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

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

BADE TONYALI

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

FEBRUARY 2014

Approval of the thesis:

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

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

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

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February, 2014, 61 pages

As the health and food relationship became important, tomato pulp was added as a functional ingredient to the extrudates. Effects of extrusion on the functional properties of the extrudates and *in vitro* bioaccessibility of lycopene after extrusion were investigated. Two different temperature set was applied during extrusion: 80 °C, 90 °C, 100 °C and 130 °C (die: 116 °C) and 80 °C, 100 °C, 130 °C and 160 °C (die: 139 °C), screw speed and feed rate were kept constant, 225 rpm and 36 ± 1 g/min, respectively. Feed moisture content was adjusted to 30 ± 1 % with the addition of tomato pulp.

Antioxidant activity and total phenolic content were found to decrease after the extrusion process. The extrudates which were obtained at 160°C last zone treatment temperature were found to have higher antioxidant activity and total phenolic content compared to samples with 130°C last zone treatment temperature. Data indicates a complete gelatinization during extrusion. High-performance liquid chromatography (HPLC) analysis indicated that lycopene

content decreased after extrusion process when feed and extruded samples were compared. *In vitro* bioaccessibility of lycopene for the extruded samples with 160°C last zone temperature was higher than the feed and extruded samples with 130°C last zone treatment temperature.

Keywords: Extrusion, Antioxidant Activity, Bioaccessibility, Lycopene

ÖZ

EKSTRÜZYONUN DOMATES POSASI EKLENMİŞ ÜRÜNLERİN FONKSİYONEL BİLEŞENLERİ VE LİKOPENİN *IN VITRO* BİYOERİŞİLEBİLİRLİĞİ ÜZERİNE ETKİSİ

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Şubat, 2014, 61 sayfa

Sağlık ve gıda arasındaki ilişkinin öneminin artması sebebiyle, domates posası ekstrüde ürünlere fonksiyonel bileşen olarak eklenmiştir. Ekstrüzyonun fonksiyonel bileşenler üzerine etkisi ve ekstrüzyon sonrası likopenin *in vitro* biyoerişilebilirliği incelenmiştir. Ekstrüzyon sırasında iki farklı sıcaklık seti kullanılmıştır; 80 °C, 90 °C, 100 °C ve 130 °C (kalıp sıcaklığı: 116 °C) ve 80 °C, 100 °C, 130 °C ve 160 °C (kalıp sıcaklığı: 139 °C), vida hızı ve besleme hızı deney boyunca sırasıyla 225 dev/dk ve 36 ± 1 gr/dk olarak sabit tutulmuştur. Besleme ürününün nem miktarı domates posası eklenerek % 30 ± 1 olarak ayarlanmıştır.

Örneklerin antioksidan aktivitesi ve toplam fenol miktarının ekstrüzyon işlemi sonrası düştüğü gözlemlenmiştir. 160°C son bölge sıcaklığında alınan ekstrüde örneklerin 130°C son bölge sıcaklığında alınan örneklerden daha fazla antioksidan aktivitesi gösterdiği ve toplam fenol miktarı içerdiği bulunmuştur. Veriler ekstrüde örneklerde jelatinizasyonun tamamlandığını göstermiştir. Yüksek Performanslı Sıvı Kromatografisi (HPLC) analizi sonucu, ekstrüde örnekler ile

ekstrüzyon işlemi görmemiş örnekler karşılaştırıldığında, likopen miktarının ekstrüzyon işlemi ile azaldığı gözlemlenmiştir. 160°C son bölge sıcaklığında alınan ekstrüde örneklerdeki *in vitro* likopen biyoerişilebilirliğinin ekstrüzyon işlemi görmemiş örneklere ve 130°C son bölge sıcaklığında alınan ekstrüde örneklere göre daha yüksek olduğu gözlemlenmiştir.

Anahtar Kelimeler: Ekstrüzyon, Antioksidan Aktivitesi, Biyoerişilebilirlik, Likopen

To my beloved family…

ACKNOWLEDGEMENTS

I would like to thank my advisor, Asst. Prof. Dr. İlkay Şensoy for her support, guidance, understanding and energy during my study. She always encouraged me.

I offer thanks to The Scientific and Technological Council of Turkey (TÜBİTAK 110O792) for financial support during my thesis.

I would like to express my thanks to my laboratory mate, Çağla Çaltinoğlu who is always there for me and made my days energic and happy. Without her, the hours in the laboratory and the tiring analysis would be boring.

I would like to say thank to Hilal Işık, Selen Caner and Tuğba Pişkin. They are always helpful and show their love to me. I can't forget their support and believe in me.

I would like to extend my thanks to Ayça Aydoğdu, Emrah Kırtıl, Hazal Turasan, Sevil Çıkrıkcı, Oğuz Kaan Öztürk, Elif Yıldız, Sezen Dinçel, Hande Baltacıoğlu, Cem Baltacıoğlu, Betül Tatar and Sinem Acar for their friendship and suggestions. Moreover, I am lucky to have a chance to study with them. I can't deny help of Ece Bulut, Elçin Bilgin, Canan Peker, Eylül Turasan, Yavuz Ergün and Sertaç Özoğlu. This work would not be completed if they did not help me. Especially, my biggest thanks go to Hazal Turasan, Oğuz Kaan Öztürk, Ayça Aydoğdu, Sevil Çıkrıkcı and Emrah Kırtıl who bear me in my stressful days, support me and make me smile.

Finally, I would like to express my biggest and deepest gratitude to my family. My father, Cemil Tonyalı, my mother Seliha Tonyalı and my sister Gökcem Tonyalı always supported me, loved me and encouraged me. Without them, I could not come so far. They always respect my decisions and are on my side at everything I choose, the words are not enough to express my gratitude and love to them. I dedicate this study to them.

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INTRODUCTION

1.1 Functional foods

In a conventional food product, important characteristics are giving pleasure, providing energy to body and contributing to the health (Guo, 2009). The term "functional foods" has a wider meaning. The expectations from the functional food are to have all these properties that conventional foods have and be able to decrease the risk of diseases (Guo, 2009).

The functional foods are called with some other names such as nutraceuticals, designer foods, pharmafoods, medifoods and vitafoods (Roberfroid, 2000). Functional foods have the characteristics of enhancing health benefits and preventing diseases; while designer foods are mostly improved nutritionally by the addition of phytochemicals or minerals (Jackson & Paliyath, 2011). Furthermore, the term "dietary supplements" which are products (mostly the pills) that contain nutrition or vitamin that is needed by body and advised to be taken into body daily can be categorized into a group called FOSHU (foods for specified health use) which was launched by Japans (Roberfroid, 2000). The functional foods are in this category with dietary supplements (Roberfroid, 2000). In general, for the functional foods, the definition can be widened that they are products that contain some ingredients or some external healthful substances which are added to these products and have an impact on human health (Roberfroid, 2000). The characteristics of being suitable for daily consumption as part of a balanced diet, containing natural substances (the added nutrients are not synthetic), providing an extra benefit with the addition substances, increasing the

life quality with improving the health and reducing the disease risks differentiates the functional foods from the rest (Roberfroid, 2000).

With increasing awareness, consumers are now more careful what to eat as it is highly related with human health, body development and preventing the illnesses. In recent years, the increase of medical care expenses, extension of the knowledge of science, the increase of expectations from life styles and lastly, the rapid development of technology to meet all these needs are the main concerns of society (Roberfroid, 2000). In fact, the expectation from nutrient in these days is actually the definition of functional food; optimization of increasing health benefit and decreasing the risks of diseases (Roberfroid, 2000).

Fruits and vegetables are known to be rich in vitamins, antioxidants, minerals and fibers, yet they provide low energy to body as compared to other foods as they contain a high amount of nondigestable carbohydrate such as cellulose and pectin (Paliyath & Shetty, 2011). Phytochemicals are found in vegetables and fruits and have the role of preventing diseases; polyphenols and carotenoids are some examples (Jackson & Paliyath, 2011).

According to The World Vegetable Center's Progress Report of 2004, the following vegetables are reported as to have high antioxidant activity that can be counted in top ten, sweetpotato leaf, ginger, amaranth, spinach, eggplant, pak-choi, leafy Chinese cabbage, tomato, Welsh onion, and kangkong (Easdown & Kalb, 2004).

1.2 Extrusion

The extrusion process has been popular for a long time. It has been used for mixing, conveying, forming and cooking in the production of many products such as macaroni, spaghetti or ready-to-eat cereals. In ready-to-eat products, the process gives energy to the feed and with high temperature and pressure, the chemical and physical changes occur. Starch gelatinization, expansion and protein

denaturation are some examples of these changes. Extruded products are popular among the society because they are ready to eat and have long shelf life. The characteristics that separate the extrusion process from other conventional processes are that it is appropriate for low moisture contents and it is a continuous process (Guy, 2001).

Extruders are machines that produce products in different size, shape or texture. They have screws that push the feed, which is mostly dough, through the rollers and pass from the die (Guy, 2001). The low moisture feeds generally less than 20% were heated over 150°C with the conversion of mechanical energy coming from the screws to heat (Harper, 1992). The feed which becomes plasticized with the heat is pushed from the die and with the rapid decrease in pressure the feed puffs while the moisture of feed turns into steam (Harper, 1992). With the usage of high temperature and shear, the processing time can be decreased and the feed can be transformed to its final shape within 30-120 seconds (Guy, 2001).

The water vapor and the melted feed during the extrusion help the extruded products to have their final structure. It is basically a foam structure that occurred with the melted feed surrounding the bubbles of water. When the superheated water in the extrusion encounter with atmospheric pressure, the bubbles enlarge. These bubbles burst when they expand too much. Due to the evaporation of water, the temperature falls quickly. With loosing moisture, the structure of product becomes rigid (Guy, 2001).

As the human beings have difficulties to digest the ungelatinized starch, extrusion cooking provides an advantage of gelatinization even with foods with low moisture content (Camire, 2001). The starch granules in feed are exposed to heat which makes them to gelatinize with the presence of water. The conditions of extrusion, pressure, high temperature and shear stress due to screws have the increasing effect on gelatinization degree (Camire, 2001). Starch digestibility is decreased by the presence of amylose-lipid complex (Camire, 2001). Screw speed of an extruder has an important role on the product by affecting the residence time

and shear stress applied on the product (Frame, 1999). With the combination of screw speed and moisture, the generated heat causes starch to melt (Frame, 1999).

The water content is really important in extrusion for the starch gelatinization, denaturation of protein components and the elastic behavior of the product (Kazemzadeh, 2012). If the moisture content of the feed is increased, the viscosity of the feed decreases and the shear due to screws is decreased (Kazemzadeh, 2012). As a result; due to decrease in shear, the starch degradation and protein denaturation are reduced (Kazemzadeh, 2012). Moreover, as the melt viscosity reduces, the product temperature decreases and product density is increased (Yacu, 2012). The effect of moisture content on the die pressure is high enough that can not to be negligible and inversely proportional, the rise in moisture content causes decrease in die pressure (Yacu, 2012).

Mostly the high temperature, but also the shear and pressure cause the enzymes and proteins denature, increase digestibility of these products (Camire, 2001). The reducing sugars occurred due to the degradation of starch, can react with lysine can lower the nutritional value of protein (Camire, 2001). If the feed has low moisture content, with the help of high temperature, formation of Maillard reactions and its products extends (Camire, 2001).

The cereals that are products of extruders are rich in starch (Moore, 1999). The starch content plays an important role in the texture and structure of the final product (Moore, 1999). Despite the high starch content of the cereals, the amounts of sugar, vitamin and mineral are low (Moore, 1999).

Extruders can be categorized by their mechanical energy generation (Frame, 1999). In low shear screw extruders, the cooking of dough is not wanted thus the mechanical energy is tried to be kept minimum to prevent cooking, this process is used in pretzels and pasta production while in high shear screw extruders, the mechanical energy is converted to heat and used for the cooking of dough which is wanted, this type of extruders are used for the production of pet foods, puffed snack foods and breakfast cereals (Frame, 1999). In the high shear extruders, the energy is highly related with the viscosity of dough, rate of screw speed and

pressure in the extruder (Kazemzadeh, 2012). To be used in the cooking of raw feed, the high shear extruder is a good option (Kazemzadeh, 2012).

Co-rotating extruders have the advantage of performing at higher screw speeds than the counter rotating ones as the distribution of radial forces are more uniform (Frame, 1999). Its advantage over the single screw extruders is the small residence time and better conveying (Frame, 1999). Between the extruder types, the co-rotating extruders have the capacity of serving wide variety of product type (Frame, 1999).

What is the main difference between single screw extruder and twin screw extruder? As the name implies, basically the twin screw has two screws. Moreover, the flow of the feed in the extruders shows differences (Yacu, 2012). The flow in single screws is based on the frictional drag force and viscous drag flow throughout the extruder where in the twin screw extruders; the frictional flow is not needed because of the second screw (Yacu, 2012). One of the disadvantages of single screw is that the heat transfer from the jacks to the barrel is poor as the mixing in the channel is inadequate and the convection heat transfer is low (Harper, 1992). The mixing and uniformity of shear rate is much better at twin screw than the single screw (Harper, 1992). The processing limit for moisture is 12-35 % for single screw whereas 6% to very high limits for twin screw (Harper, 1992). The twin screw extruders have the ability of working with different size of grinding (Riaz, 2001). Also, the materials with high content of oil, high viscosity and moisture content can be worked with twin screw extruders (Riaz, 2001).

Among the twin screw extruders; in the counter-rotating twin screw extruders, the feed is kept between the screws, the flow of the feed is not free as in co-rotating extruders and within the limited space, the feed is allowed to flow in C shape (Yacu, 2012). Although the die pressure is more tolerable in these extruders, the mixing of feed is not fully due to C shapes (Yacu, 2012). On the other hand, in the co-rotating twin screws, the flow of feed can be described as 8 shape with changing the direction and surface renewal (Yacu, 2012). This 8 shape has the advantages of providing uniform heat distribution, good mixing, shear stress

distribution, high melting capacity and finally good melting temperature control (Yacu, 2012). These features are not applicable for counter rotating, the shear stress is not uniform as the feed is trapped between screws (Yacu, 2012). As a result of this, the counter rotating extruders are worked at low speed and high pressure (Yacu, 2012). This limited parameter conditions of this kind of extruders – counter rotating- made them mainly in the usage of production of low viscous high pressured products such as candy or liquorish (Yacu, 2012).

Before the evolution to twin screw extruders, the single screw extruders were already in use. One of the reasons of choosing twin screw over single screw is that the operational capability of twin screw extruders is much better than the single screw extruders (Harper, 1992). With this feature, the twin screw can be used for confectionary area such as carmels, toffees, clear hard candies, wine gums and licorice, expanded cereal bases for candy production and conching chocolate (Harper, 1992). Furthermore, it is possible to use twin screw in cookie or cracker baking line which results in the decrement in equipment cost and energy (Harper, 1992).

If the extrusion feed samples are classified according to the moisture contents, there will be 3 groups; low moisture feed which is maximum 25% moisturized, intermediate group which is between 25-50% moisturized and lastly the high moisture feed that is above 50% (Bhattacharya, 2012). Mostly, the ready-to-eat cereals are categorized in the low moisture feed group. The processed products of intermediate moisture feed expand as they go out from the die; however collapses after a while forms a high density product (Bhattacharya, 2012). The products of this group may need extra drying because the moisture content is not low to prevent the microbial growth (Bhattacharya, 2012).

The advantages of ready-to-eat (RTE) cereals are being easy to transport, having long shelf life and lightweight (Fast, 2000). There are two techniques to cook RTEs, the first one is injection of steam directly to rotating vessels which contain grains and secondly, the continuous extrusion cooking (Fast, 2000).

The materials used in cereal industry are generally corn, wheat or rice, they mostly contribute to the diet in the form of protein and carbohydrates (Henry, 2001). The carbohydrates that are found in cereal grains are in the form of simple sugar, polysaccharide (starch) and oligosaccharide (fructan) (Henry, 2001). The starch has the lead role in the nutritional quality as the snacks are dense in starch content (Henry, 2001). Moreover, the starches that are not digested in gut, resistant starches, are known to be effective in the prevention of certain diseases such as heart diseases (Henry, 2001). The soluble and insoluble fibers are separately important, soluble fibers decrease the risk of heart diseases, the insoluble fibers prevent the risk of colonic cancers (Henry, 2001).

Corn is the most used cereal in the confectionary area. The low cost of corn and having good expansion capacity makes the corn popular (Moore, 1999). The corn grit is a logical choice to use in the extrusion as the cost is low, availability and processing are easy (Bhattacharya, 2012). The protein content of the corn grit can vary between 6% and 9% and fat and ash content are considerably small (Bhattacharya, 2012). The component data for corn grit is given in Table 1.

Table 1: The component percentages of corn grit adapted from Caldwell et al., (2000).

Component	**Corn grit**
Moisture, %	11.7
Protein, %	7
Fat, %	0.6
Crude Fiber, %	0.2
Ash, %	0.2
Starch, %	78.3
Other polysaccharides, %	2.0

1.3 Tomato

The tomato *(Solanum lycopersicum)* is recorded in the Solanaceae family in which some other popular vegetables like pepper, eggplant and potato belongs (Diez & Nuez, 2008). For the consumption of tomato as a vegetable (non-processed), both the internal and external characteristics are important for consumer (Diez & Nuez, 2008). The external characteristics can be classified as size, shape and color while the internal characteristics have two subgroups (Diez & Nuez, 2008). One of these subgroups is organoleptic which is related with flavor, aroma, firmness, texture or acidity (Diez & Nuez, 2008). The second subgroup is the nutritional quality of tomato. With the help of the parameters of °Brix and pH, the nutritional value of a tomato can be analyzed (Diez & Nuez, 2008). The content of organic acid and reducing sugar directly affects the flavor (Diez & Nuez, 2008). On the dry basis, reducing sugars are in the percentage of 50, while the organic acids like malic and citric are 10% where the citric acid is much more than the malic acid (Diez & Nuez, 2008; Henriques da Silva et al., 2008). The remaining part is composed of proteins, pectin, cellulose, hemicellulose, minerals, pigments, vitamins and lipids (Henriques da Silva et al., 2008). The tomato is rich in aroma with having 400 volatile compounds, 30 of these 400 are dominant of the aroma development (Diez & Nuez, 2008). The color development is accompanied with red color of lycopene and orange-yellowish color of β-carotene (Diez & Nuez, 2008). A tomato is mostly composed of water, the remaining dry part, which is about 5-10 %, have soluble substances in the 75 % whereas the skin and seeds have about 1-3 % (Shi & Le Maguer, 2000). The water content of tomato is about 93-95% (Henriques da Silva et al., 2008). The tomato is known to be rich in antioxidants. The following table (Table 2) shows the composition of chemical substances of tomato (Guo, 2009):

Table 2: The chemical composition of tomato adapted from Guo (2009).

Constituent	Range
Moisture (%)	93.1-94.2
Protein (%)	0.7-1.0
Ash (%)	0.40-0.52
Ascorbic acid (mg/100g)	16-24.2
Vitamin E (mg/100g)	0.80-1.22
β-carotene (mg/100g)	0.30-0.52
γ-carotene (mg/100g)	0.04-1.61
Phenolic (mg/100g)	8.4-17
Lycopene (mg/100g)	0.90-9.30
Lutein (mg/100g)	0.04-0.10
Phytoene (mg/100g)	0.49-2.80
Na (mg/kg)	102-186
K (mg/kg)	2158-3192
Ca (mg/kg)	38.4-58.0
Mg (mg/kg)	63.3-96.1
Fe (mg/kg)	0.44-2.58
Cu (mg/kg)	0.19-0.71
Zn (mg/kg)	0.67-1.01
Mn (mg/kg)	0.45-0.67
pH	4.06-4.22
Brix degree	4.50-6.62
Refractive index	1.3395-1.3427
Acidity (%)	0.48-0.56

The tomatoes have low composition of fat, calories and cholesterol; however they are rich in fiber, protein, vitamin A and C, lycopene, β-carotene and potassium (Shi & Le Maguer, 2000). Although the vitamin A is in high concentration, vitamin B_1, B_2, B_3, B_6, niacin, folic acid, biotin, vitamin C (ascorbic acid) and

vitamin E (α-tocopherol) are not less important (Henriques da Silva et al., 2008). The lycopene is known to be predominant (Henriques da Silva et al., 2008).

1.4 Lycopene

The red color of tomato comes from lycopene; moreover among the total carotenoid content, lycopene leads with the percentage of 75-83 (Henriques da Silva et al., 2008). Lycopene can be in high amounts in a few fruits or vegetables (Henriques da Silva et al., 2008). The characteristic that makes tomato and its product to distinguish from other fruits and vegetables is that the lycopene content (Guo, 2009). Of course, the tomato is not the only source of lycopene but without a doubt it is the best source. Other fruits such as watermelon, pink guava, pink grapefruit, strawberry or papaya which are the other sources for lycopene have less lycopene when they are compared with tomato (Guo, 2009).

It is indicated that intake of lycopene in moderate amounts decreased the risk of cardiovascular disease, prostate cancer and gastrointestinal tract cancer (Guo, 2009). According to Health Professional Follow-up Study, the comparison between no serving and 2-4 servings of raw tomato is showed that with the consumption the risk for prostate cancer decreased by 26% (Guo, 2009). Furthermore, the study for processed tomato products indicates that the consumption of these products have a decreasing effect on the risk too (Guo, 2009). The carotenoid composition in a tomato is shown in Table 3.

Table 3: The composition of different carotenoid species in a tomato given by Zhang et al., (2008).

Carotenoid Species	Composition (%)
Lycopene	80-90
α-carotene	0.03
β-carotene	3-5
γ-carotene	1-1.3
ξ-carotene	1-2
Phytoene	5.6-10
Phytofluene	2.5-3.0
Neurosporene	7-9
Lutein	0.011-1.1

The lycopene content of the tomatoes can show differences among the different cultivars but in general, it is stated to be 48 to 141 mg kg^{-1} in peel whereas in pulp the amount is 20 to 69 mg kg^{-1} on a fresh weight base (Zhang et al., 2008).

It is known that the carotenoids help the color formation, it is due to their conjugated double bonds (deMan, 1990). Increase in the number of conjugated double bonds causes the color to be more red (deMan, 1990). Even for development of yellow color, the needed number of double bond is seven (deMan, 1990). The double bonds can be in different chemical configuration, trans or cis. The carotenoids in the foods usually show trans configuration (deMan, 1990). Configuration is important because the color of fruit is affected with this orientation (deMan, 1990). Trans bonds have the effect of darkening the color while cis bonds gave the lighter color to foods (deMan, 1990). With some external modifications such as heat, light and acid, the bonds can change from cis to trans or vice versa (deMan, 1990).

Carotenoids are not only important for color properties but also they are important for the diet; such as having vitamin A activity (Cemeroğlu et al., 2001). As the

fruits and vegetables mature, the level of activity of provitamin A increases (Cemeroğlu et al, 2001). For example, the samples from matured tomato have higher total carotenoid than the ones from semi-mature (Liu & Luh, 1977).

As the carotenoids are the substances which contain double bond, they can easily be oxidized (Cemeroğlu et al., 2001). The tendency of carotenoids in the living tissue to oxidize is different from the carotenoids in the solution (Cemeroğlu et al., 2001). The reason is that with the help of some preservatives in the tissues and limited permeability of the cell wall, the carotenoids in the living tissues are more resistant (Cemeroğlu et al., 2001).

Lycopene has no provitamin-A activity (Nguyen et al., 2001). In fresh tomato, lycopene is mostly found in the all-*trans*-lycopene form (Shi & Le Maguer, 2000). Lycopene is unstable to the heat and light. Like β-carotene, lycopene is also affected negatively from the oxidation and isomerization. During the processes, the energy is given and all-*trans*-lycopene is converted to *cis*-lycopene, which is the form of unstable and high energy (Shi & Le Maguer, 2000). The applied heat during process causes this conversion, moreover as the time and temperature increase, the *cis*-isomers increases (Shi & Le Maguer, 2000). It is mentioned that absorption of *cis* lycopene is easier than *trans* lycopene in the body (Guo, 2009).

The bioavailability of lycopene is dependent on many factors. In contrast to the stability, bioavailability of cis form is higher than the trans form (Shi & Le Maguer, 2000). Furthermore, the bioavailability of unprocessed tomato products is lower than the processed products (Shi & Le Maguer, 2000). The reason could be the damage of the cell wall which results in loose bonds between the lycopene and tissue, consequently lycopene becomes more accessible (Shi & Le Maguer, 2000). Furthermore, the structure and texture of the food material affects the bioavailability (Shi & Le Maguer, 2000).

The lycopene content of the tomato and its products changes in a wide range. Mainly the content depends on whether they are processed or not. In Table 4, the lycopene contents of various tomato products are depicted.

Table 4: The tomato products and their related lycopene amounts (Guo, 2009).

Tomato products	Lycopene (mg/g weight)
Fresh tomatoes	8.8-42
Cooked tomatoes	37
Tomato sauce	62
Tomato paste	54-1500
Tomato soup (condensed)	19.9
Tomato powder	1126-1264.9
Tomato juice	50-116
Pizza sauce	127.1
Ketchup	99-134.4

The lycopene is reported to be in highly concentrated in the skin and pericarp of tomato (D'Souza et al., 1992). When the amounts of lycopene in the skin and whole tomato are compared, it was seen that the skin has 12 mg lycopene/100 g skin (wet basis) where whole tomato has 3.4 mg lycopene/100 g (wet basis) (Al-Wandawi et al., 1985). Thus, it can be concluded that the skin is richer in the lycopene amount.

1.5 Phenols

Phenolic substances contain an aromatic ring that one or more hydroxyl group is attached to that ring (Vermerris & Nicholson, 2008). These substances are weak acids due to their aromatic ring and they are mostly found in fruits (Vermerris & Nicholson, 2008). In plants, the phenolic substances take the role to defend the plant against environmental and biological stress (Vattem & Shetty, 2006). Furthermore, they have a very important role about reproduction and growth of plant (Shahidi & Naczk, 1995). But their importance to the consumers is that they provide health benefits and they are found in the fruits and vegetables in large

amounts (Shahidi & Naczk, 1995). Apples, cranberries, cabbages and barley are some examples of food materials that are rich in phenolic compounds (Vattem & Shetty, 2006). Flavonols are mostly found phenolic substances in fruits where the cereals and legumes contain flavonoids, tannins and phenolic acids (Vattem & Shetty, 2006). Besides flavonols, fruits and vegetables are also rich in polyphenolic compounds (Vattem & Shetty, 2006). Flavonoids such as quercetin are the most known and widely found group of phenolic phytochemicals (Vattem & Shetty, 2006).

The phenol compounds in the tomatoes are mostly conjugate forms of flavonoids (quercetin and kaempferol) and these substances are mostly found in the skin of tomato (Kaur & Kapoor, 2008). Beside the flavonoids, water soluble phenolics and caffeic and ferulic acids can also be found in the skin (Kaur & Kapoor, 2008).

Lycopene is counted as the most effective antioxidant within the carotenoid family (Guo, 2009). However, the tomato does not contain only lycopene, the other antioxidants such as flavonols are also high in concentration (Guo, 2009). The preform of flavonols is quercetin that is found in tomatoes. However the main flavonol in tomato is rutin (Guo, 2009).

With the application of some processes such as frying or boiling, the quercetin level in tomato is decreased by 35-78%, the reason of the decrement can be the degradation of flavonols or diffusion of flavonols to water (Guo, 2009). However, the flavonols are in high amount in tomato juice and puree (Guo, 2009). In these processing procedures, the level of quercetin increases (Guo, 2009).

Among the cereal flours, the corn flour is known to have highest phenolic content (Shahidi & Naczk, 1995). The total amount of total phenolic acid in corn is about 309.1 μg/g which the ferulic acid contributes the most (Shahidi & Naczk, 1995).

The phenolic compounds are helpful with the prevention of oxidation (Camire, 2001). They are important for both oxidation prevention and microbial safety (Shahidi & Naczk, 1995). They show antioxidant characteristics.

1.6 Antioxidants

Oxygen is the most important thing for humans. With the oxidation reaction in human body, the oxygen-derived free radicals can be formed which are not good for the cell (Guo, 2009). Free radicals damage some cellular enzymes and destruct the cell by damaging the proteins, membrane lipids or even DNA which result in finishing the respiration of cell (Guo, 2009).

The term autoxidation is defined as the chain reaction that causes the degradation of hydrocarbons in the lipids, proteins, DNA and some other materials in the living organisms (Guo, 2009). When the oxygen and organic molecules meet, the reaction takes place, the peroxy radicals are formed and these radicals lead to autoxidation (Guo, 2009). In the food, the naturally present antioxidants in the system play the role of guard against oxidation to some level (Guo, 2009). However, if the food is processed or stored, these antioxidants are generally degraded (Guo, 2009). With the loss of antioxidants, the lipids which are now vulnerable to oxidation are easily degraded with the presence of heat, light, ionizing radiation, trace metals, metallo-proteins and enzymatically by lipoxygenase (Guo, 2009).

All the unwanted effects of oxidation can be prevented by some level with the consumption of antioxidants. The antioxidants are mostly used to inhibit the off-flavor, rancidity and to keep the nutritional value as high as possible (Guo, 2009).

Moreover, the adverse effect of oxidation is not limited with lipids. The inhibiting result of oxidation to DNA and protein can be more dangerous than the oxidation of lipids especially in gastrointestinal tract and the tissues of body (Guo, 2009). The reason is actually maybe the biggest health problem in these days: cancer. The reflected result of damaged DNA to the body is more serious than the oxidized lipids (Guo, 2009). The damaged DNA is highly responsible for development of cancer cells (Guo, 2009).

The DNA damage is generally counted as having a major role in the tumor occurrence (Guo, 2009). Thus; the antioxidants are important because they have the ability to prevent the cancer. Consuming the antioxidants from an external source and absorbing them is an easy way to access them.

The benefits of antioxidants can be counted in four ways. The first one is the antioxidants protect the vegetable or fruit itself from the oxidation reaction (the antioxidants in spices reduce the oxidation in the foods), the second one is with the absorption of antioxidants, the oxidation reaction in the body is delayed. Thirdly, they can be absorbed by the body even in the gastrointestinal tract. Lastly, they can be used as anti-inflammatory, anti-ischemic and antithrombotic agents; such as in the herbal medicines (Guo, 2009).

1.7 Food Digestion

The "digestion" is basically breaking the large food molecules into the smaller compounds which makes the absorption of nutrients easier. The digestion of a food starts in the mouth, continues in stomach, and finally ends in the small intestine. In mouth, the food is mechanically divided into small portions with mastication. These small portions have higher surface area for the saliva to interact and start digestion. The enzymes in the saliva can reach more areas of food and releasing of substances that are trapped into the food matrix becomes easier (Sensoy, 2014). The esophagus which links the mouth to stomach, transfers the pre-digested food in mouth to the stomach. The stomach contains acids and enzymes and thus, the digestion of food continues. In stomach, mostly the breaking down procedure is carried on (Sensoy, 2014). The food which is mostly disrupted is ready to be absorbed. The absorption of nutrients and other compounds in the food matrix occurs in the small intestine (Sensoy, 2014).

The definition of bioavailability can be given as the amount or rate of consumed matter (mostly the beneficial food material) can expand its effect to related tissue

or site of action (D'Archivio et al., 2010). The bioavailability is difficult to examine because it can be affected by various factors. The list of these factors is given below Table 5 (D'Archivio et al., 2010).

Table 5: The list of factors that affect the bioavailability (D'Archivio et al., 2010).

External factors	**Environmental factors, food availability**
Food processing related factors	Thermal treatments, homogenization, cooking and culinary preparation, storage
Food related factors	Food matrix, presence of positive or negative effectors of absorption
Interaction with other compounds	Bonds with proteins or with polyphenols with similar mechanism of absorption
Polyphenols related factors	Chemical structure, concentration in food, amount introduced
Host related actors	Intestinal factors (enzyme activity, colonic microflora, intestinal transit time) Systemic factor (gender and age, disorders and pathologies, physiological condition)

Bioaccessibility is defined to be the proportion of consumed substance which is available for the absorption in the gastrointestinal tract (Stahl et al., 2002). The consumed material is not generally 100% absorbed by the body, because this

requires full transform from the food matrix without any loss (Stahl et al., 2002). In fact, the substance may interact with other components which lead to be unabsorbable material (Stahl et al., 2002). Physical properties of food matrix affect the physical, enzymatic, and chemical digestion processes that result in affecting both bioaccessibility and bioavailability (Parada & Aguilera, 2007).

In order for body to use the antioxidants, phenols or carotenoids, these compounds should be released from the food matrix they are trapped in (Stahl et al., 2002). The absorption process depends on many parameters such as the state of food (processed or raw), particle size, the process of size reduction (cell rupture), enzymes that contribute to digestion, composition of meal, presence of bile acids or salts and lastly the time (Stahl et al., 2002). In the case of carotenoids, the physical form and location have more impact than the chemical form on the bioaccessibility (Stahl et al., 2002).

For the digestion model, there are two subgroups; static models and dynamic models. In static models, the physical processes applied to the food are not performed. In dynamic models, mechanical, physical and temporal processes are taken into account as *in vivo* digestion environment is tried to be simulated (Kamiloğlu et al., 2013). For *in vitro* digestion model, salts and enzymes are used while adjusting the pH of the environment to model the stomach and small intestine (Kamiloğlu et al., 2013).

1.8 Objectives of the Study

The aim of the project was to add tomato pulp as a functional ingredient to corn grits. The effect of extrusion on the functional properties of the tomato pulp added extrudates was investigated. In addition, the change in the *in vitro* bioaccessibility of lycopene subsequently to extrusion was studied.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

The corn grit was supplied by Teknik Tarım (Manisa, Turkey). Tomatoes used in the experiment were purchased from local groceries (Ankara, Turkey). Pulps were prepared from whole tomatoes after removing the stem and the seeds. Halogen moisture analyzer at 160 °C (MX-50, AND, Japan) was used to determine the moisture contents of the samples. Samples were prepared by mixing corn grits (12.23 ± 0.06 % moisture) with tomato pulps (95.27 ± 0.13 % moisture) to the moisture content of 30 ± 1% with the help of a mixer (Kitchen Aid, Ariston, USA). 30 % moisture content was chosen to maximize the tomato pulp content in the feed. The prepared feed was left at the refrigerator (4 °C) overnight. Before the extrusion process, the samples were allowed to equilibrate at the room temperature for two hours.

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

A laboratory scale co-rotating twin-screw extruder (Feza Gıda Müh. Makine Nakliyat and Demir Tic. Ltd. Şti. İstanbul, Turkey) with computer control and data acquisition system was used for the study. The die diameter and the barrel length to diameter ratio (L/D) were 3 mm and 25:1, respectively. The screw configuration of extruder was shown below, Table 6.

Table 6: Screw configurations of the extruder

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

Screw diameter (*D*) = 25 mm.

One kneading element = 0.25 D.

The extruder had four heating zones controlled by electrical heating and water cooling. The barrel zone temperatures and rotor speed were controlled by the computerized data acquisition system. The feed was fed to the extruder with a twin-screw volumetric feeder that was built into the extruder system. Flow rate of the feed was 36 ± 1 g/min. The working conditions for screw speed was kept constant at 225 rpm when the barrel temperature zones were set at 80 °C, 90 °C,

100 °C and 130 °C (die: 116 °C) and 80 °C, 100 °C, 130 °C and 160 °C (die: 139 °C). After reaching the steady state conditions, samples were taken only when actual measured barrel zone temperatures and die temperatures varied only ± 2°C from the set temperatures.

2.2.2 Extraction

The extraction method of Anton et al. (2009) was applied with some modifications. The extruded samples were grinded. Grinded samples were sieved from 425 microns mesh sieve (Laboratory Test Sieve, Endecotts LTD. England). 500 mg of feed and grounded extrudates were mixed with 12.5 mL of acetone-water mixture (4:1) (v/v). The mixture was stirred at a magnetic stirrer (JeioTech-Multichannel Stirrer, MS-52 M) for 2 hours. The samples were centrifuged at 3000xg for 12 minutes (Sigma, 2-16 PK, Germany). Lastly, the supernatant was collected and filtered through 0.45 μm syringe type filter (Syringe Filter, PTFE 25 mm) for total phenolic content and antioxidant activity analysis.

2.2.3 Total Phenolic Content

Total phenolic contents of samples were determined colorimetrically using Folin–Ciocalteau reagent, as described by Anton et al. (2009) with some modifications. The color change occurs due to reduction of Folin-Ciocalteau reagent with the help of sodium carbonate in the presence of phenolic compounds. 0.2 mL of acetone-water extract of the samples was mixed with 1.5 mL of Folin-Ciocalteau reagent (Merck, Germany) which was diluted 10-fold. After 5 minutes of delay, 1.5 mL of Na_2CO_3 (60 g/L) was added. Reagents were allowed to react at room temperature for 90 minutes in dark. The absorbance was read at 725 nm by using

UV-Visible spectrophotometer (Shimadzu, UV-Visible Spectrophotometer, UV-1700, Japan).

A standard curve was prepared with gallic acid (3,4,5-Trihydroxybenzoicacid, Sigma–Aldrich, Germany). The results were expressed in mg gallic acid equivalent (GAE) /gr dry weight.

2.2.4 Antioxidant Activity

Antioxidant activities of the feed and extrudates were determined with a stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Anton et al. (2009) with some modifications. DPPH has the characteristic of the changing color with the presence of antioxidant substances. 200 μL of acetone-water extract was added to 3.8 mL of 63 μM DPPH (Sigma–Aldrich, Germany). The extracts were left at dark for 40 minutes. The absorbance was read at 517 nm with UV-Visible spectrophotometer. A standard curve was prepared with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid from Sigma–Aldrich, Germany), a synthetic, hydrophilic vitamin E analogue, as an external standard with a range of concentrations from 10μM to 100 μM. The results were expressed as trolox equivalent (TE) /gr dry weight.

2.2.5 Protein Analysis

1 gr of homogenized tomato pulp and corn grit samples were weighed into the Kjeldal tubes. Potassium sulfate (K_2SO_4) and Copper (II) sulfate ($CuSO_4$) were added as catalyst. 15 mL of Sulfuric acid (H_2SO_4) was added to each tube. After that, the tubes were placed into the Kjeldal burning unit. The burning period lasted about 3-4 hours. After that, they were left at room temperature to cool. 0.1 N of NaOH was used in the distillation period. 50 mL of distilled water was

added to the tubes. The tubes were placed into the distillation unit (Şimşek Laborteknik Ltd. Şti. DES-1), meanwhile the erlenmayers which contain 50 mL of boric acid (4%) placed in the unit. The erlenmayer flasks which were taken out from the distillation units were titrated with 0.1 N HCl.

N % = (mL of standard acid- mL of blank) * 0.1*1.4007
Protein % = (N %) * 6.25

6.25 was chosen as the conversion factor.

2.2.6 Ash

3-5 gr of tomato pulp and corn grit were weighed into the crucibles that were previously left at muffle furnace at 105°C overnight and cooled in a desiccator. The temperature was set to 550°C for 8 hours. Then crucibles were cooled in the desiccator and then weighed.

Ash in wet weight % = (ash/ weighed sample)*100

2.2.7 Pectin

Pectin analysis was completed by the method described in Monsoor & Proctor (2001) with slight modifications. 60 mL of 0.05 M HCl was added to 10 grams of homogenized tomato pulp. The mixture was stirred at 50 rpm for 1 hour at 90 °C. Afterwards, the samples were left at room temperature to cool about 30°C. The cooled samples were centrifuged at 4000xg for 12 minutes (Sigma, 2-16 PK, Germany). The supernatant was collected and separated. Equal volume of 2-proponal was added to the supernatant. The new mixture was left to stand for 6

hours. Then, the mixture was centrifuged at 7000xg for 12 minutes. This time, the precipitate was collected, dispersed in 50 mL of 2-Proponal and stirred for 1 hour. This centrifugation, dispersion and stirring were repeated two more times. After the last stirring, the mixture was centrifuged again at 7000xg for 12 minutes. The precipitate was dried at 60°C for 5 hours. The dried weight of precipitate was measured as pectin amount.

2.2.8 *In vitro* Digestion Analysis

In vitro digestion analyses were performed at the Food Engineering Department of Aegean University, İzmir, Turkey. 0.5 gram of samples were weighted and put into the centrifuge tubes. 0.5 mL of saliva was added on the samples and on the blank sample tube. The centrifuge tubes were vortexed. After the addition of 500 µL distilled water to the tubes, 500 µL of saliva was added again. The pH of the tubes were checked, adjusted to 7 and vortexed again. 0.2 mL pepsin solution and 0.8 mL simulated gastric fluid were added, respectively. The tubes were adjusted to pH 3. In orbital shaker, the tubes were incubated at 37°C for 2 hours. The shaking was set to the values of gastric rate (gastric digestion model). The tubes were removed from the orbital shaker and the gastric digestion model was completed. The pH of the tubes was set to 5.5. The dialysis membranes that contain 6.25 mL of 0.5 N $NaHCO_3$ were placed into each tube. The tubes' pH were arranged to pH 7. The solution of pancreatin-bile was prepared with dissolving 8 grams of pancreatin and 24 grams bile into the pancreatic juice and completing to 100 mL. From this solution, 2 mL was taken and added to the tubes. Again, the tubes were left at orbital shaker at 37°C for 2 hours; during this incubation pH was checked and kept at 7. The dialysis membranes were taken from the tubes. The membranes were opened and the inside matter was transferred to another tube which were frozen at -20 °C until the HPLC analysis. The solution outside of the dialysis membranes were also put into new tubes and frozen at -20 °C until the HPLC analysis.

The dialysis membranes which were used after incubation were prepared as follow. The membranes (Spectrum Laboratories, Inc.; Spectra/Por Dialysis Membrane, Molecular porous, Membrane tubing) (MWCO: 6-8,000, Flat Width: 23 mm, Length: 30m/100 ft, Diameter: 14.6 mm, Vol/Length: 1.7 ml/cm) were cut in the length of 10 cm and left to hot water in order to expand. When they were enlarged, they were washed with cold water. The washed membranes were tied at one end, and then 6.25 mL of 0.5 N $NaHCO_3$ was added to the membranes. The air inside the membranes was removed and the other ends were tied.

2.2.9 Extraction of Carotenoids

The extraction method was based on the method by O'Connell et al. (2007). The extrudates were grinded (KSW 445 CB, Bomann, Germany) and sieved from a mesh sieve, 425 microns (200 M.M. B.S., Endecotts Ltd, London) prior to analysis. 10 gram of homogenized samples from both extrudates and feed were taken and approximately 30 mL of distilled water was added onto them. The water added mixtures were left at 4 °C overnight. The equilibrated samples were homogenized (Witeg, HG-15A, Germany) at 13500 rpm for 2 minutes. From the homogenized samples, 4 grams were taken and 0.4 gram Calcium carbonate ($CaCO_3$) was added. The samples were mixed with 25 mL of hexane:acetone:ethanol (50:25:25) (v/v). They were stirred at magnetic stirrer at 250 rpm for 20 minutes. After 20 minutes, the supernatant part was separated from the rest and put in another beaker. The residue part was again mixed with 25 mL of hexane:acetone:ethanol (50:25:25) (v/v). The mixture was stirred at 250 rpm for 20 minutes. Afterwards, both supernatants were combined and centrifuged at 9500xg at 4°C for 20 minutes. The hexane layer formed after centrifugation was dried under the stream of nitrogen at 37°C with nitrogen evaporator. The dried samples were immediately dissolved with THF and mobile phase and prepared for HPLC analysis.

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

2.2.10 HPLC Analysis

The HPLC analysis was performed as described by O'Connell et al. (2007). The extracts obtained from the feed and extruded samples which were dried under nitrogen, were dissolved with 100 µL THF and then diluted with 900 µL mobile phase. Dried extracts obtained from digested feed and extruded samples were dissolved in 50 µL THF and diluted with 450 µL mobile phase. The tubes were vortexed. The samples were filtered through the 0.45 µm syringe filter (Syringe Filter, PTFE 13 mm). The HPLC system (Thermo Scientific, Finnigan Surveyor) with a UV visible detector (Finnigan Surveyor, UV-Vis Plus Detector) was used for lycopene quantification. The colon was reverse phase C-18 column (Inertsil ODS-2, 4.6x250 mm, 5 µm). The column temperature was set to 20°C. The wavelength used was at 450 nm. The flow rate was 1 mL/min while the injection volume was 25 µL. The mobile phase was the mixture of acetonitrile: methanol: dichloromethane (75:20:5) (v/v/v) which contain 10 mmol/L ammonium acetate, 4.5 mmol/L buthylated hydroxytoluene and 3.6 mmol/L triethylamine. The filtration of mobile phase was provided by 0.45 µm filter (Filtration Membranes, Membrane Disc PVDF 47 mm, 0.45 µm) and degassing was completed with ultrasonic agitation.

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

$$\textit{In vitro}\ \text{Bioaccessibility}\ (\%) = \frac{(\text{Dialyzed portion}) + (\text{Not dialyzed portion})}{\text{Amount before } \textit{in vitro} \text{ digestion}}$$

2.2.11 DSC

The thermal properties and gelatinization of the corn grit-tomato pulp feed and extrudates were determined with differential scanning calorimetry method (DSC). The analysis was conducted with Perkin Elmer DSC 4000 with Intracooler. Indium and zinc were used for the calibration of the instrument where the reference was an empty pan.

The samples which were kept at the refrigerator at -25 °C after the extrusion were grounded and sieved from 425 μm mesh. The grounded samples and feed were weighed to the pans (30 μL, Perkin- Elmer) around 8 mg. About 16 mg of distilled water was added to the pan. The pans were sealed with a sample-encapsulating press system. The closed pans were allowed to equilibrate in the refrigerator overnight. The pans were heated from 0°C to 130°C at the rate of 10°C/min. The nitrogen flushing was set to 20 ml/min. Gelatinization onset temperature (T_0), peak temperature (T_p), enthalpy of the gelatinization (ΔH) and end temperature (T_c) were calculated with the Pyris software (Version 11.0.0.0449).

2.2.12 Statistical analysis

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

CHAPTER 3

RESULTS AND DISCUSSION

Determined protein, pectin and ash content of feed materials, corn grit and tomato were given in Table 7.

Table 7: The protein, pectin and ash content of tomato and corn grit (wet basis).

	Tomato (%)	**Corn grit (%)**
Protein	0.80 ± 0.09	8.39 ± 0.99
Pectin	1.03 ± 0.05	-
Ash	0.42 ± 0.02	0.49 ± 0.05

3.1 Total Phenolic Content

The total phenolic content of the feed was higher compared to extrudates treated with 130°C and 160°C last zone temperature (Table 8).

Table 8: Total Phenolic Content results of feed and extrudate samples where last zone temperatures were 130°C and 160°C.

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

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

Several researches indicated a decrease in total phenolic content after extrusion (Anton et al., 2009; Sharma et al., 2012). Anton et al. (2009) stated that extrusion had decreasing effect on both total phenol content and antioxidant activity.

The high temperature during the extrusion process may cause the phenolic compounds to decompose as these compounds are known to be heat sensitive especially at the temperatures above 80°C (Sharma et al., 2012). Other reasons apart from the change of molecular structure can be the inadequate extraction caused by the polymerization and decrement of chemical reactivity (Altan et al., 2009). The phenols may be exposed to decarboxylation with the temperature of the extrusion process and moisture content; this transformation can cause

polymerization of phenols which means the extraction of these phenols and the activity of antioxidants would be limited (Obiang-Obounou & Ryu, 2013).

In this study, extruded samples with higher treatment temperature (160°C last zone temperature) had higher phenol content compared to samples with lower treatment temperature (130°C last zone temperature). With applied high temperature during extrusion, the higher proportion of phenol release and extractability could be achieved.

According to Masatcioglu et al. (2013), increasing the content of tomato powder in corn flour extrudates gave an increasing total phenolic content. When the temperature of extrusion was elevated, the total phenol content was increased significantly. According to Masatcioglu et al. (2013), increased phenol content with increasing temperature was because of high temperatures, the phenolics which were in bound form before process could be released. Thus, the extraction of this phenolics would be easier.

Nayak et al. (2011) studied on the phenol content of the different potato and pea flour and their mixtures before and after extrusion. Generally, they have seen that at die temperature of 130°C, except one formulation, all extrudates had lower total phenol content than their feeds. They claimed that polyphenol substances degraded to phenol substances or other than phenol substances; when they were exposed to heat and high temperatures of extrusion (Nayak et al., 2011). On the other hand, they have not seen significant difference between the total phenol contents of extrudate samples treated at different die temperatures of 130°C or 140°C.

In the study of Gujral et al. (2012), it was mentioned that the extrusion caused the total phenol content of three different cultivar of brown rice to decrease. Moreover, in that study, the same cultivar samples were applied two different temperatures, 100°C and 120°C. The results of total phenol content at 120°C were lower than of at 100°C (Gujral et al., 2012). The increase in temperature led decrease in the total phenol content which was explained with the components are being labile to heat.

In the study of Dlamini et al. (2007), the total phenol of samples was found to decrease after extrusion in an important amount when the analysis was applied to tannin sorghums both; whole and decorticated form.

It was indicated that the unextruded samples had higher total phenol content than the extruded samples where honey was added to barley (Kumar et al., 2013). The results were expressed with the reasons that the phenolic compounds are sensitive to heat. The total phenol content showed an increase with the increasing the honey content. In their study, to the temperature limit of die temperature 150°C, the increase in the moisture content showed an increase in the total phenol content (Kumar et al., 2013).

In the study of Sharma et al. (2012), the total phenol content decreased with increasing the moisture content from 15% to 20% at constant temperature of 180°C. However, the total phenol content increased with increasing moisture content from 15% to 20% at constant temperature of 150°C. They stated that the moisture content gives more damage when it is combined with high temperature (Sharma et al., 2012). While keeping the moisture content constant (15%), as the temperature was increased from 150°C to 180°C; a significant increase in the total phenol of contents was seen. However, at moisture content of 20%, as the temperature was increased from 150°C to 180°C , the total phenol contents were decreased significantly with the study of barley grits (Sharma et al., 2012).

In Sompong et al. (2011)'s study, the extrusion decreased the total phenol content of the both black rice and red rice significantly. The moisture content of the feed samples influenced the total phenol content change during extrusion. In black rice extrudates, the extrudate sample from feed having 16 % moisture content had higher total phenol content than the extrudate sample from feed having 12% moisture content. However, in red rice extrudates, the extrudate sample from feed having 16 % moisture content had lower total phenol content than the extrudate sample from feed having 12% moisture content (Sompong et al., 2011).

In some studies, the total phenol content was reported not to be related with the screw speed, moisture content and feed rate (Özer et al., 2006). In addition, no

significant difference in the total phenol content between the extruded samples and non-extruded samples was seen (Özer et al., 2006).

In Altan et al. (2009)'s study, the extrusion decreased the total phenol content when the extruded samples of barley flour compared with untreated flour. Between the samples treated with different die temperatures (136°C, 140°C, 150°C, 160°C and 164°C) and screw speeds (140, 150, 175 and 210 rpm), the highest total phenol content was found in the samples of 175 rpm screw speed, 150°C die temperature and, 200 rpm screw speed, 160°C die temperature. Altan et al. (2009) stated that the extruded samples of tomato pomace added barley flour had significantly less total phenol content than the feed material. However, the effect of temperature and screw speed could not be explained with a trend.

In some cases, the phenol content and antioxidant activity can be increased after extrusion. The reason was suggested to be the release of these substances from the cell as the result of extrusion process (Brennan et al., 2011).

3.2 Antioxidant Activity

The antioxidant activity of the feed was found to be higher compared to extruded samples with last zone temperatures of 130°C and 160°C (Table 9). Extrudates with 160°C last zone temperature had higher antioxidant activity compared to extrudates with 130°C last zone temperature. The reason could be that more Maillard products might have been formed and extractability of phenols could be higher. The antioxidant activity and total phenolic content showed a high correlation with $R^2 > 0.99$.

Table 9: The antioxidant activities of feed and extrudate samples where last zone temperatures were 130°C and 160°C.

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

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

Masatcioglu et al. (2013) found that with increasing tomato powder, the antioxidant activity increased. Generally, the high temperatures caused the antioxidants to decompose. In their study, the increase in temperature caused antioxidant activity to increase significantly. The reason of this situation was attributed with the Maillard reaction, as the high temperature could result in more Maillard reaction products leading to higher activity (Masatcioglu et al., 2013). The reason of rise in the antioxidant activity at 160°C compared to 130°C could be the Maillard reaction in our study. Maillard reaction mostly takes place in the conditions of high temperature and low moisture. In this reaction, the reducing sugars and amino acids are included and the product is mostly in brown color which provides many sensory properties to the food. Furthermore, these products are counted to have a role in antioxidant activity (Masatcioglu et al., 2013).

The substance is tested with DPPH for its scavenging stable radicals. The method is based on the measuring the antioxidants with methanol medium. The antioxidants are expected to donate hydrogen. With this reaction, the color is turned to yellow while the stock solution of DPPH is dark purple (Sharma et al., 2012). In the research of Sharma et al. (2012), when the samples were investigated for the antioxidant activities, it was seen that the samples which were

treated with extrusion had higher antioxidant activity than the raw samples. It was possible that with high temperatures, Maillard reaction could occur in the samples, thus the formation of brown pigments can contribute to the antioxidant activity (Sharma et al., 2012).

For the two moisture content of the feed sample (15% and 20%), where the moisture content was kept constant and the temperature was increased from 150°C to 180°C, the antioxidant activity of the samples decreased significantly (Sharma et al., 2012). In the same study, at constant temperatures of 150°C and 180°C, where the moisture content of the samples were shifted from 15% to 20%, the antioxidant activity of the samples were increased significantly.

Gujral et al. (2012) stated that the extrusion caused a decrease in the antioxidant activity in the samples where three paddy cultivars were extruded. The decrease was observed at 100°C, moreover, when the temperature was raised to 120°C, the results showed more reduction. The reason of decrease in this level was attributed to the destruction due to high temperature treatment of extrusion. However, Grela et al. (1999) stated that increasing temperature from 100°C, 160°C and 200°C had no significant effect on the antioxidant activity with the study of grass peas.

In the study of Kumar et al. (2013), the extrudates had higher antioxidant activity than the barley flour feed which was enriched with honey. They stated that the honey was rich in antioxidant so the addition of honey to barley flour might cause the antioxidant activity to increase. As the amount of honey increased, the antioxidant activity increased. Moreover, Maillard reaction during extrusion process might have happened, the products of Maillard had the antioxidant properties. Kumar et al. (2013) mentioned that the increasing moisture content increased the antioxidant activity in their research. When the moisture content of the sample was low, the temperature of die had no significant effect on the antioxidant level however if the moisture content was high, as the die temperature increased the antioxidant activity decreased (Kumar et al., 2013).

Nayak et al. (2011) mentioned that the raw samples did not have significantly different antioxidant capacity than the extruded products at 130°C die

temperature. The extrusion could lead to the polyphenols to decompose to phenols which show antioxidant activity, with applied heat the phenols can react with proteins and lastly, formation of substances due to Maillard reaction can occur (Nayak et al., 2011). The extrudate samples from 130°C die temperature and 140°C die temperature were not statistically different from each other. However, in 35% and 65% purple potato flour added samples, the raw samples had higher antioxidant activity than the extrudated sample at 140°C.

The antioxidant activity of extruded samples decreased by 60-68 % as a result of the extrusion process (Altan et al., 2009). The extrusion is known to be high temperature process which leads the antioxidant compounds to decompose at these temperatures. Moreover, besides degrading of these substances, evaporation can be another reason to decrease (Altan et al., 2009). It was found out that when the screw speed was set constant to 150 rpm, with increasing temperature from 140°C to 160°C, the antioxidant activity was seen to decrease significantly. However, if the screw speed was set to 200 rpm and the same temperature shift was applied, no significant difference observed within the results of 140°C and 160°C. When the temperature was set to 150°C and the screw speed was changed, the antioxidant activity decreased with increasing screw speed. In general, they concluded that there was no certain relationship between antioxidant activity and screw speed and temperature (Altan et al., 2009).

It was stated that the extrusion had no effect on total antioxidant activity of samples where 10% brewer's spent grain added to corn flour and wheat flour (Stojceska et al., 2009). It was mentioned that the antioxidant activity was increased with extrusion in the red cabbage added samples. They indicated the reason of this result may be due to the temperature, water stress and wounding of vegetables with the extrusion process. Furthermore, the Maillard reaction was stated as another effect, the new antioxidants were developed with this reaction and non-enzymatic browning can cause some inactive antioxidants to be active (Stojceska et al., 2009).

It was stated that there was a correlation between the total phenol content and the antioxidant activity, as the results showed the similar pattern (Anton et al., 2009). They stated that the extrusion caused the total phenol content and antioxidant activity to decrease.

In the Stojceska et al. (2009)'s study, the antioxidant level of extruded products were not significantly different from the unextruded samples of mixture of corn, wheat and brewer's spent grain. However, the extrusion increased the antioxidant level of corn, wheat and red cabbage mixture (Stojceska et al., 2009).

In Altan et al. (2009)'s study, they mentioned that there was no correlation between the total phenol content and antioxidant activity. The reason was explained as that possibility of some phenolic substances which were not included in the total phenol content but affected the antioxidant activity results. In the study, they processed barley with different pomace types such as tomato and grape. They mentioned that when the addition of tomato pomace was small, increase in screw speed caused the antioxidant activity to decrease. They stated that although the extrusion decreased the antioxidant activity, as the amount of pomace increases, the activity increases (Altan et al., 2009). According to Anton et al. (2009), when the corn starch-bean mixtures were extruded, the significant reduction in the antioxidant activities were seen. According to the study of Obiang-Obounou & Ryu (2013), extrusion increased the antioxidant activity in the chestnut samples.

In the study of Özer et al. (2006), the antioxidant activity was affected from the feed rate, moisture content of feed and screw speed. In these three elements, moisture content is not dominant on the antioxidant activity as the others. They stated that when the feed rate is low, increasing screw speed lead antioxidant level to decrease. As the speed of screw increases, the residence time decreases. Thus, samples were exposed to less heat and pressure. However, the increase in screw speeds cause the mechanical energy to increase which effects the antioxidant structure more than the residence time (Özer et al., 2006). The increase in moisture content results the antioxidant activity to increase, the effect of extrusion

on the less moisturized samples would be more detrimental for the antioxidant substances. According to Özer et al. (2006), the moist helps the process to be gentler.

3.3 Starch Gelatinization

In our study, the extrudates treated at both 130°C and 160°C last zone temperatures were shown a straight line, giving no peak at DSC which was an indication of complete gelatinization (Figure 1, 2 and 3). The temperature of extrusion was high above the gelatinization temperature of the corn grits. When the temperature of the extrusion was increased from 130°C to 160°C, no change was seen in thermograms of DSC. The reason could be that even 130°C was high enough for fully gelatinization that no difference could be appeared above this temperature.

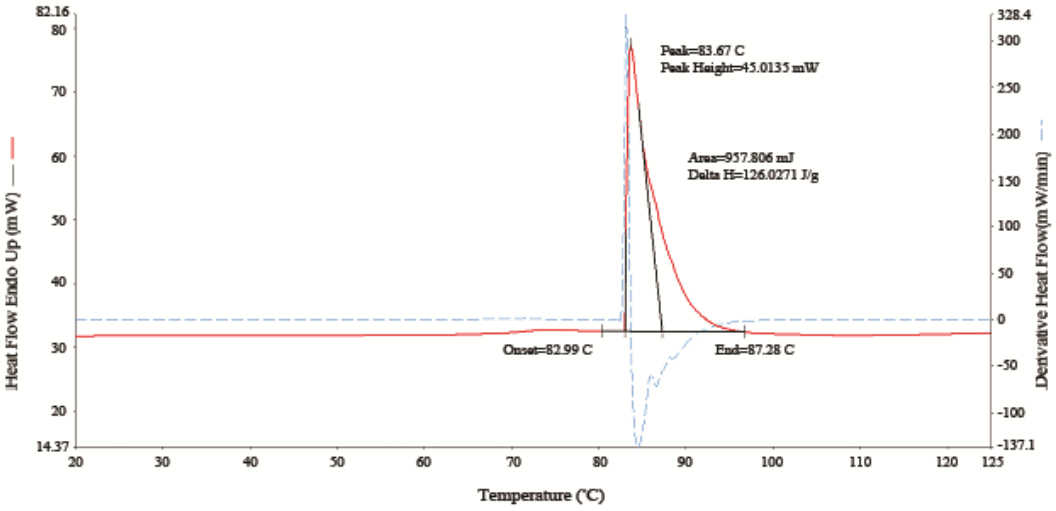

Figure 1. DSC diagram for feed (tomato-corn grit mixture)

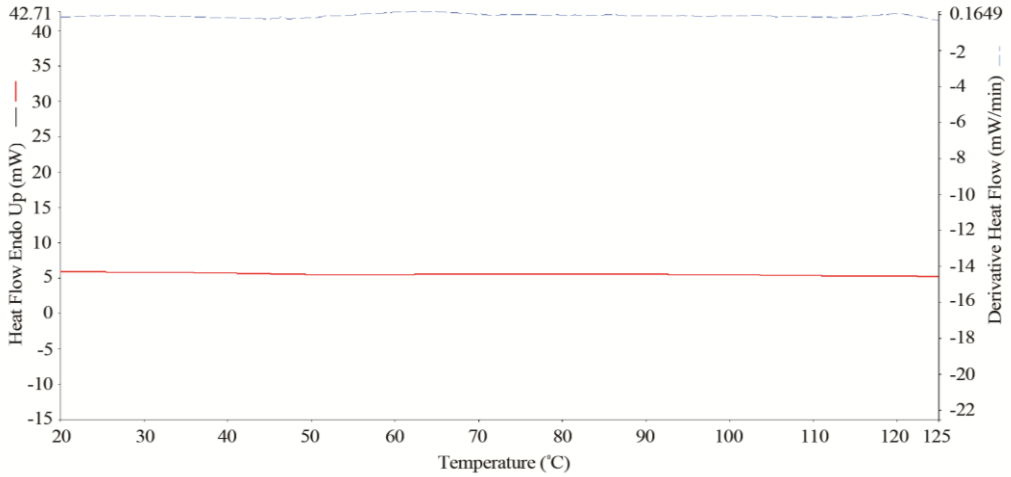

Figure 2. DSC diagram for extruded sample at 130°C last zone temperature

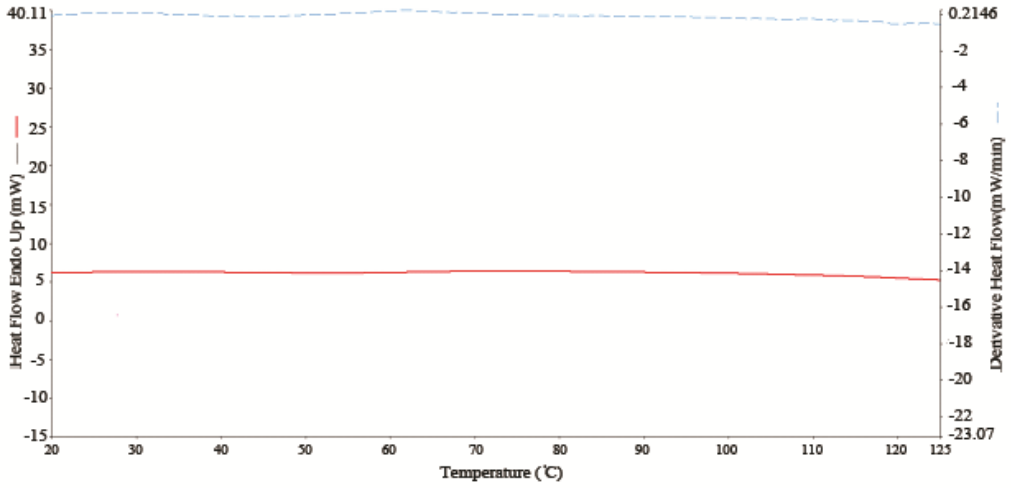

Figure 3. DSC diagram for extruded sample at 160°C last zone temperature

The starch molecules absorbed the moisture in the sample and got in to the amorphous region (Gomez & Aguilera, 1984). With proper heating (temperature in the extrusion) these amorphous structures went through the gelatinization reaction. If the moisture content was not enough, these molecules become unchanged, mostly ungelatinized. If the temperature was not high, this time the gelatinization, again, would not occur; furthermore the shear process would cause these structures to dissociate (Gomez & Aguilera, 1984).

Aguilar-Palazuelos et al. (2012) found that the maize showed a peak between 62-80°C. There was no peak at the thermograms of the extruded samples. It was related with the gelatinization that already occurred during the extrusion process (Aguilar-Palazuelos et al., 2012). It was mentioned that the gelatinization level would be higher within the high temperature process conditions.

Chinnaswamy & Hanna (1990) studied on the physical and gelatinization properties of four types' corn starches which have different amylose content (0, 25, 50 and 70 %) and these were compared with the extruded samples. DSC analysis showed that the gelatinization temperature of these samples were not same which was related to their amylose content. The temperature changed between 71.3 - 97°C and the increase trend was seen with increasing amylose content (Chinnaswamy & Hanna, 1990). When the extruded samples were run at DSC, the peaks from the raw materials were not seen which supported the idea that the starch was gelatinized during extrusion (Chinnaswamy & Hanna, 1990).

In the study of Gomez & Aguilera (1984), the raw corn starch was showed a peak in DSC, where all extruded products showed none. The temperature of the extruded products was 90-130°C which depended on the moisture content of the samples. Even, the products exposed to lowest temperature did not give a peak (Gomez & Aguilera, 1984). The reason was said to be that the amount of ungelatinized starch molecules were so small that it was below the limits of DSC detection (Gomez & Aguilera, 1984).

It was mentioned that the corn starch and corn grit would show the highest gelatinization degree in the presence of moisture content at about 27-28% (Gomez & Aguilera, 1984). In our study, the moisture content was set to 30 ± 1%.

Gomez & Aguilera (1984) stated that in the low moisture feeds; the starch would be extensively degraded, showing no peak at DSC thermograms. However, the no peak situation could also mean that the low amount of starch that was left at the sample could be out of the limits of DSC detection, not necessarily meaning fully degradation of starch (Gomez & Aguilera, 1984).

3.4 Lycopene Content and *In vitro* Bioaccessibility of Lycopene

Absorption of carotenoids can be achieved in two ways; by harming the cell with mechanical damage or extraction to a lipophilic environment (Gartner et al., 1997). With extrusion technique, feed material was treated with both thermal and mechanical process. The thermal process has the same effect as mechanical disruption on the cells (Gartner et al., 1997). Thus, the lycopene could be more easily absorbed by the body after processing.

In the study, both extrudates with the last zones temperatures of 130°C and 160 °C had lower lycopene content than the feed (Table 10). The high shear and high temperature of extrusion process could destroy the lycopene in the samples which are known to be labile to heat and mechanical disruption. It was seen that extrudates with the last zone temperature of 160°C showed higher lycopene content than the extrudates with last zone temperature of 130°C. This could be due to the destruction of cell walls with high temperature and enhanced extractability of lycopene from the sample (Gartner et al., 1997; Dehghan-Shoar et al., 2011).

In vitro bioaccesibility of lycopene was higher for the sample with 160 °C last zone temperature compared to feed and extrudates with last zone temperatures of 130°C (Table 10). With the extrusion process fiber compounds in the cell wall

structure are broken down, thus, the accessibility of lycopene can increase through the digestion process. The reason for higher *in vitro* bioaccessibility of lycopene for the samples that have last zone temperature of 160°C compared to the samples that have last zone temperature of 130°C could be that the damage at higher temperature result in more lycopene accessibility (Colle et al., 2010). Similarly, Colle et al. (2010) stated that with increasing treatment temperature, *in vitro* bioaccessibility of lycopene increased for tomato pulp heat treated for 30 min, where the significant difference was observed at temperatures of 130°C and 140°C.

Table 10: Lycopene content of the samples before and after *in vitro* digestion model

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

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

In the study of Gartner et al. (1997), it was concluded that the bioavailability of lycopene that was absorbed from tomato paste showed significantly higher values than the bioavailability of lycopene from fresh tomato. It was stated that the cell walls were damaged due to cooking thus making lycopene more reachable.

Boileau & Erdman, Jr. (2004) stated that when the bioavailability of foods was categorized, the raw materials was minimum, slightly processed foods was higher where the oily mixtures had the highest bioavailability.

The fresh tomato had higher lycopene concentration, and the all-*trans* lycopene contributes the most of it (Boileau et al., 1999). The fiber constituent or protein and lipid structure could protect the lycopene in the tomato to transform to cis

lycopene (Boileau et al., 1999). When the tomato was treated with heat, some of trans-lycopene was converted to cis form but this conversion is not high (Gartner et al., 1997). The reason of increasing the bioavailability of lycopene in the heat treated products could be attributed to this conversion, however Gartner et al. (1997) and Stahl & Sies (1992) found that the bioavailability increased with heat but increase due to the cis isomer transformation was minimal, not significantly contributed. However, Boileau et al. (1999) found that the cis lycopene concentration in tissues was higher in human as the bioavailability of it was higher than the trans form. The heat treatments enhance the carotenoid isomerization (Shi & Le Maguer, 2000).

The increasing bioavailability with slightly heating was acquired in Stahl & Sies (1992)'s study with tomato juice. It was mentioned that heat could disrupt the cell wall concluding lycopene coming out of cell (Stahl & Sies, 1992; Parada & Aguilera, 2007).

In Dehghan-Shoar et al. (2011)'s study, the energy and torque of the extrusion had a negative impact on the lycopene content of the tomato added corn grit extrudates. Even though the lycopene amount decreased, the bioaccessibility of lycopene increased. The feed of extrusion had bioaccessibility between 16-56 % where the extruded products gave a result between 19-105 % (Dehghan-Shoar et al., 2011). In the sample of 20% tomato paste added corn grit mixture, the lycopene content of extruded product and its proportion of bioavailability were found to be 233.99 ppm and 21.15, respectively (Dehghan-Shoar et al., 2011).

Dehghan-Shoar et al. (2011) stated that the destructive effect of heat and mechanical energy of extrusion caused the fibers and other substances within the tomato to decompose, making easier for lycopene to be released from the cell. The different amount of tomato paste addition showed that when the amount was increased, the lycopene amount in extrudates was increased however the bioaccessibility decreased (Dehghan-Shoar et al., 2011). This decrease was explained with decrease of applied force to the feed material due to the added amount, resulting in reduced destruction of cells and less release of lycopene from

the cell (Dehghan-Shoar et al., 2011). When the samples containing 5%, 10% and 20% tomato paste added were compared, the minimum lycopene bioaccessibility was seen in 20% (Dehghan-Shoar et al., 2011). Furthermore, they indicated that the increase in the bioaccessibility due to heating was due to increase in the cis lycopene proportion with respect to trans form. The applied heat in the extrusion caused the trans isomers transform to the cis isomers (Dehghan-Shoar et al., 2011). Gartner et al. (1997) and Stahl et al. (2002) mentioned that the bioaccessibility was higher in the processed or heated samples but the reason was not the isomerization of lycopene where Dehghan-Shoar et al. (2011) stated that the increase might be due to this isomerization. The cis isomers are known to be more soluble in oil, thus through the digestion system, the accessibility would be easier (Dehghan-Shoar et al., 2011).

Colle et al. (2010) found that the raw tomato pulp (without any additional treatment) contained 1300 μg/g DW (dry weight) lycopene. When the samples were exposed to heat treatment below 130°C, the total lycopene content of these samples were not significantly decreased (Colle et al., 2010). When the temperature was set to higher value like 140°C, the lycopene content was seen to decrease as a result of degradation of lycopene (Colle et al., 2010). Still, the content was around 74% of original value. The all-trans lycopene degraded mostly as its contribution to total amount was extremely high (Colle et al., 2010). Colle et al. (2010) stated that lycopene bioaccessibility of untreated tomato pulp was about 15 % where the result of tomato pulp treated at temperatures between 60°C and 120°C was higher than the raw, but not significantly different. However, a statistically significant increase was seen at 130°C and 140°C treated samples' all trans lycopene as the results were around 20% and 25%, respectively (Colle et al., 2010). At the same temperatures, cis lycopene showed higher results, around 30% and 35% (Colle et al., 2010). According to their results, the *in vitro* bioaccessibility of lycopene was enhanced with the heat treatment (Colle et al., 2010).

Shi & Le Maguer (2000) said that the promoted bioavailability due to damages to cell wall was a result of the broken bonds between lycopene and its surrounding

matrix. It was mentioned that this breakage did not only enhance the bioavailability but also triggered the isomerization of trans-cis forms (Shi & Le Maguer, 2000). The oily environment or substance could trigger this formation (Unlu et al., 2007).

Honest et al. (2011) mentioned that the cis isomer of lycopene is shorter in length which makes them to go into the micelle easily. The cis lycopene had higher bioavailability than the all trans form (Honest et al., 2011). Parada & Aguilera (2007) mentioned that cis form was more bioavailable as the solubility of this compound was higher in bile acid micelles which were used in the digestive stimulation method. Before the absorption process, the lycopene was released from the food matrix which was disrupted during digestion (Parada & Aguilera, 2007). Moreover, the damage to the food matrix could help the isomerization of lycopene (Parada & Aguilera, 2007).

Unlu et al. (2007), found an increase in cis-lycopene amount by 5% as a result of heat treatment at 75°C. Further increase to 45% of total lycopene content was obtained by improving the conditions to 127°C for 40 minutes (Unlu et al., 2007).

Within the different geometrical isomerization of lycopene, trans isomers which were more abundant was less bioavailable than the cis isomers which had low concentration (Shi & Le Maguer, 2000). The highly solubility characteristic and easy absorption into the cell, made the cis form more bioavailable (Shi & Le Maguer, 2000). It was indicated that the processed products had small concentration of cis isomers; nevertheless further processing could took this amount to higher levels (Shi & Le Maguer, 2000).

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

The total phenolic content of the feed material was significantly decreased with the extrusion process. When the temperature was increased from 130°C to 160°C, the obtained result showed that the extrudate samples of 160°C last zone treatment temperature had significantly higher total phenolic content compared with 130°C last zone treatment temperature.

The raw material had significantly higher antioxidant activity than the extrudates. The extrudates of 160°C last zone treatment temperature had higher antioxidant activity than the extrudates of 130°C last zone treatment temperature.

The extrudates had lower lycopene content compared to feed. *In vitro* bioaccessibility of extrudates with last zone temperature of 130°C was not different from feed while *in vitro* bioaccessibility of lycopene of extrudates with last zone temperature of 160°C was significantly higher than feed and extrudates with last zone temperature of 130°C.

For future study, tomato powder can be used as a functional ingredient to be able use higher concentration of functional ingredient in the feed. The effect of screw speed on the functional components can also be investigated. The cis form of lycopene can be analyzed to investigate the isomerization through extrusion.

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APPENDIX A

CALIBRATION CURVES

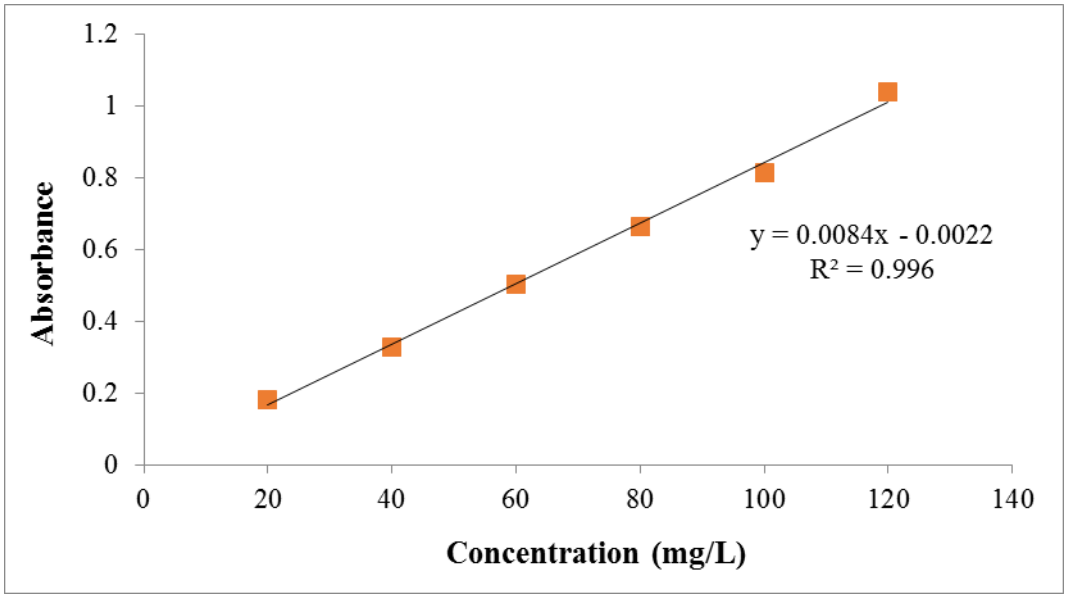

Figure 4. Calibration curve prepared by Gallic acid in ethanol for total phenolic content analysis.

$$\text{ABS (at 725 nm)} = 0.0084 \left(\frac{\text{mg GA}}{\text{L}} \right) - 0.0022 \qquad R^2 = 0.996$$

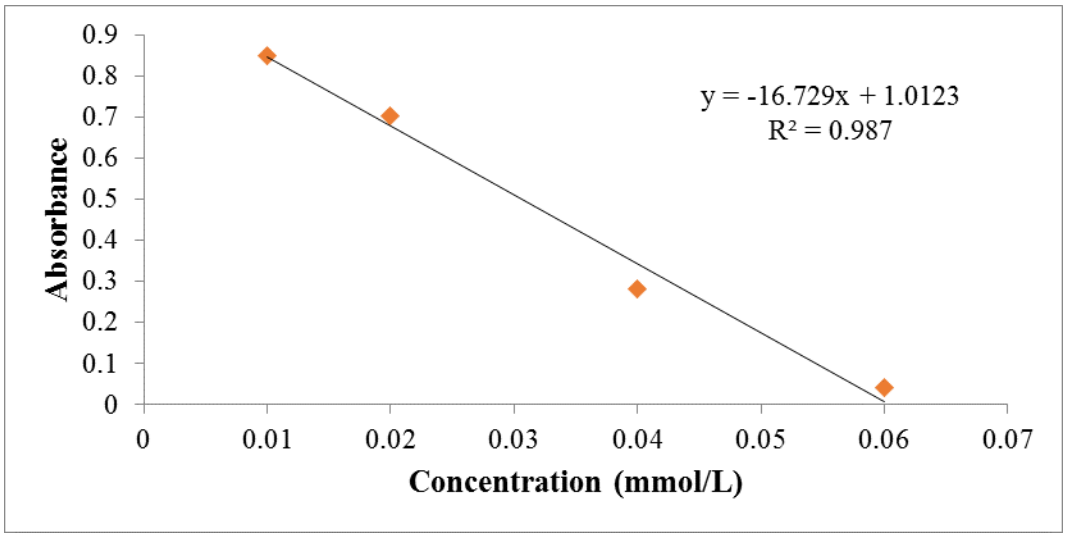

Figure 5. Calibration curve prepared by Trolox in methanol for antioxidant activity analysis.

$$\text{ABS (at 517 nm)} = -16.729 \left(\frac{\text{mmol Trolox}}{\text{L}}\right) + 1.0123 \qquad R^2 = 0.987$$

APPENDIX B

HPLC CHROMATOGRAM

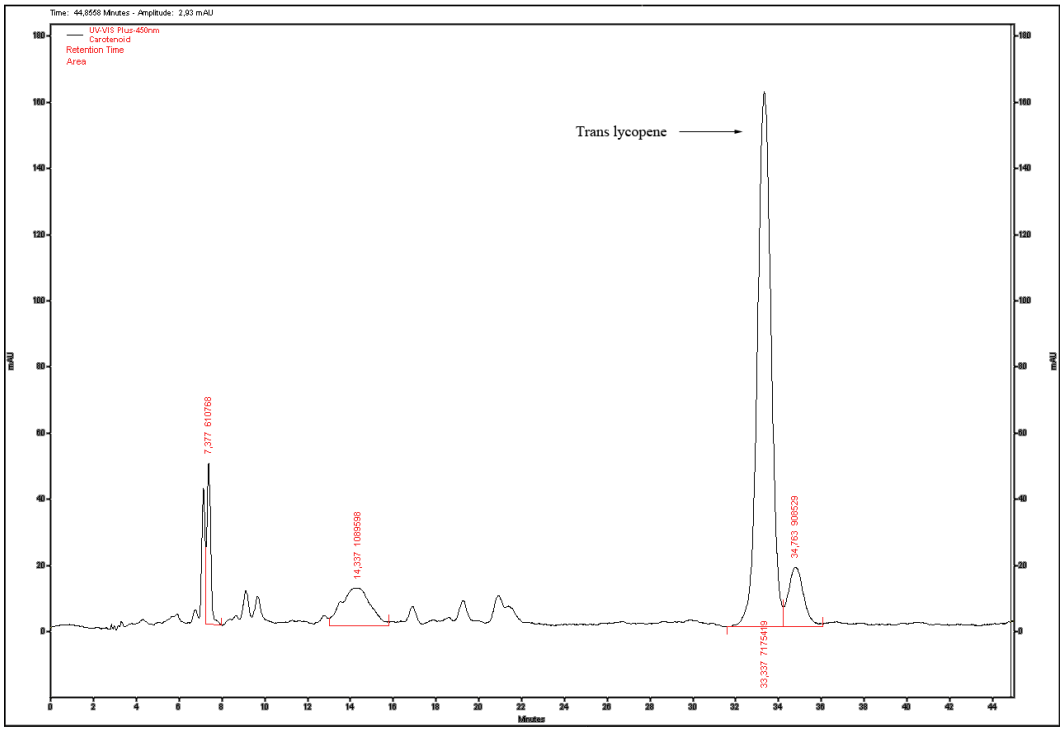

Figure 6. HPLC Chromatogram for the feed sample.