spinach, broccoli lettuce, egg yolk (Socaciu, 2007). Lutein and zeaxanthin are non-provitamin A carotenoids (Rodriguez-Amaya and Kimura, 2004). Müller (1997) found zeaxanthin in some vegetables and fruits including parsley (leaf), spinach, white cabbage, red paprika, carrot (med-sized and young), potato, blackberry, strawberry, raspberry, black currant, grape, nectarine, avocado, apricot, papaya, peach, pear and banana.

Zeaxanthin and lutein exist in the retina at the macula lutea, plasma, ovaries and adipocyte tissue and they give yellow color of the macula (Krinsky and Johnson, 2005).

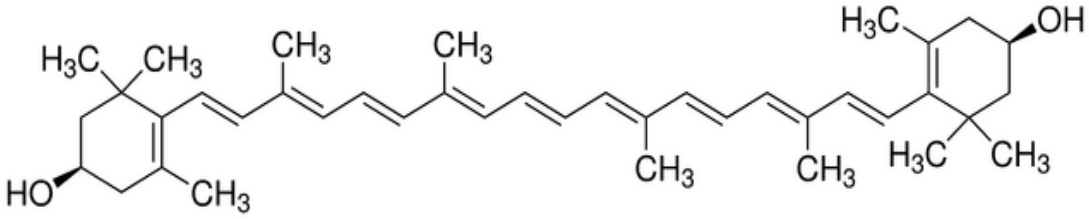

Figure 2. The structure of the zeaxanthin ($C_{40}H_{56}O_2$, mol wt 568.87).

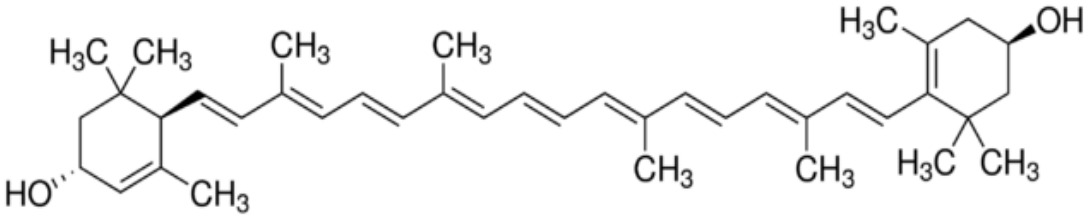

Figure 3. The structure of the lutein ($C_{40}H_{56}O_2$, mol wt 568.87).

1.8.5 Stability of Carotenoids

Carotenoids are naturally protected by the cellular structure in plant tissues. Shredding, cutting and pulping of fruits and vegetables cause increasing exposure to oxygen and coming together enzymes and carotenoids that catalyze oxidation of

carotenoids (Rodriguez-Amaya and Kimura, 2004). Carotenoids have conjugated double bond; for this reason, they are instable towards oxygen, light and heat. Therefore, to ensure maximum retention and prevent loss of carotenoids some precautions should be taken during handling and storage (Oliver and Palou, 2000).

Degradation of labile nutrients such as carotenoids is caused by processing. Carotenoids are susceptible to degradation because of the conjugated double bonds at high temperature, presence of light and reactive oxygen species and at low pH. Degradation cause decreasing of the active carotenoids, transforming into cis isomers and changing properties with different colors. In general, trans isomers are thermodynamically more stable than their cis forms due to an increased tendency to crystallization (Socaciu, 2007).

1.9 Bioavailability

Bioavailability is known as the ratio of the ingested compound absorbed and available in the bloodstream for utilizing in normal physiological function or storage (Jackson, 1997). A nutrient has to be bioavailable to be beneficial to health; that is, it becomes bioaccessible or released from the food matrix during digestion (Faulks and Southon, 2005).

Bioavailability includes three *in vivo* processes. The first one is bioaccessibility that is the release of the compound from food and its solubilization in the gut lumen. The second one is absorption that is uptake of the compound by the intestinal cell followed by its secretion into the blood circulation. The last one is circulation of the compound in the bloodstream and its delivery to the tissues to be utilized and stored for biological activities. Compounds can be broken down into products of degradation used for specific actions in the body (Socaciu, 2007).

Several distinct *in vitro* and *in vivo* methods can be used in order to access the bioavailability of carotenoid which is a food pigment. *In vivo*, balance, total plasma

responses, postprandial chylomicron responses, isotopic labeling techniques methods can be used. *In vitro*, Caco-2 cell model, *in vitro* digestion approach and *in vitro* digestion and Caco-2 cell model combination model can be used (Socaciu, 2007).

1.9.1 Bioaccessibility

Bioaccessibility (micellarization) is known that the amount of the ingested compound available in the gastrointestinal tract for absorption (Hedren et al., 2002; O'Connell et al., 2007).

There are different factors affecting bioaccessibility of carotenoids and affecting the micellarization of the compound in the gut. The factors of physicochemical properties of compound are configuration, degree of linkage and lipophilic character. The factors for release of compound from food matrix are subcellular location of compound, food processing and type of food matrix. Furthermore, there are intraluminal factors which are bile salts, pH, microflora, and nutrients such as lipids, fibers and other carotenoids (Socaciu, 2007).

1.10 Objectives of the Study

The objective of this study was to investigate the effect of extrusion on functional components of red pepper pulp added corn grits and *in vitro* biaccessibility of β-cryptoxanthin and zeaxanthin.

CHAPTER 2

MATERIAL AND METHODS

2.1 Materials

In this study, feed was prepared from corn grits (*Zea mays* L.) and sweet red pepper (*Capsicum annuum* L.) pulp. Corn grits were gained from Teknik Tarım Ürünleri Ltd. Şti. in Manisa in Turkey. Sweet red peppers were purchased from a local grocery market in Ankara in Turkey.

Red peppers were washed with distilled water, dried and cleaned from their stems and the seeds. Each one was cut longitudinally and put into a blender (Cuisinart CBT700E, Stamford). The pulp was prepared by running blender for about 5 minutes. The moisture contents of the corn grits, pulp and feed were determined with a halogen moisture analyzer (MX-50, AND, Japan) at 160°C. The feed were prepared from corn grits (12.52 ± 0.03% moisture) and red pepper pulp (92.29 ± 0.05% moisture) in a proportion to reach the moisture content of 25 ± 0.5% by mixing in a mixer for 20 minutes (Kitchen Aid, Ariston, USA).

The feed was stored in black plastic bags at 4°C overnight before the extrusion process. The feed material was allowed to equilibrate for two hours at room temperature before extrusion.

All the reagents that were used in the analyses except the ones in HPLC analysis were of analytical grade where the reagents used in HPLC analysis were HPLC grade.

2.2 Methods

2.2.1 Extrusion Process

A laboratory scale co-rotating twin screw extruder with computer control and data acquisition system produced by Feza Gıda Müh. Makine Nakliyat and Demir Tic. Ltd. Şti. in İstanbul in Turkey was used. Extruder, screws and die of the extruder that were used in this study were shown in Figure 4, 5, and 6, respectively. The die diameter and the barrel length to diameter ratio (L/D) were 3 mm and 25:1, respectively. Screw configurations of this extruder was as follows 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 and die. Screw diameter was equal to 25 mm (1 D) and one kneading element was equal to 0.25 D.

Figure 4. Laboratory scale co-rotating twin screw extruder used in the study.

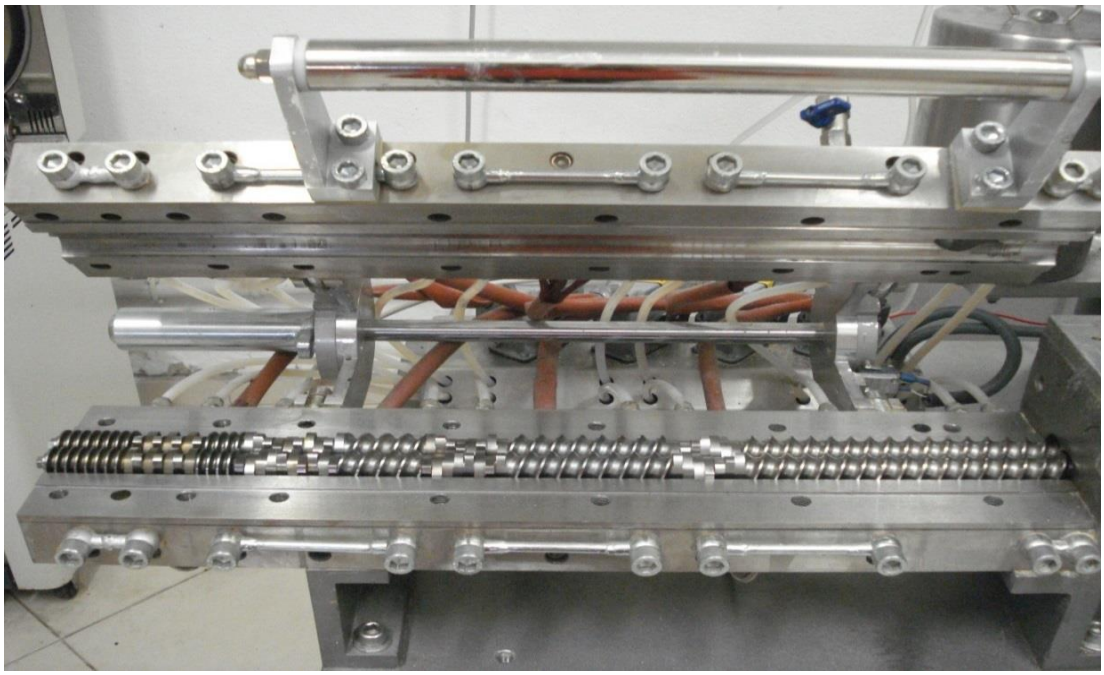

Figure 5. The screws of the extruder used in the study.

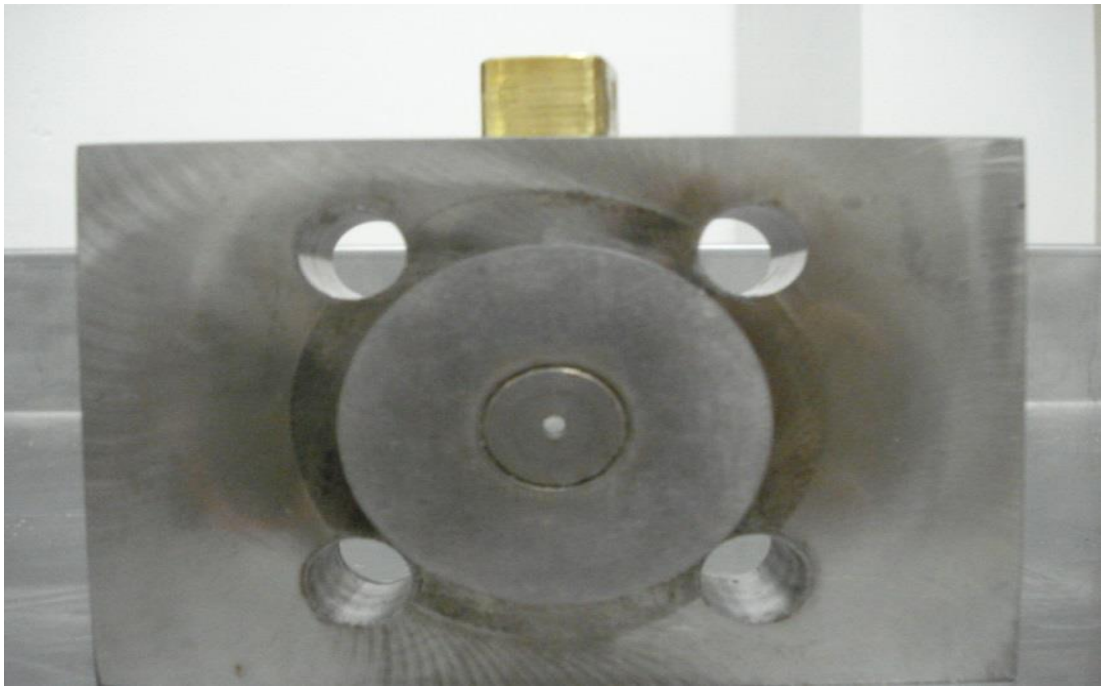

Figure 6. The die of the extruder used in the study.

The extruder has four heating zones which are controlled by electrical heating and water cooling. Computerized data acquisition system was used in order to control the barrel temperatures and screw speed. The feed was given to the extruder by a twin screw volumetric feeder which is a part of the extruder.

Two different temperature profiles were used for the barrel zones during the experiments: 80°C, 90°C, 100°C and 130°C (die: 121°C) and 80°C, 105°C, 130°C and 160°C (die: 142°C). Screw speed was 225 rpm and feed flow rate was 36 g/min for both temperature profile set. After the steady-state condition was achieved, extrudates were collected only when actual measured barrel zone temperatures and die temperatures varied only ± 2°C from the set temperatures. After production, extrudates were left to cool down for an hour.

The diameters of the extrudates were measured with a digital caliper. The moisture contents of collected extruded samples were measured in a halogen moisture

analyzer (MX-50, AND, Japan) after 2 hours from the extrusion process. Only the samples for the starch gelatinization analyzes were dried at 50°C for 15 minutes in an incubator (Nuve Sanayi Malzemeleri İmalat ve Tic. A. Ş. ES500, Turkey) after extrusion. The extruded samples were grinded with a blender (Waring 8011ES, USA) for 2 minutes and passed through 425 μm mesh sieve (Laboratory Test Sieve, Endecotts Ltd. England). Dried and ground samples were kept in black polyethylene bags at room temperature for starch gelatinization analysis by DSC. Ground samples were stored at -22°C in a freezer (Arçelik, Turkey) until the chemical analysis were done.

2.2.2 Protein Content Analysis

Kjeldahl method described by Cemeroglu (2010) was used with some modifications to measure protein content of the samples. 1 g of red pepper pulp and corn grit samples were weighed into the Kjeldahl tubes. 5 g potassium sulfate (K_2SO_4) and 0.3 g copper (II) sulfate ($CuSO_4$) were added as Kjeldahl catalyst into the each tube. Also, 15 mL of sulfuric acid (H_2SO_4) was added into the tubes. The tubes were placed into the Kjeldal burning unit. The burning period was about 3-4 hours. After burning, tubes were left at room temperature to cool. 0.1 N of NaOH was used for distillation. 50 mL of distilled water was added to each tube. Afterwards, the tubes were put into the distillation unit (Şimşek Laborteknik Ltd. Şti. DES-1, Turkey) and the erlenmeyer flasks containing 50 mL of boric acid (4%) placed in the unit. Lastly, erlenmeyer flasks in the distillation units were titrated with 0.1 N HCl. Nitrogen content was determined by a conversion factor of 6.25 in order to estimate protein content.

Calculations were done according to the formulations below;

N % = (mL of standard acid - mL blank) * 0.1 * 1.4007

Protein % = (N %) * 6.25

2.2.3 Ash Content Analysis

Ash content analysis method given by Cemeroglu (2010) was used with slight modifications. Porcelain crucibles were left at muffle furnace at 105°C overnight. Then, the crucibles were cooled in a desiccator for 30 minutes. After that, 5 g of red pepper pulp and corn grit samples were weighed into the crucibles. The crucibles were kept in the furnace at 550°C for 8 hours until the ash color turns to gray. Then, the crucibles were cooled down in the desiccator and weighed.

Calculations were done according to the formulations below;

Ash in wet weight % = $(m_2/m_1) * 100$

m_1: amount of sample weighed, g = 5 g

m_2: amount of ash after burning, g

2.2.4 Pectin Content Analysis

The pectin content of red pepper was determined by the method described in Monsoor and Proctor (2001) with slight modifications. 10 g of red pepper pulp were weighed and 60 mL of 0.05 M HCl was added into the pulp. The mixture was stirred in a stirrer at 50 rpm and 90°C for an hour. Then, the samples were left at room temperature in a water bath in order to cool to about 30°C and samples were centrifuged (Sigma, 2-16 PK, Germany) at 4000xg for 12 minutes. The supernatant was collected and separated. 2-proponal was added to the supernatant in equal volume. Pectin was precipitated by adjusting the pH of the dispersion medium to 3.5 and mixture was left to stand for 6 hours. Then, the mixture was centrifuged again at 4000xg for 12 minutes. The precipitate was collected, dispersed in 50 mL of 2-proponal and stirred for 1 hour. This centrifugation, dispersion and stirring steps were repeated two more times. After the last stirring step, the mixture was centrifuged at 4000xg for 12 minutes. The precipitate was collected and dried at

60°C for 5 hours. After drying, the weight of precipitate was measured and it was taken as the pectin amount in the samples.

2.2.5 Extraction Procedure for Antioxidant Activity and Total Phenol Content Determination

Extractions were done by the method described by Anton et al. (2009) for total phenol content analysis and antioxidant activity of corn grits, feed and extruded samples. 400 mg of raw and extruded finely ground samples were mixed with 10 mL of acetone/water mixture (4:1 v/v). The mixture was put into a 25 mL glass beaker and glass beaker was wrapped by paraffin and aluminum foil. Then, it was stirred with a magnetic stirrer (JeioTech-Multichannel Stirrer, MS-52 M, Korea) at 200 rpm for 2 hours. The samples were centrifuged (Sigma, 2-16 PK, Germany) at 3000xg and 20°C for 12 minutes. The supernatant was transferred to 5 mL syringe and filtered through 0.45 μm sterile syringe type filter (Syringe Filter, PTFE 25 mm).

2.2.6 Total Phenolic Content Analysis

Total phenolic contents of the raw and extruded samples were determined by using the Folin-Ciocalteau method as described by Anton et al. (2009) with some modifications. 0.8 mL extract of the samples was put into a 25 mL glass beaker and was mixed with 6 mL of Folin-Ciocalteau reagent (Merck, Germany) freshly diluted 10-fold. After 5 minutes waiting, 6 mL of sodium carbonate solution prepared by Na_2CO_3 and distilled water in proportion to 60 g/L was added. Reduction of Folin-Ciocalteau reagent causes color changes in the presence of sodium carbonate and phenolic compounds. During analysis, glass beaker was wrapped by paraffin and aluminum foil. Reagents were allowed to incubate at room temperature for 90 minutes in dark. Acetone-water solution (80:20 v/v) was prepared as a blank in

spectrophotometer. After that, absorbance was read at 725 nm by using a UV-Visible spectrophotometer (Shimadzu, UV-1700, Japan) at 21°C.

The calibration curve was prepared with gallic acid (3,4,5-trihydroxybenzoicacid, Sigma-Aldrich, Germany). The linear range of the calibration curve was 20-200 mg/L (R^2 = 0.998). The results were expressed as mg gallic acid equivalents (GAE) per g dry weight of samples.

2.2.7 Antioxidant Activity Analysis

Antioxidant activities of the raw and extruded samples were determined with a stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Anton et al. (2009) with some modifications.

0.8 mL of extract of the samples was put in a 25 mL glass beaker wrapped by paraffin and aluminum foil and combined with 15.2 mL of DPPH (Sigma-Aldrich, Germany) solution (6.34 x 10^{-5} M in methanol) to react with each other for 30 minutes. DPPH changes the color in the presence of antioxidant substances. Absorbance of the samples at 517 nm was measured at room temperature in the dark with a UV-Visible spectrophotometer (Shimadzu, UV-1700, Japan) at 21°C. The control was prepared with 0.8 mL acetone/water mixture (4:1 v/v) and 15.2 mL of DPPH solution. Methanol was used as a blank.

The calibration curve was prepared with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, Germany) which is a synthetic, hydrophilic vitamin E analogue. The linear range of the calibration curve was 10 μM to 100 μM (R^2 = 0.99). The results were expressed as μmol of trolox equivalents per g dry weight of samples.

2.2.8 Measurements of Starch Gelatinization by DSC

The thermal properties and gelatinization of feed and extrudates were analyzed by using differential scanning calorimeter (DSC). The analysis was conducted with PerkinElmer DSC 4000 with intracooler. Indium and zinc were used for the calibration of the instrument.

Ground samples of about 7 mg were weighed on aluminum pans (30 µL, PerkinElmer, The Netherlands) and about 14 mg of distilled water was added into pans by using a micro-syringe. The pans were sealed with a sample-encapsulating press system and allowed to equilibrate overnight at 4°C in a refrigerator.

Samples were heated at the rate of 10°C/min from 0°C to 130°C with nitrogen flushing (20 mL/min). A sealed empty pan was used as a reference. Gelatinization onset temperature (T_o), peak temperature (T_p), conclusion (end) temperature (T_c), and the enthalpy (ΔH) of the endotherm for gelatinization were calculated with the Pyris software, version 11.0.0.0449 (PerkinElmer Life and Analytical Sciences, Shelton, Conn., U.S.A.).

2.2.9 Extraction of Carotenoids

The extraction method of Cemeroglu (2010) was used with slight modifications. 10 grams of homogenized samples from both finely ground extrudates and feed were taken in a glass beaker and 30 mL of distilled water was added. The beakers were wrapped by paraffin and aluminum foil and kept in a refrigerator at 4°C overnight for rehydration. The equilibrated samples were homogenized with homogenizator (Witeg, HG-15A, Germany) at 13500 rpm for 2 minutes. 4 grams of homogenized samples were weighted into a glass beaker, 0.4 g calcium carbonate ($CaCO_3$) was added on them to neutralize any organic acids. After that, the samples were mixed with 25 mL of hexane/acetone/ethanol (50:25:25 v/v/v). The beakers were wrapped by paraffin and aluminum foil and mixture was stirred with a magnetic stirrer

(JeioTech-Multichannel Stirrer, MS-52 M, Korea) at 250 rpm for 20 minutes. After stirring, the supernatant was separated and put into a glass beaker. The residue part was again mixed with 25 mL of hexane/acetone/ethanol (50:25:25 v/v/v) mixture. Extraction was performed in two steps to make the sample colorless and to extract carotenoids completely. The beakers were wrapped by paraffin and aluminum foil and mixture was stirred again at 250 rpm for 20 minutes. After stirring, the supernatant was separated and combined with previous supernatant in a centrifuge tube. Then, 10 mL distilled water added to supernatants and they were centrifuged at 9500xg at 4°C during 20 minutes to separate hexane layer. After centrifugation, the hexane layer on top of the solution was separated into glass tubes with a micro-syringe and dried under the stream of nitrogen at 37°C with nitrogen evaporator at the Prof. Dr. Levent Bayındırlı Food Analysis Laboratory in the Food Engineering Department of the Middle East Technical University.

The carotenoids of the digested raw and extruded products were extracted by the same method with the minor modifications for *in vitro* bioaccessibility analysis. The digested samples were thawed at room temperature. Then, 25 mL of hexane/acetone/ethanol (50:25:25 v/v/v) mixture was added to the samples. This time, extraction in one step was enough to extract all carotenoids from samples. The samples were stirred at 250 rpm for 20 minutes and supernatants were collected. Then, 5 mL distilled water was added on supernatants and centrifuged at 9500xg at 4°C for 20 minutes. The hexane layer was separated into glass tubes with a micro-syringe and dried under the stream of nitrogen at 37°C with nitrogen evaporator.

2.2.10 *In Vitro* Digestion Model

In vitro digestion analysis was conducted at nutrition laboratory in Food Engineering Department of Ege University, Izmir, Turkey. To simulate human gastrointestinal digestion, the procedure described by Gil-Izquierdo et al. (2002) was applied with some modifications. Simulated salivary and gastric fluids were prepared according to

Kopf-Bolanz et al. (2012) with some modifications. Urea was not added to simulated salivary and gastric juices, and glucuronic acid, glucosamine and galactose were not added to simulated gastric juice. Pepsin solution was prepared as weighing 3.05 g (495 units/mg solid) pepsin from porcine gastric mucosa powder (Sigma-Aldrich P7012, Germany) in a volumetric flask and adding simulated gastric fluid until 10 mL. The pepsin solution was mixed by the heat-stirrer (Stuart CC162, UK). Before the analysis, protein contents of the samples were measured by Kjeldahl method with conversion factor of nitrogen into protein 5.75. Moisture contents of the samples were measured with a moisture analyzer device (Denver Instrument, NY, USA). Samples from feed, 130°C and 160°C extrudates were crushed roughly in porcelain mortar with hand enough to disintegrate in the mouth during chewing. Then, they were accurately weighed as 0.50 g in plastic centrifuge tubes by using a precision scales device (Denver Instrument S1-234, NY, USA). Then, 0.5 mL distilled water and 1.0 mL simulated salivary fluid (Table 2) added into samples and a blank was prepared by adding distilled water and salivary fluid into tubes without samples. The contents in tubes were adjusted to pH 7.0 and were mixtured by a vortex. After that, 0.2 mL pepsin solution and 0.8 mL simulated gastric fluid (Table 2) were added into tubes and pH was set to 3.0. The tubes were incubated at 37°C in a shaking water bath (Memmert, Type SV 1422, Schwabach, Germany) for 2 hours. After simulated gastric digestion, the pH was increased to 5.5. During incubation period, dialysis bag (Spectra/Por Dialysis Membrane MWCO: 6-8000, Spectrum Laboratories, Inc.) including 6.25 mL 0.5 N $NaHCO_3$ was prepared and after incubation one membrane was put into a tube when pH was 5.5. The pH was increased to 7.0. The mixture of pancreatin and bile extract was prepared by dissolving 8.0 g pancreatin (Sigma-Aldrich P1750, Germany) and 24.0 g bile extract porcine (Sigma-Aldrich B8631, Germany) in pancreatic juice brought to 100 mL, and 2 mL of this solution added to the tubes. The tubes were again incubated at 37°C at the same speed in orbital shaker for 2 hours. During incubation period of the intestinal phase of the *in vitro* digestion process, pH was measured continuously to maintain constant pH at 7.0. Dialysis membranes were taken from the tubes and they were opened and the inside matter

was transferred into 15 mL plastic tubes. Aliquots of the digested samples were frozen at -20°C overnight. Also, the solution outside of the dialysis membranes were transferred into other tubes and frozen at -20°C overnight. After that, frozen samples were transported to Food Engineering Department of the Middle East Technical University in Ankara for HPLC analysis of carotenoids (β-cryptoxanthin and zeaxanthin).

The dialysis membranes were prepared as follows. The membranes (Spectrum Laboratories, Inc.; Spectra/Por Dialysis Membrane, MWCO: 6-8000) were cut 10 cm length and put into hot distilled water in order to expand. After that, they were washed with cold distilled water. The washed membranes were tied at one end, and then 6.25 mL of 0.5 N $NaHCO_3$ at pH 7.0 was added to the membranes. The air inside the membranes was removed and the other ends were tied. During *in vitro* analysis, pH was set by using 0.1 N HCl and 0.5 N $NaHCO_3$.

Table 2. Preparation of the digestion juices (saliva and gastric juice) (Kopf-Bolanz et al., 2012).

Saliva			Gastric Juice		
Volume (mL)	Compound	Stock (g/L)	Volume (mL)	Compound	Stock (g/L)
10	KCl	46.72	28	KCl	46.72
1	KSCN	40	0.9	KH_2PO_4	68
20	KH_2PO_4	68	6.5	$NaHCO_3$	168
4	$NaHCO_3$	84	10	NaCl	120
1	NaCl	120	2	$MgCl_2(H_2O)_6$	30
1	$MgCl_2(H_2O)_6$	30	1	NH_4Cl	27.28

2.2.11 Carotenoid Content Determination with HPLC Analysis

The HPLC method described by O'Connell et al. (2007) was used with some modifications. Dried carotenoids of feed and extrudate samples in glass tubes were reconstituted in 100 µL tetrahydrofuran (THF) to split from glass completely and then dissolved in 900 µL mobile phase. The digested feed and extrudate samples were reconstituted in 50 µL THF and then dissolved with 450 µL mobile phase because of the low concentrations of carotenoids. The content of the tubes were mixed with a vortex. The mixtures were filtered through the 0.45 µm syringe filter (Syringe Filter, PTFE 13 mm) into brown glass vials. Then, the carotenoid contents were identified by a reverse-phase HPLC. The HPLC system (Thermo Scientific, Finnigan Surveyor) consisted of an UV visible detector (Finnigan Surveyor, UV-Vis Plus Detector), which was at the Prof. Dr. Levent Bayındırlı Food Analysis Laboratory in the Food Engineering Department of the Middle East Technical University, was used for β-cryptoxanthin and zeaxanthin quantification. Column system consisted of a guard column connected to Inertsil ODS-2, 4.6 x 250 mm, 5 µm reverse-phase C18 column. The visible detector was set at 450 nm and the column temperature was set to 20°C. The isocratic flow rate was 1 mL/min and the injection volume was 25 µL. Mobile phase included acetonitrile/methanol/ dichloromethane (75:20:5 v/v/v) containing 10 mmol/L ammonium acetate, 4.5 mmol/L buthylated hydroxytoluene, and 3.6 mmol/L triethylamine. The mobile phase was filtered through 0.45 µm filter (Filtration Membranes, Membrane Disc PVDF 47 mm, 0.45 µm) and degassed with ultrasonic agitation.

The standard curves of β-cryptoxanthin (Sigma-Aldrich C6368, ≥ 97% (TLC), Germany) and zeaxanthin (Sigma-Aldrich 14681, ≥ 97% (TLC), Germany) were prepared with a range of concentrations from 0.0025 µg/mL to 0.1 µg/mL. All solvents used in this procedure were HPLC grade.

Calculations of *in vitro* bioaccessibility of feed and extrudate samples were as follows:

In vitro bioaccessibility % = [(carotene content in dialyzed portion) + (carotene content in not dialyzed portion)] / (carotene content in the sample before *in vitro* digestion)

2.2.12 Statistical Analysis

Analysis of variance (ANOVA) was used for analyzing the results by using data analysis function of the Microsoft Office Excell 2010. Differences at $p < 0.05$ were considered as significant difference.

CHAPTER 3

RESULT AND DISCUSSION

3.1 Proximate Analyses of Ingredients

Determined moisture, protein, pectin and ash contents of the corn grit and red pepper pulp were shown in the Table 3.

Table 3. Moisture, protein, pectin and ash contents of the corn grit and red pepper pulp.

Sample	Moisture (%)	Protein (%)	Pectin (%)	Ash (% wet weight)
Corn Grits	12.52 ± 0.03	6.87 ± 0.88	-	0.41 ± 0.17
Red Pepper Pulp	92.29 ± 0.05	0.57 ± 0.09	0.80 ± 0.10	0.45 ± 0.02

Results are means ± SD (n = 3).

3.2 Total Phenolic Content and Antioxidant Activity

The results of total phenolic content and antioxidant activity analyses were shown in Table 4.

Table 4. Total phenolic content and antioxidant activity results of corn grit, feed and extrudate samples.

Sample	mg GAE/g dry sample	μmol TE/g dry sample
Corn Grits	1.59 ± 0.03 [a]	0.52 ± 0.003 [a]
Feed	1.51 ± 0.08 [b]	0.83 ± 0.002 [b]
Extrudate 130°C	0.88 ± 0.00 [c]	0.59 ± 0.002 [c]
Extrudate 160°C	0.79 ± 0.01 [d]	0.64 ± 0.009 [d]

Results are means ± SD (n = 3); values of the same column followed by the different letters indicate significant differences ($p < 0.05$).

Total phenolic content of the corn grit was found as 1.59 ± 0.03 mg GAE/g dry sample while it was 1.51 ± 0.08 mg GAE/g dry sample for feed. It can be seen that total phenolic content was slightly decreased by adding red pepper pulp into corn grit to prepare the feed. It can be concluded that corn grits had higher total phenolic content than red pepper pulp in gram dry sample. Extruded product processed under 130°C last zone temperature had a 0.88 ± 0.00 mg GAE/g dry sample while total phenolic content of extrudate processed under 160°C last zone temperature was 0.79 ± 0.01 mg GAE/g dry sample. Reduction in total phenolic content was seen after extrusion. Increasing the temperature decreased the total phenolic content further. The reason may be the moist heat that is highly disruptive and it may produce a synergistic effect with high temperature. The other reason of decrease in total phenolic content may be decarboxylation of phenolic acids since phenolic compounds are less resistant to heat (Sharma and Gujral, 2011). These compounds are heat labile, and their nature may be destroyed or altered by heating over 80°C (Zielinski et al., 2001). The reasons of the reduction in total phenolic content may be the destruction of phenolic compounds because of the alteration in molecular structure of them or high temperature. They may cause a decrease in the chemical reactivity of phenolic compounds or reduction on their extractability in consequence

of certain degree of polymerisation (Altan et al., 2009). The flavonoids and phenolic compounds may not show their actual value because of the fact that they may be interacted with the proteins (Arts et al., 2002).

Antioxidant activity of corn grit, feed, 130°C extrudate and 160°C extrudate were 0.52 ± 0.00, 0.83 ± 0.00, 0.59 ± 0.00 and 0.64 ± 0.00 μmol TE/g dry sample, respectively. It can be shown that antioxidant activity increased with adding red pepper pulp into corn grit. High temperature of extrusion cooking caused a decrease in antioxidant activity. Rising temperature from 130°C to 160°C caused an increase in antioxidant activity of extrudates. The Maillard browning products formed during extrusion may be the reason of the increase in antioxidant activity due to they enhance the antioxidant activity (Rufian-Henares and Delgado-Andrade, 2009) of extrudates processed at 160°C last zone temperature. During extrusion process high barrel temperatures and low moisture promote the reaction of amino acids and reducing sugar, Maillard reaction (Guy, 2001). It is known that the thermal processing generate more antioxidants which contribute to antioxidant activity. Maillard browning pigments could have produced by changing extrusion temperature and moisture (Manzocco et al., 2001). Many factors which are reactant concentration, temperature, water activity and reaction time influences Maillard browning reaction (Stojceska et al., 2009). Increasing of the antioxidant activity as a result of thermal processing has also been observed in some studies (Nicoli et al., 1997; Dewanto et al., 2002). In this study, high last zone temperatures (130°C and 160°C) and low moisture content (25%) were used. Therefore, Maillard browning pigments may be produced after extrusion.

The natural antioxidants are labile to high temperatures; therefore, heating above 80°C damages their antioxidant activities (Zadernowski et al., 1999). Antioxidants and phenolic compounds are known to be sensitive to the heat, oxygen and light. Antioxidants and phenolic compounds were labile to heat; therefore, the high temperatures used in this research could cause changes in their structure. Riedl and Hagerman (2001) stated that grain proteins are denatured during extrusion and this

leads to open lose structures after that tannin-protein complexes are formed retaining antioxidant activity. Caltinoglu et al. (2013) investigated that the effects of extrusion on the functional components of the extrudates made with tomato pulp added corn grits. They found a decrease in antioxidant activity of the extruded tomato pulp added corn grit samples. That is, extrusion caused a significant decrease in antioxidant activity of extrudates. Similar result was reported for the red pepper pulp added corn grits extrudates in this study.

Some studies reported that antioxidant activity increased in extrudates and increased when barrel temperature was increased. White et al. (2010) extruded cranberry pomace mixed with corn starch in various ratios at 150°C, 170°C, 190°C and found an increase about 16-30% in oxygen radical absorbance capacity (ORAC) values with increased temperatures during extrusion at 170°C and 190°C. Products formed during Maillard reaction might be the reason of the increase in ORAC values. Gumul and Korus (2006) observed a high antioxidant potential of rye bran extruded products processed at a temperature of 120°C and 180°C. They observed that the high molecular weight Maillard products acted as antioxidants formed at higher temperatures during extrusion. They stated that these Maillard products may be one of the reasons of the high antioxidant potential. Maillard reaction products have stronger antioxidant properties than common food antioxidants (Liu et al., 2010). In addition, Shih et al. (2009) observed that DPPH radical scavenging activity of the sweet potato extruded products was significantly increased after extrusion. In the study of Yagci and Gogus (2009), extrudates including higher partially defatted hazelnut flour and fruit waste content produced at 150-175°C showed an increase in the antioxidant activity and total phenolic content of the samples. Sharma et al. (2012) observed an important increase in antioxidant activity of barley extrudates upon extrusion.

Sharma et al. (2012) observed the non-enzymatic browning index (NEB) was significantly increased in all barley extrudates after extrusion cooking. Rising the temperature from 150°C to 180°C at constant moisture level of feed (20% and 15%)

caused a significant increase in NEB index. Formation of Maillard pigments during extrusion causes higher NEB index (Sharma and Gujral, 2011).

Many researchers reported that similar trend with this research for the total phenolic content and antioxidant activity of extrudates after extrusion (Korus et al., 2007; Dlamini et al., 2007; Anton et al., 2009; Delgado-Licon et al., 2009; Altan et al., 2009; Limsangouan et al., 2010; Gujral et al., 2012). Korus et al. (2007) investigated the effect of extrusion cooking on the antioxidant activity and phenolic content of dry beans. They stated a significant reduction in antioxidant activity and polyphenol content. Dlamini et al. (2007) studied the effects of extrusion on the total phenolic content and antioxidant activity of sorghums. They observed a decrease in total phenolic content and antioxidant activity for extruded products after extrusion cooking. Anton et al. (2009) observed that a reduction in total phenolic content and antioxidant activity of the corn and bean flours mixture after the extrusion cooking. Furthermore, Delgado-Licon et al. (2009) stated a significant reduction in the total polyphenols and antioxidant activity of bean and corn mixture after the extrusion process. Similarly, Altan et al. (2009) observed that antioxidant activities and total phenolics significantly decreased after extrusion by 60-68% and 46-60%, respectively, in all extrudates made with barley flour and tomato or grape pomace. They also found that total phenolic content was not correlated with antioxidant activity. Limsangouan et al. (2010) investigated the influence of extrusion on the functional properties of extruded foods produced from cereal, legumes, and the by-products from vegetables and herbs. They observed that extrusion cause a slight decrease the antioxidant capacity and phenolic content of extruded products. Gujral et al. (2012) observed a reduction in the total phenolic content and antioxidant activity of the brown rice products after the extrusion process. Moreover, their study showed that raising the extrusion temperature from 100°C to 120°C caused a reduction in the antioxidant activity total and phenolic content.

Moreover, El-Hady and Habiba (2003) observed a significant reduction in total phenolic content of extruded products made with different kinds of beans and peas.

This reduction was commonly attributed to the effect of feed moisture and barrel temperature; however, an interaction effect of the temperature and moisture on the total phenolic content was not observed.

Anton et al. (2009) stated an important decrease of about 70% in total phenolic content of corn starch with red bean extrudates, about 10% decrease for mixture of corn starch and navy bean extrudates and 100% reduction starch based extruded products. In the study of Sharma (2012), an important reduction in total phenolic content and total flavonoid content and an important increase in antioxidant activity were found after extrusion. Also, total phenolic content of the barley extruded samples moisturized to 20% level was significantly decreased by increasing temperature from 150°C to 180°C.

Ozer et al. (2006) stated that extrusion processing and the screw speed did not cause a change on the phenolic content of extruded snacks. Total phenolic content was not affected with the feed rate and moisture content of feed at constant barrel temperature (110°C) while antioxidant activity was decreased by extrusion (Ozer et al, 2006).

High temperature can change molecular structure of phenolic compounds and decrease their chemical reactivity or reduce their extractability because of the certain degree of polymerization (Alonso et al., 2000). This polymerization causes the reduction of antioxidant properties of them (Zadernowski et al., 1999). Therefore, antioxidant activity and phenolic compounds of the red pepper pulp added corn grits may be reduced chemical reactivity and decreased their extractability due to high temperature extrusion cooking.

The temperature has a significant influence on the stability of bioactive compounds because they are temperature sensitive compounds. Decarboxylation of phenolic compounds may occur because of the high temperature during extrusion process. Also, high moisture level may assist polymerization of phenols causing reduced antioxidant activity and extractability (Dlamini et al., 2007; Repo-Carrasco-Valencia

et al., 2009). Sensoy et al. (2005) concluded that changes in polar compounds occurred during extrusion process. The mechanical energy which is supplied by the extruder may influence the nature of some complexes formed between the flour components and the decomposition of starch.

Some researchers were found an increase in total phenolic content unlike this study and the studies mentioned above. Shih et al. (2009) found a significant decrease in anthocyanin and an increase in total phenolic content for extruded potato products. A similar increase in total phenolic content of cereals including vegetables after extrusion cooking was reported by the study of Stojceska et al. (2008). The contents of certain phenolic acids in extrudates were increased by the release from cell wall after extrusion cooking.

3.3 Starch Gelatinization

DSC thermograms of the feed and extrudates evaluated by DSC are presented in Appendix as Figures 9, 10 and 11. The results of starch gelatinization analyses were shown in Table 5.

Table 5. Gelatinization characteristics of red pepper pulp added feed and extrudates.

	Gelatinization transition			
Sample	T_o(°C)	T_p(°C)	T_c(°C)	ΔH(J/g)
Feed	69.40 ± 0.34	75.07 ± 0.89	80.56 ± 1.62	-2.20 ± 0.31
Extrudate 130°C	-	-	-	-
Extrudate 160°C	-	-	-	-

Results are means ± SD (n = 3); values of the same column followed by the different letters indicate significant differences ($p<0.05$).

Gelatinization is known as the conversion of raw starch to a digestible and cooked starch by the presence of water and heat. Water molecules are absorbed and bound to the starch molecules. Therefore, starch granule structure alters (Ding et al., 2005; Ding et al., 2006).

Starch of maize has a gelatinization temperature around 62-72°C (Delcour and Hoseney, 2010). Coral et al. (2009) studied on the influence of the moisture and the grain size on the gelatinization temperature of starch from four industrial maize flours. They reported that gelatinization temperature was found between 70-75°C for all studied samples. In this study, gelatinization peak temperature was found 75.07 ± 0.89°C.

No peak was detected for red pepper pulp added extrudates processed at 130°C and 160°C last zone temperatures indicating 100% gelatinization (Figure 10 and 11). Gomez and Aguilera (1984) claimed that there was no peak in the range of 25°C to 115°C for extrudates and the amount of native starch in extrudates was minimal and was not detected by the DSC. Chanvrier et al. (2007) stated that DSC thermograms did not show a residual gelatinization enthalpy for wholemeal products processed at 110°C during extrusion. Nevertheless, they claimed that some crystallinity residue, which was not detected by DSC, was detected by X-ray diffraction method. According to Altan et al. (2009), no peak was detected by DSC for extrudates produced with barley, tomato pomace or grape pomace mixtures. However, they observed that there was a native starch granule by measurements of iodine complexing method and the degree of gelatinization of barley extrudates was not more than 90%. It was stated that cereal starches containing amylose and natural fatty acid form amylose-lipid complex after extrusion process (Mercier et al., 1980).

Feed moisture and temperature have significant effect on gelatinization (Lawton et al., 1972). The degree of gelatinization of maize grits extrudates was increased with increasing temperature. The reaction of starch gelatinization would be influenced with the mechanical energy during extrusion through shearing effects (Ilo et al., 1996).

3.4 Carotenoid Contents and *In Vitro* Bioaccessibility of Carotenoids

Amount of zeaxanthin of feed and extrudates in dialysate and *in vitro* bioccessibility results of zeaxanthin are shown in Table 6.

Table 6. Amount of zeaxanthin of feed and extrudates in dialysate and *in vitro* bioaccessibility of zeaxanthin.

Zeaxanthin (μg/g dry sample)				
Sample	Before digestion	Non-dialysable fraction in the small intestine	Dialysated fraction in the small intestine	*In vitro* Bioaccessibility (%)
Feed	2.97 ± 0.05^{a}	1.95 ± 0.24^{a}	ND	65.69 ± 7.78^{a}
Extrudate 130°C	1.49 ± 0.14^{b}	1.21 ± 0.04^{b}	ND	81.73 ± 5.36^{a}
Extrudate 160°C	1.78 ± 0.21^{c}	1.48 ± 0.02^{c}	ND	84.07 ± 10.90^{a}

Results are means ± SD. Two parallels and two replicates were conducted. Different letters indicate significant differences at the 0.05 level ($p < 0.05$). ND, non-detectable.

The amount of zeaxanthin decreased after extrusion when compared with the feed where decrease was higher for lower temperature treated extrudates. There were not statistically significant difference between the *in vitro* bioaccessibility of zeaxanthin of feed and extrudates.

Table 7. Amount of β-cryptoxanthin of feed and extrudates in dialysate and *in vitro* bioaccessibility of β-cryptoxanthin.

β-cryptoxanthin (μg/g dry sample)				
Sample	Before digestion	Non-dialysable fraction in the small intestine	Dialysated fraction in the small intestine	*In vitro* Bioaccessibility (%)
Feed	0.97 ± 0.04 [a]	0.44 ± 0.03 [a]	ND	45.75 ± 3.24 [a]
Extrudate 130°C	0.80 ± 0.01 [b]	0.35 ± 0.01 [b]	ND	43.52 ± 1.75 [a]
Extrudate 160°C	0.69 ± 0.08 [c]	0.38 ± 0.02 [b]	ND	56.17 ± 7.71 [a]

Results are means ± SD. Two parallels and two replicates were conducted. Different letters indicate significant differences at the 0.05 level ($p < 0.05$). ND, non-detectable.

Amount of β-cryptoxanthin of feed and extrudates and *in vitro* bioaccessibility results of β-cryptoxanthin are given in Table 7. Extrusion decreased the β-cryptoxanthin content. Increasing the last zone temperature from 130°C to 160°C caused slight decrease in the β-cryptoxanthin content. There were not statistically significant difference between *in vitro* bioaccessibility of β-cryptoxanthin of feed and extrudates similar to the *in vitro* bioaccessibility of zeaxanthin. Therefore, *in vitro* bioaccessibilities of β-cryptoxanthin and zeaxanthin were not affected significantly by increasing temperature from 130°C to 160°C and extrusion. The cell walls are mechanically disrupted during extrusion and released the cell compounds into the starchy matrix. Deterioration of the cell walls may improve the bioaccessibility of β-cryptoxanthin and zeaxanthin; however, it can expose the labile β-cryptoxanthin and zeaxanthin molecules to the high temperature and shear during processing that result in their loss to some extent.

It was found that zeaxanthin more bioaccessible than β-cryptoxanthin. The similar results were found in the study of O'Sullivan et al. (2010) where bioaccessibilities of red bell pepper carotenoids were as follows; 87.6%, 54.3%, 33.1% and 6.2% for zeaxanthin, lutein, β-cryptoxanthin and β-carotene, respectively. Similarly, O'Connell et al. (2008) and Ryan et al. (2008) found that % bioaccessibility of red bell pepper carotenoids were 81.3-97.7%, 77.1%, 29.7-65.7%, and 13.3- 21.2% for lutein, zeaxanthin, β-cryptoxanthin and β-carotene, respectively.

Dehghan-Shoar et al. (2011) observed that the bioaccessibility of lycopene associated with the fibrous tissue of the cell walls as a result of the breakdown of resistant cell structures was improved after extrusion. Moreover, their study showed that the lycopene was not completely destroyed after exposure of lycopene to high shear and heat during extrusion, and was available for uptake by the cells.

Extrusion process and the presence of some food components affect the bioavailability of carotenoids. For instance, soluble fibers including pectin and hemicelluloses exist in red peppers can decrease shear and temperatures that degradation of cell walls are decreased (Lillford, 2008).

The information on β-cryptoxanthin and zeaxanthin retention during extrusion is scarce; however, many researches have been conducted on the retention of β-carotene (Berset et al., 1989; Shih et al., 2009), lycopene (Dehghan-Shoar et al., 2010; Dehghan-Shoar et al., 2011) and anthocyanins during extrusion (Camire et al., 2002; Camire et al., 2007). The retention values were found by them changed from 10% to 75% according to the type of the pigment and the food matrix. Shih et al. (2009) found an important reduction in β-carotene for yellow and orange sweet potatoes during extrusion. Dehghan-Shoar et al. (2010) found that lycopene retention in extruded corn, wheat and rice snacks including tomato paste was much lower than extrudates including tomato paste because of that initial concentration of lycopene was higher in the tomato paste.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In summary, the total phenolic content of the feed was significantly decreased after extrusion cooking and increasing the last zone temperature caused further decrease. Total phenolic content of the samples were found to decrease about 41.72% and 47.68% after 130°C and 160°C last zone temperature extrusion process, respectively. Antioxidant activity of the feed was decreased with the extrusion process. Antioxidant activity of the samples were found to decrease about 28.92% and 22.89% after 130°C and 160°C last zone temperature extrusion process, respectively. Extrudates processed at higher temperature had higher antioxidant activity than extrudates processed at lower temperatures in the studied range.

No gelatinization peak was observed on DSC thermograms after extrusion whereas a peak was observed before extrusion.

Although decrease in amounts of β-cryptoxanthin and zeaxanthin was observed after extrusion process, β-cryptoxanthin and zeaxanthin were retained in the extrudates to some extent. A decrease in β-cryptoxanthin content about 17.53% and 28.87% was seen whereas a reduction was observed in zeaxanthin content about 49.83% and 40.07% after 130°C and 160°C last zone temperature extrusion process, respectively. *In vitro* bioaccessibilities of β-cryptoxanthin and zeaxanthin were not affected significantly by extrusion.

Information gained in this research would help the food manufacturers to develop new extruded products. Sweet red pepper pulp can be added to design new extruded products. For future study, red pepper powder can be added into feed as a functional ingredient to be able to increase concentration of functional ingredient of extrudates. The effect of screw speed on the functional components can also be investigated.

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APPENDIX A

COMPOSITION OF RAW MATERIALS

A.1 Composition of Red Peppers

Table A. 1. The composition of sweet red pepper (*Capsicum annum* L.)*.

Nutrient	Unit	Value per 100 grams
Proximates		
Water	g	92.21
Energy	kcal	31
Energy	kJ	129
Protein	g	0.99
Total lipid (fat)	g	0.30
Ash	g	0.47
Carbohydrate, by difference	g	6.03
Fiber, total dietary	g	2.1
Sugars, total	g	4.20
Glucose (dextrose)	g	1.94
Fructose	g	2.26
Minerals		
Calcium, Ca	mg	7
Iron, Fe	mg	0.43
Magnesium, Mg	mg	12
Phosphorus, P	mg	26
Potassium, K	mg	211
Sodium, Na	mg	4
Zinc, Zn	mg	0.25
Copper, Cu	mg	0.017
Manganese, Mn	mg	0.112
Selenium, Se	µg	0.1

Table A.1 (Continued)

Vitamins		
Vitamin C, total ascorbic acid	mg	127.7
Thiamin	mg	0.054
Riboflavin	mg	0.085
Niacin	mg	0.979
Pantothenic acid	mg	0.317
Vitamin B-6	mg	0.291
Folate, total	µg	46
Folate, food	µg	46
Folate, DFE	µg	46
Choline, total	mg	5.6
Betaine	mg	0.1
Vitamin A, RAE	µg	157
Carotene, beta	µg	1624
Carotene, alpha	µg	20
Cryptoxanthin, beta	µg	490
Vitamin A, IU	IU	3131
Lutein + zeaxanthin	µg	51
Vitamin E (alpha-tocopherol)	mg	1.58
Tocopherol, beta	mg	0.05
Tocopherol, gamma	mg	0.14
Tocopherol, delta	mg	0.01
Vitamin K (phylloquinone)	µg	4.9
Tocopherol, beta	mg	0.05
Tocopherol, gamma	mg	0.14
Tocopherol, delta	mg	0.01
Vitamin K (phylloquinone)	µg	4.9
Lipids		
Fatty acids, total saturated	g	0.027
Fatty acids, total monounsaturated	g	0.003
Fatty acids, total polyunsaturated	g	0,070
Amino Acids		
Tryptophan	g	0.012
Threonine	g	0.040
Isoleucine	g	0.021
Leucine	g	0.036
Lysine	g	0.036
Methionine	g	0.006
Cystine	g	0.019
Phenylalanine	g	0.050
Tyrosine	g	0.009

Table A.1 (Continued)

Valine	g	0.031
Arginine	g	0.036
Histidine	g	0.017
Alanine	g	0.026
Aspartic acid	g	0.284
Glutamic acid	g	0.211
Glycine	g	0.028
Proline	g	0.024
Serine	g	0.050
Flavonoids		
Flavones		
Luteolin	mg	0.6
Flavonols		
Quercetin	mg	0.2

*(Table adapted from USDA National Nutrient Database for Standard Reference, Release 26 February 2014)

A.2 Composition of Corn Grits

Table A. 2. The composition of yellow corn (*Zea mays* L.)*.

Nutrient	Unit	Value per 100 grams
Proximates		
Water	g	10.37
Energy	kcal	365
Energy	kJ	1527
Protein	g	9.42
Total lipid (fat)	g	4.74
Ash	g	1.20
Carbohydrate, by difference	g	74.26
Fiber, total dietary	g	7.3
Sugars, total	g	0.64
Minerals		
Calcium, Ca	mg	7
Iron, Fe	mg	2.71
Magnesium, Mg	mg	127
Phosphorus, P	mg	210
Potassium, K	mg	287
Sodium, Na	mg	35
Zinc, Zn	mg	2.21
Copper, Cu	mg	0.314
Manganese, Mn	mg	0.485
Selenium, Se	µg	15.5
Vitamins		
Thiamin	mg	0.385
Riboflavin	mg	0.201
Niacin	mg	3.627
Pantothenic acid	mg	0.424
Vitamin B-6	mg	0.622
Folate, total	µg	19
Folate, food	µg	19
Folate, DFE	µg	19
Vitamin A, RAE	µg	11
Carotene, beta	µg	97
Carotene, alpha	µg	63
Cryptoxanthin, beta	µg	0
Vitamin A, IU	IU	214
Lutein + zeaxanthin	µg	1355
Vitamin E (alpha-tocopherol)	mg	0.49

Table A.2 (Continued)

Vitamin K (phylloquinone)	µg	0.3
Lipids		
Fatty acids, total saturated	g	0.667
Fatty acids, total monounsaturated	g	1.251
Fatty acids, total polyunsaturated	g	2.163
Amino Acids		
Tryptophan	g	0.067
Threonine	g	0.354
Isoleucine	g	0.337
Leucine	g	1.155
Lysine	g	0.265
Methionine	g	0.197
Cystine	g	0.170
Phenylalanine	g	0.463
Tyrosine	g	0.383
Valine	g	0.477
Arginine	g	0.470
Histidine	g	0.287
Alanine	g	0.705
Aspartic acid	g	0.655
Glutamic acid	g	1.768
Glycine	g	0.386
Proline	g	0.822
Serine	g	0.447
Flavonoids		
Flavones		
Luteolin	mg	0.6
Flavonols		
Quercetin	mg	0.2

*(Table adapted from USDA National Nutrient Database for Standard Reference, Release 26 February 2014)

APPENDIX B

IMAGES OF THE EXTRUDATES

Figure 7. Images of the extrudates extruded with 25 ± 0.5% moisture content of feed at 130°C forth zone temperature and at 225 rpm with feed flow rate of 36 g/min (14.57 ± 0.01% moisture and 8.52 ± 0.61 mm diameter).

Figure 8. Images of the extrudates extruded with 25 ± 0.5% moisture content of feed at 160°C forth zone temperature and at 225 rpm with feed flow rate of 36 g/min (14.27 ± 0.02% moisture, 6.96 ± 0.53 mm diameter).

APPENDIX C

C. DSC THERMOGRAMS

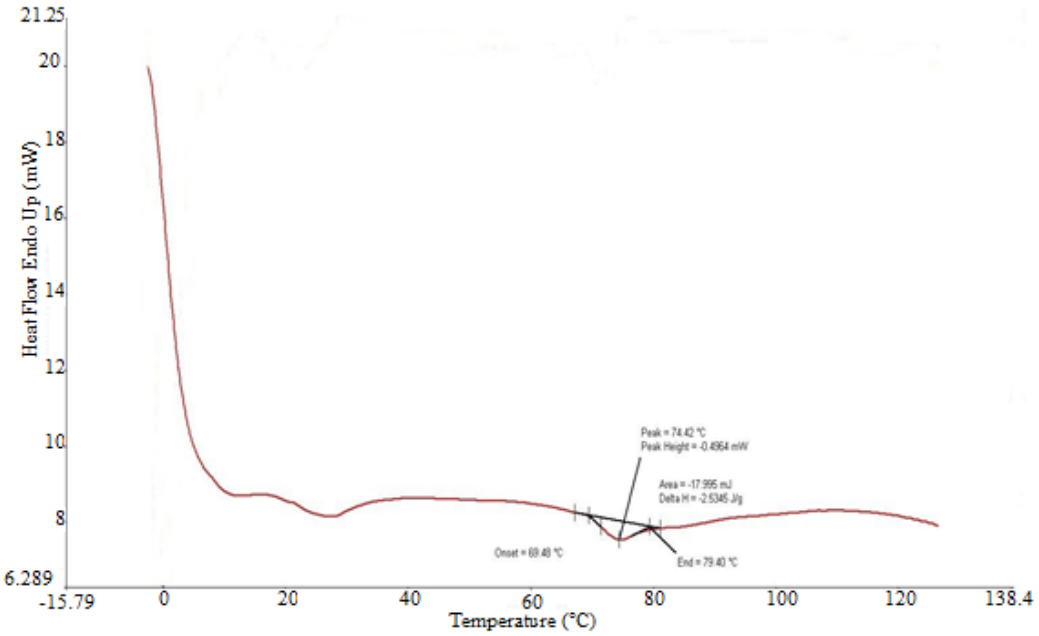

Figure 9. DSC thermogram of feed sample.

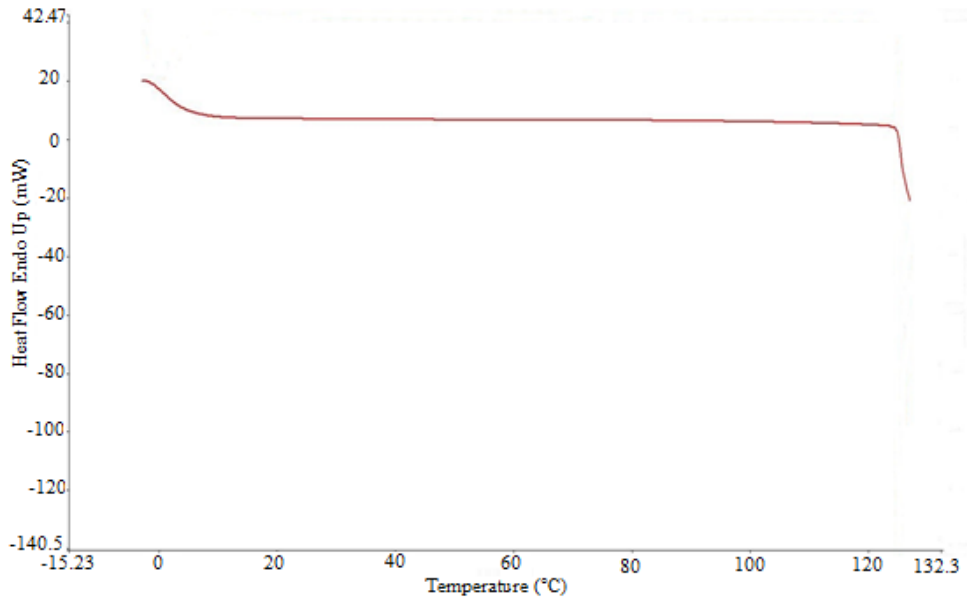

Figure 10. DSC thermogram of extrudate sample at 130°C last zone temperature.

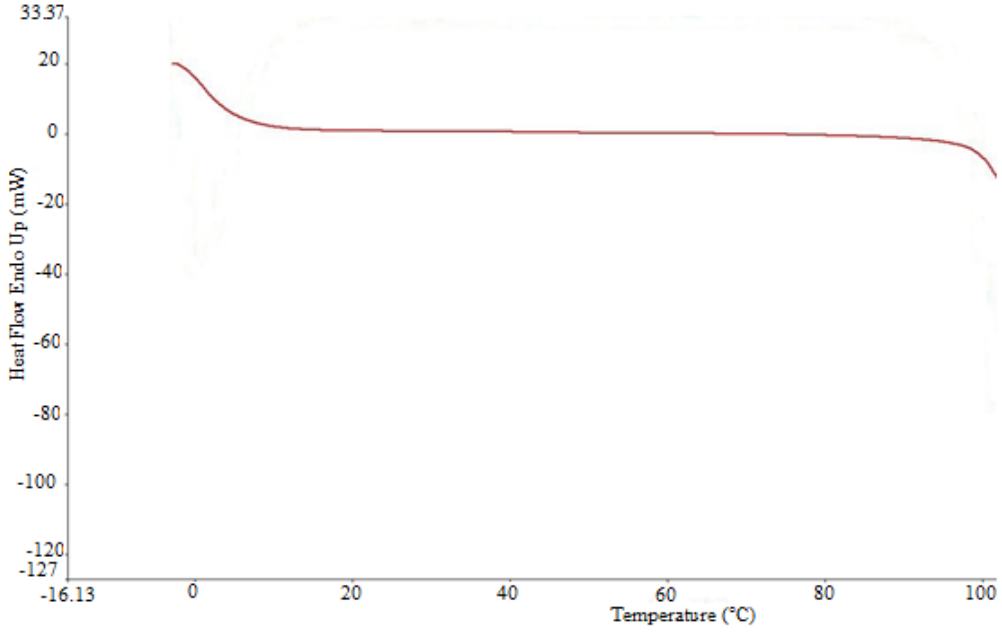

Figure 11. DSC thermogram of extrudate sample at 160°C last zone temperature.

APPENDIX D

HPLC CHROMATOGRAMS

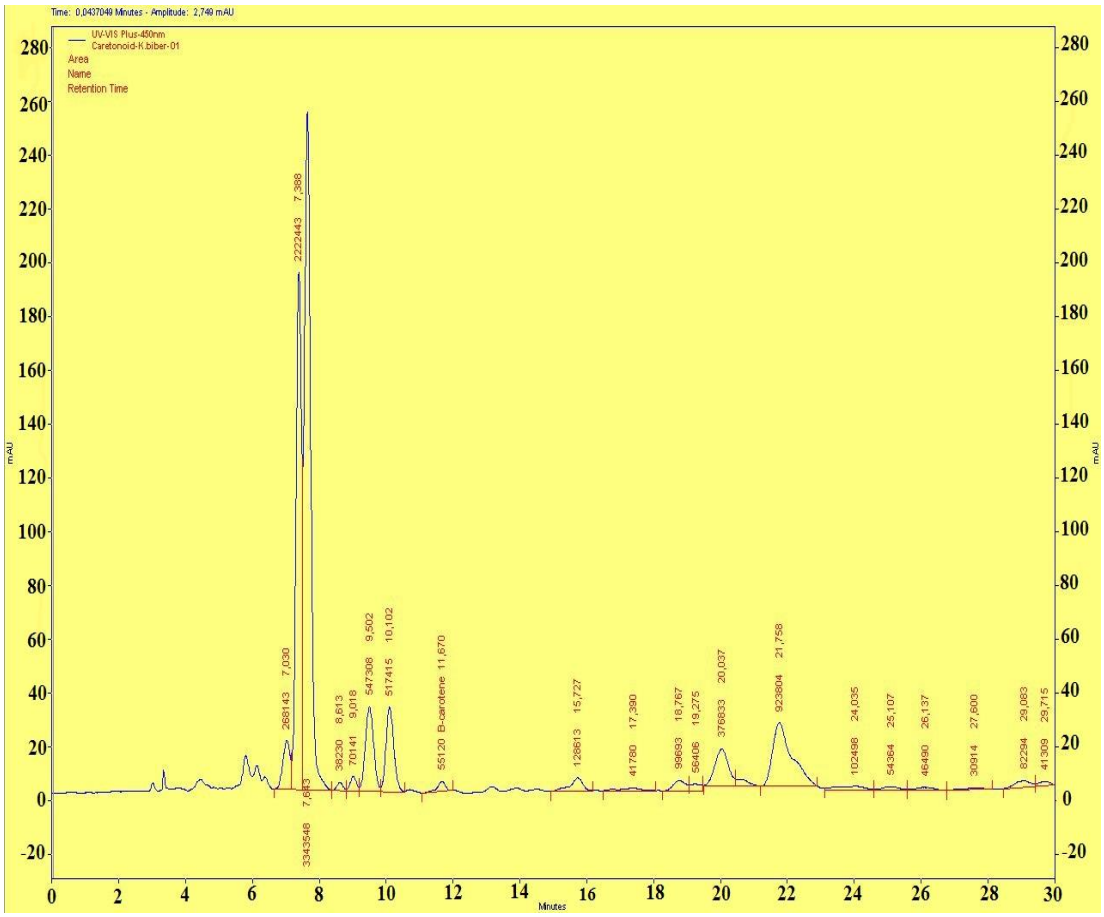

Figure 12. HPLC chromatogram for the feed sample of before digestion.

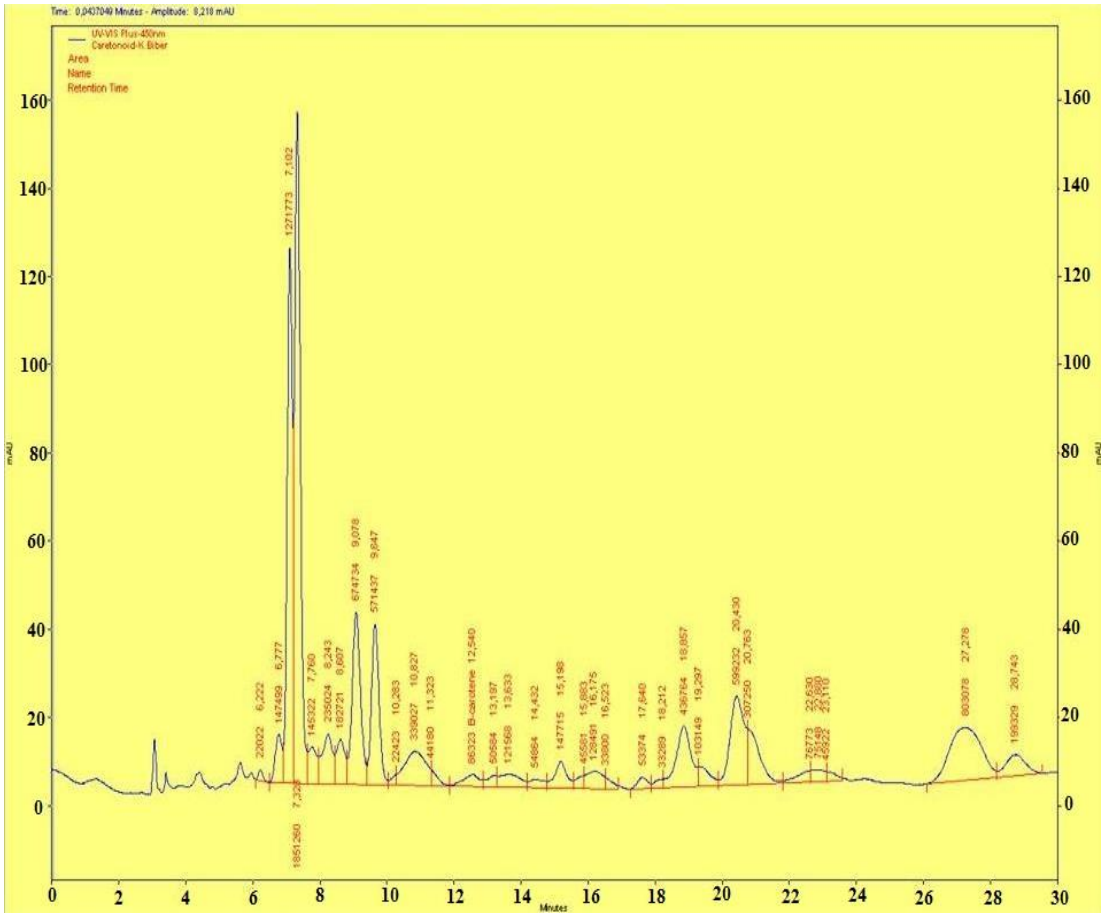

Figure 13. HPLC chromatogram for the extrudate sample at 130°C last zone temperature before digestion.

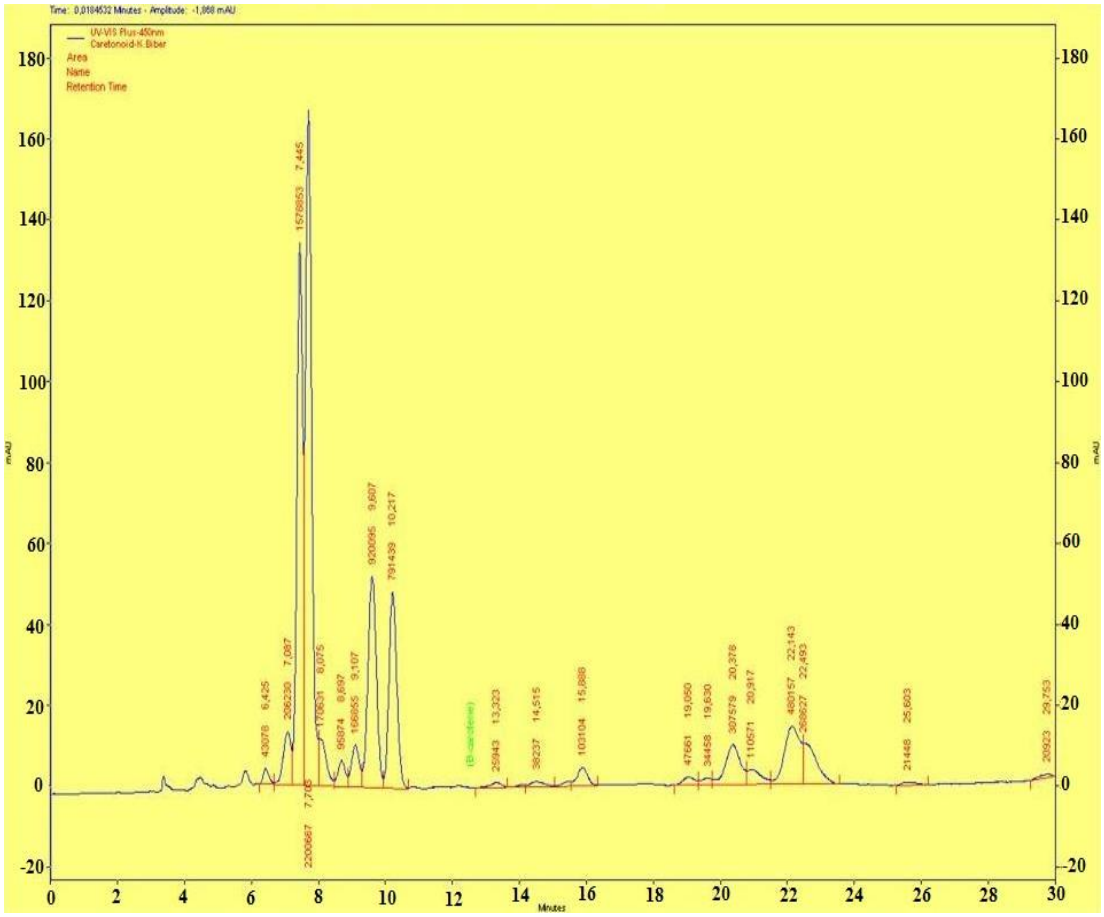

Figure 14. HPLC chromatogram for the extrudate sample at 160°C last zone temperature before digestion.

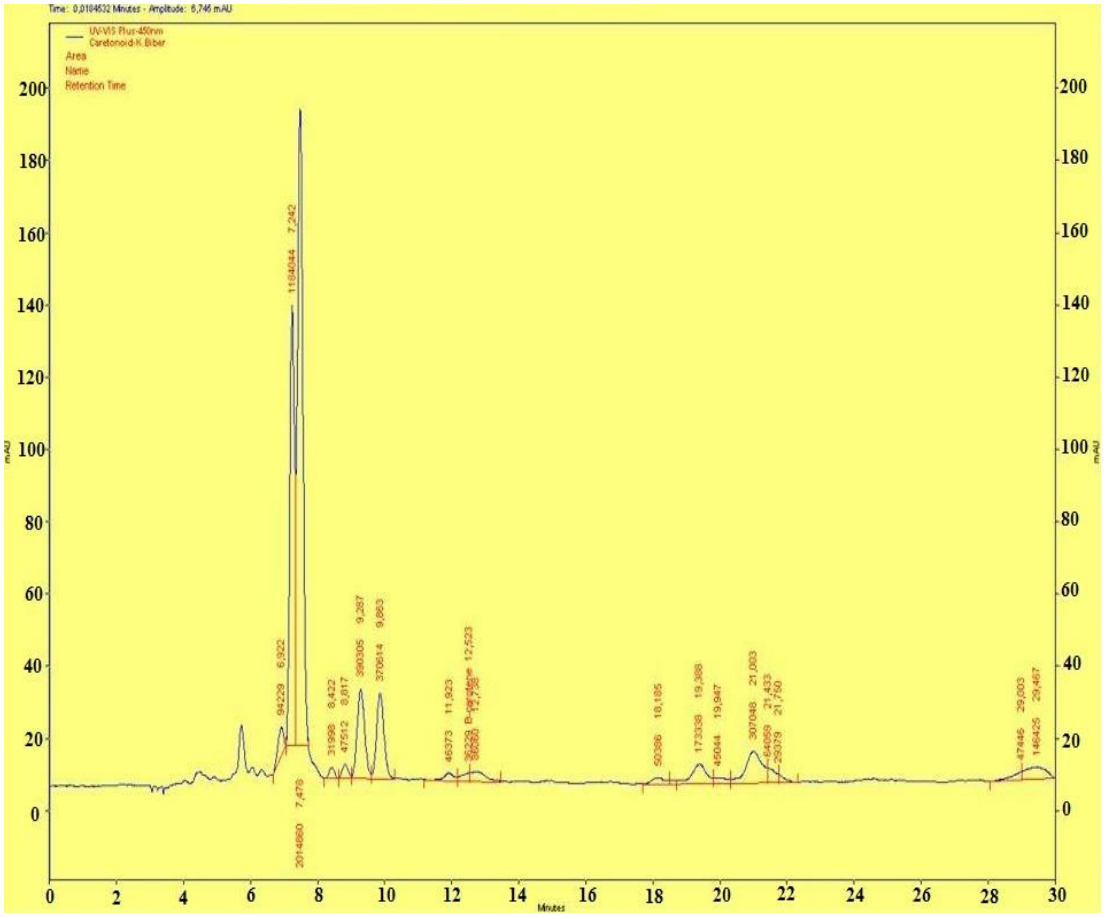

Figure 15. HPLC chromatogram for the feed sample of non-dialysable fraction in the small intestine.

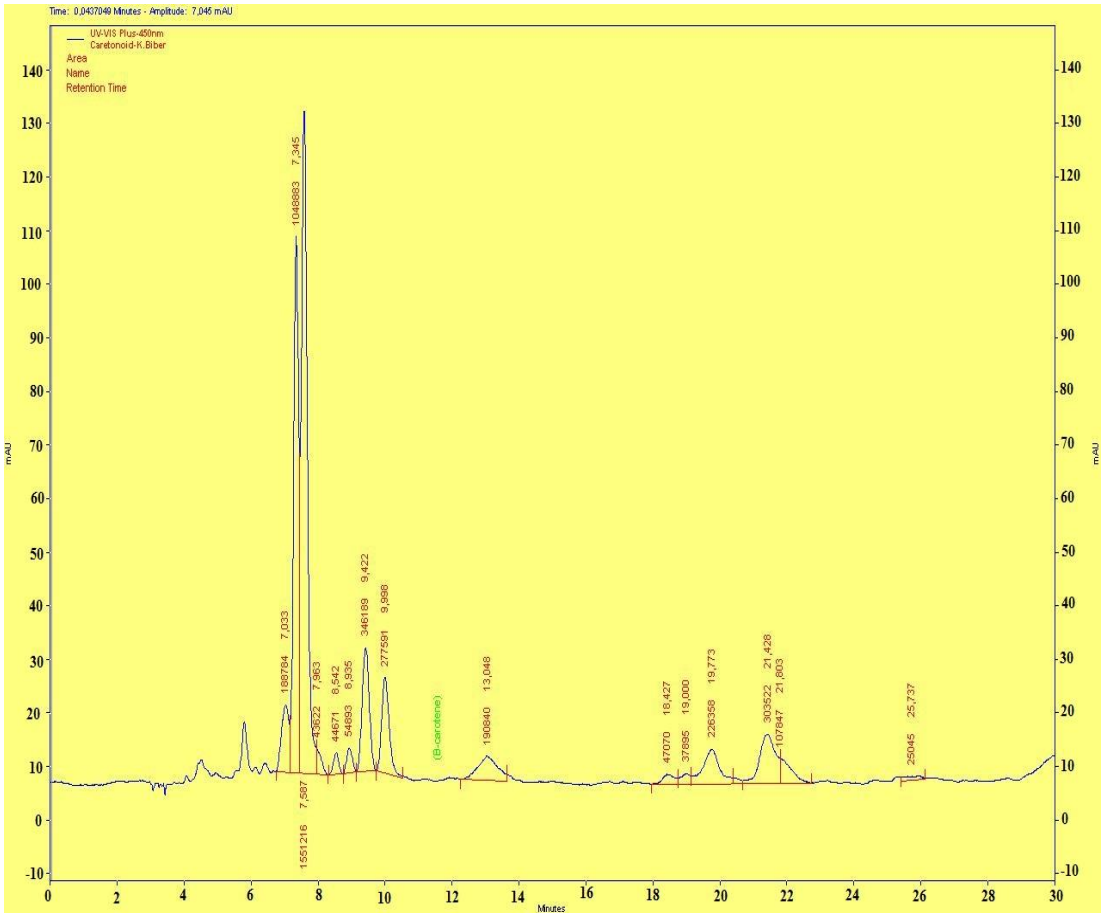

Figure 16. HPLC chromatogram for the extrudate sample at 130°C last zone temperature of non-dialysable fraction in the small intestine.

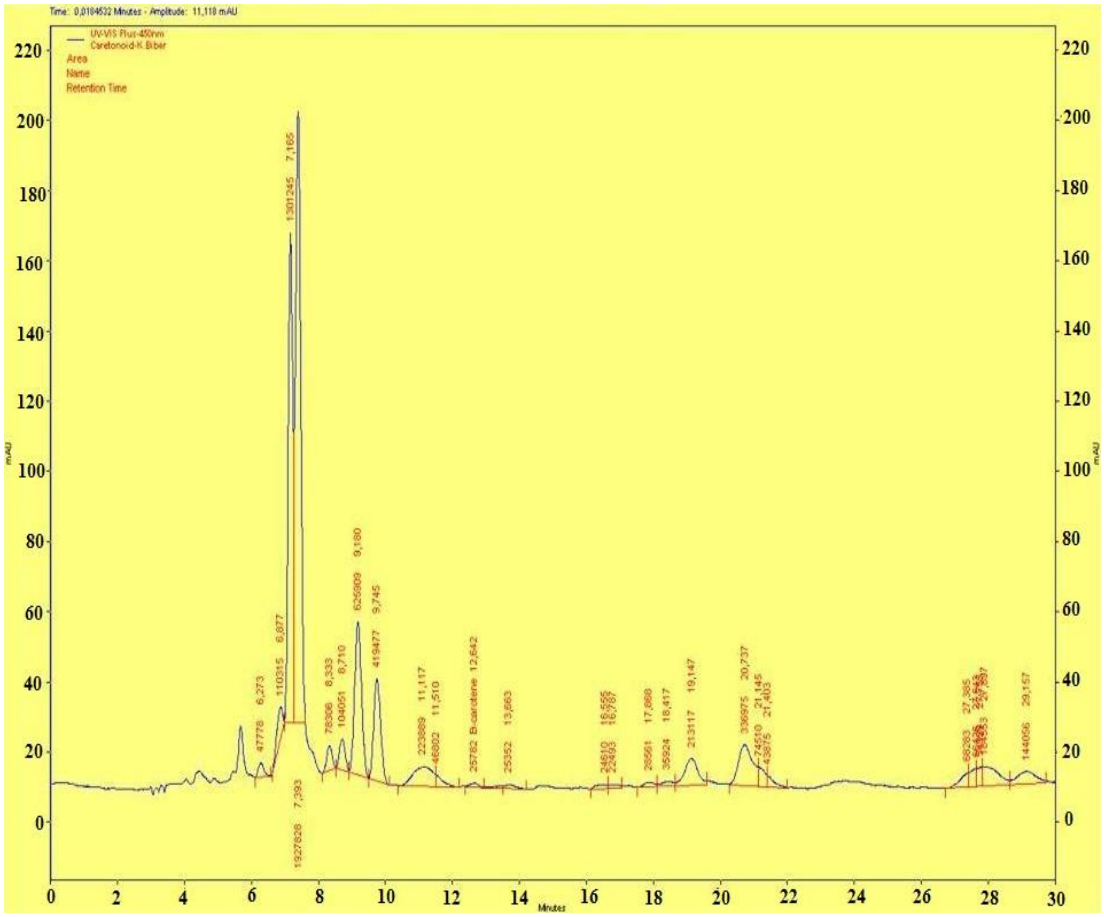

Figure 17. HPLC chromatogram for the extrudate sample at 160°C last zone temperature of non-dialysable fraction in the small intestine.

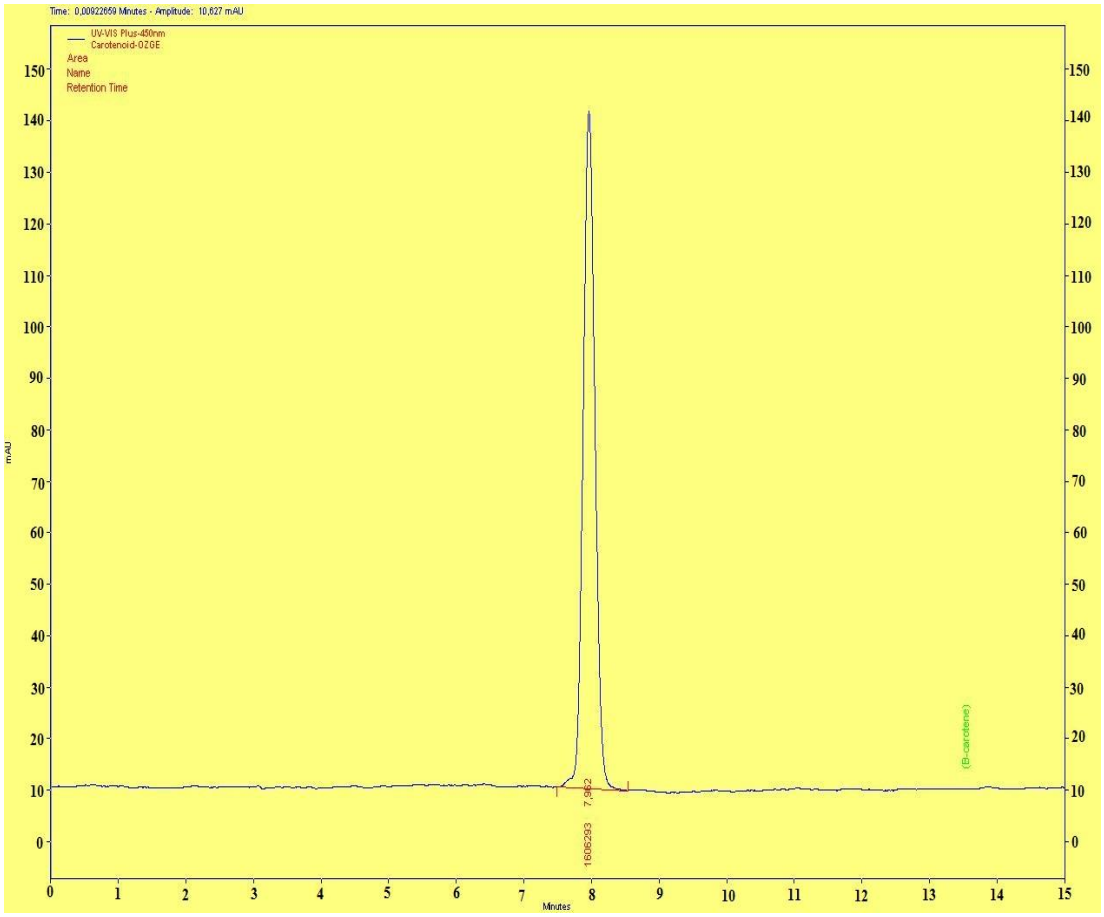

Figure 18. HPLC chromatogram for the standard zeaxanthin sample.

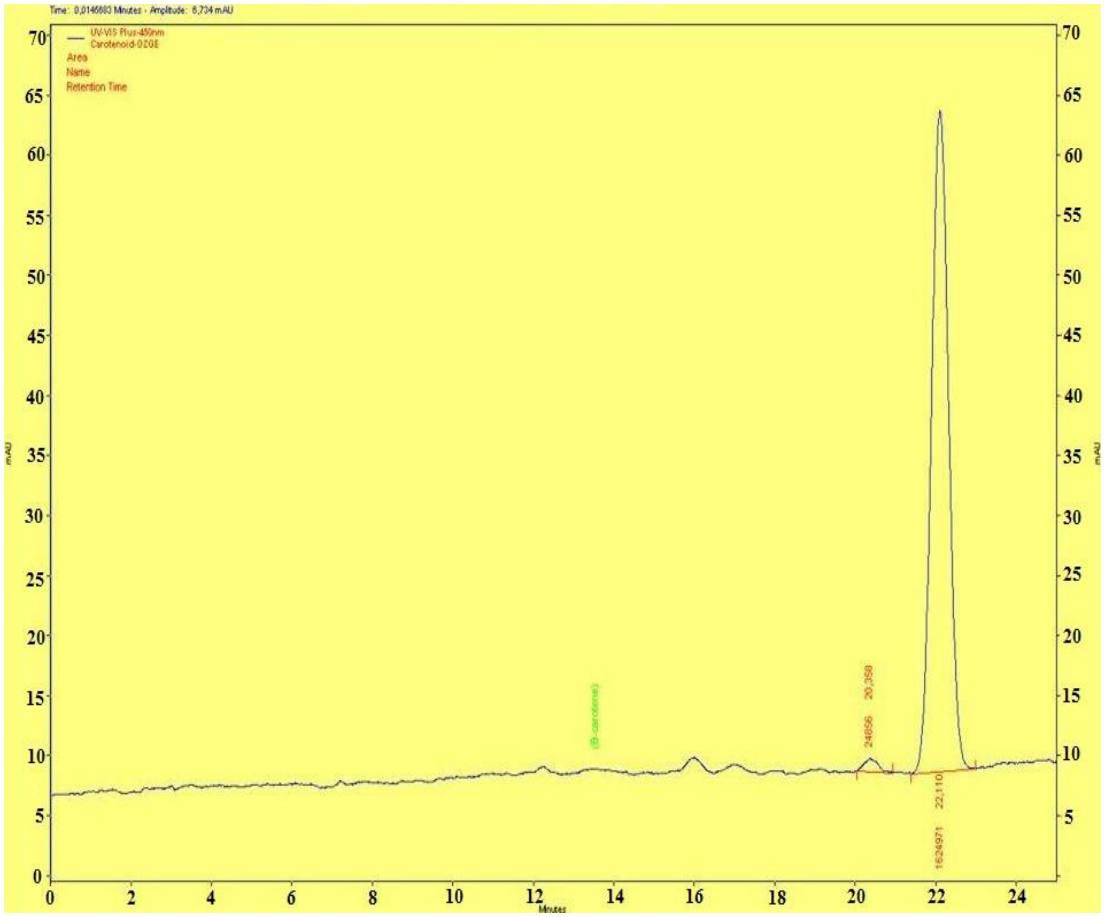

Figure 19. HPLC chromatogram for the standard β-cryptoxanthin sample.

APPENDIX E

STANDARD CURVES

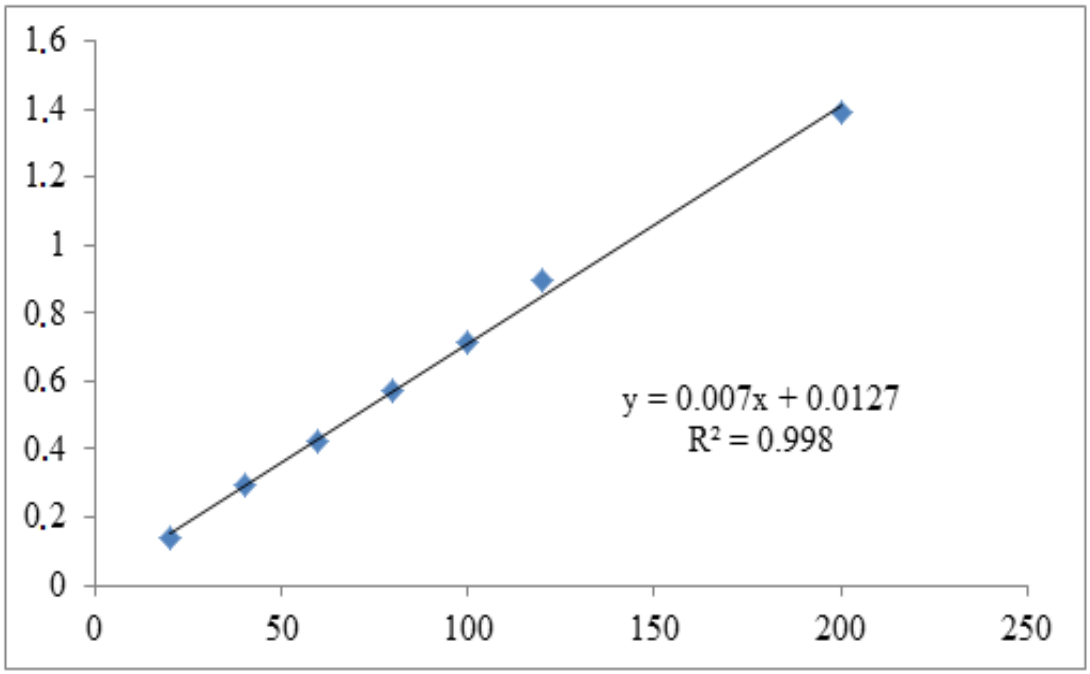

Figure 20. Calibration curve prepared by Gallic acid in ethanol for total phenolic content analysis.

ABS (at 725 nm) = 0.007 (mg GA / L) + 0.0127 $R^2 = 0.998$

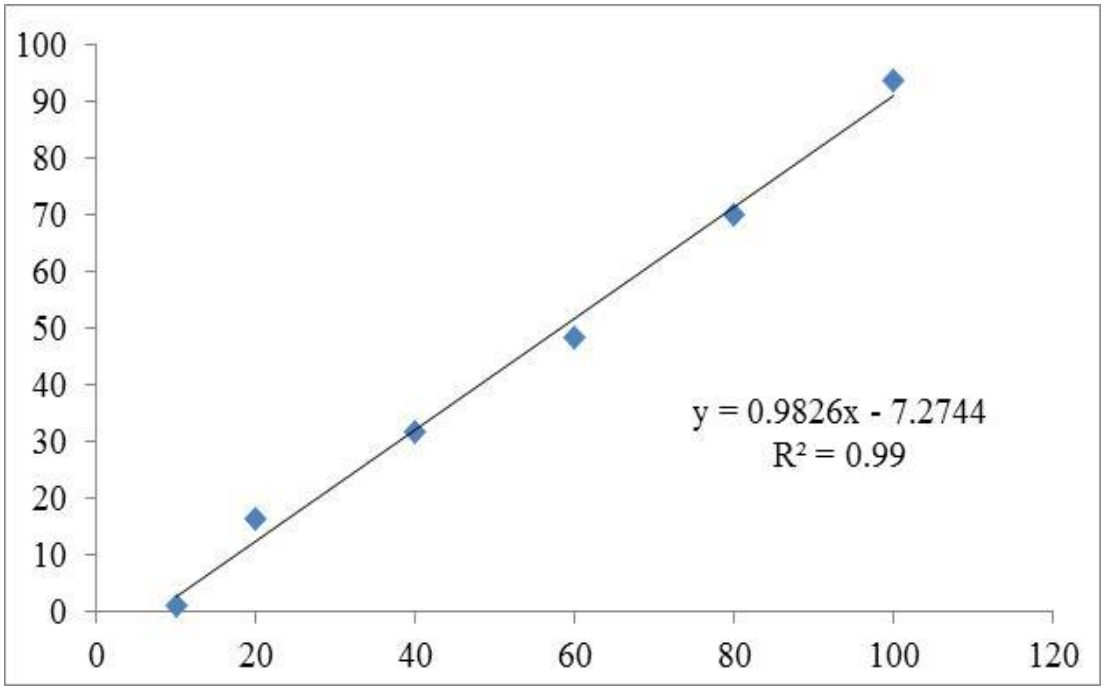

Figure 21. Calibration curve prepared by Trolox in methanol for antioxidant activity analysis.

Inhibition % = 0.9826 (μM trolox) - 7.2744 $R^2 = 0.99$

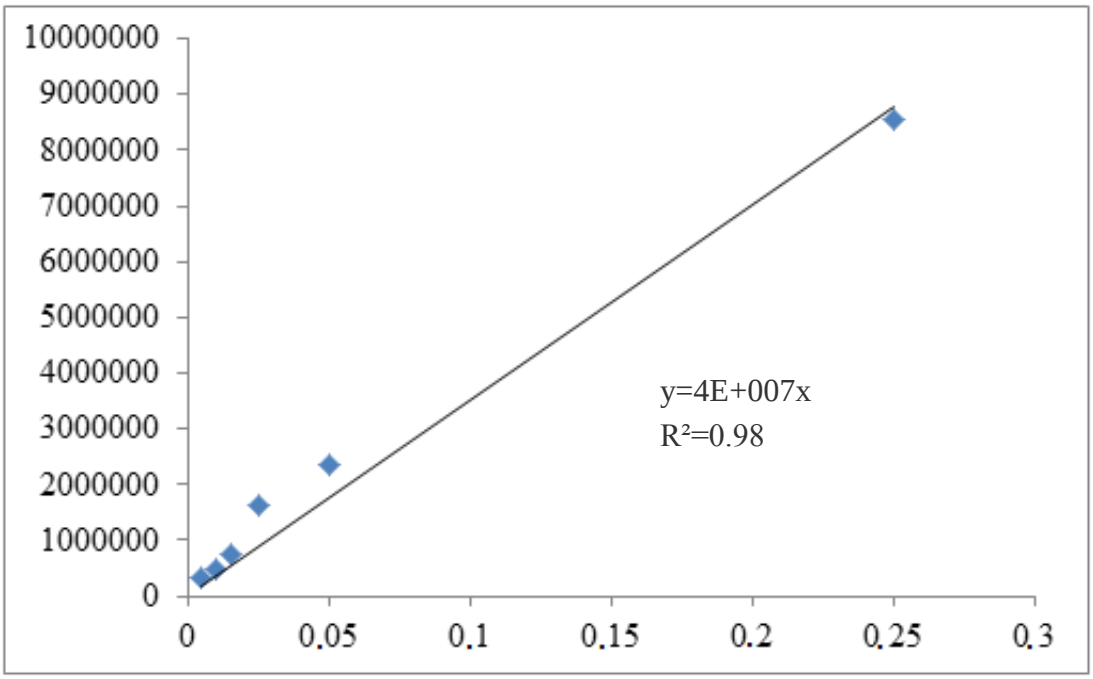

Figure 22. The standard curve of β-cryptoxanthin.

Area = $4*10^7$ (microgram β-cryptoxanthin) $R^2 = 0.98$

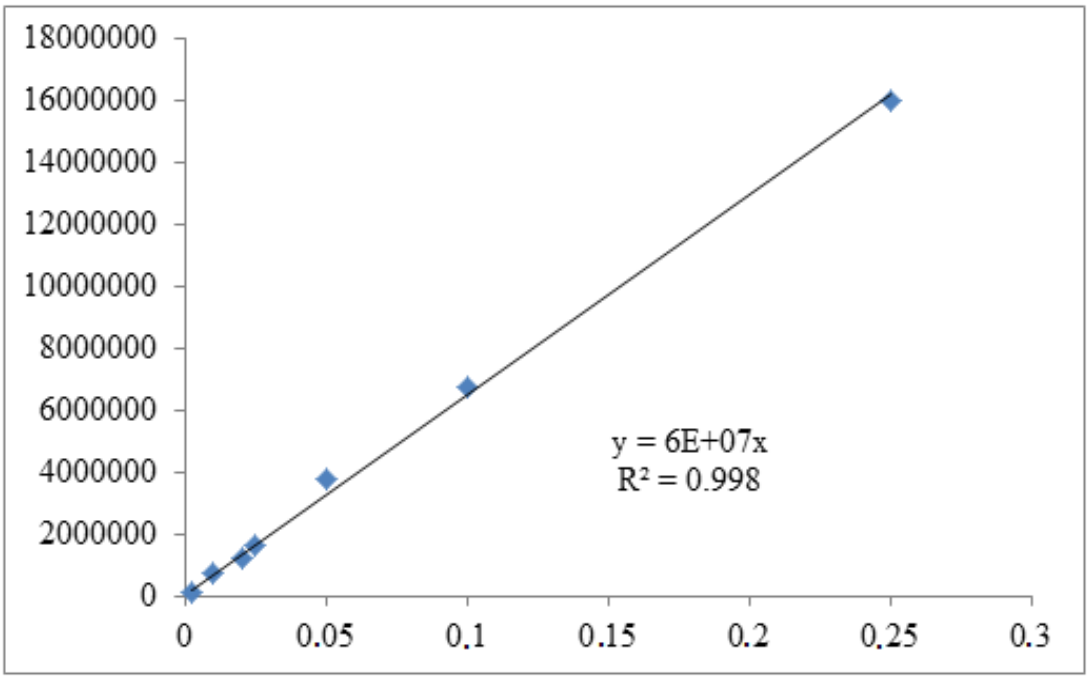

Figure 23. The standard curve of zeaxanthin.

Area = $6*10^7$ (microgram zeaxanthin) $R^2 = 0.998$