EFFECT OF ADDED INGREDIENTS ON THE ACRYLAMIDE LEVEL AND QUALITY OF EXTRUDATES

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ABSTRACT

EFFECT OF ADDED INGREDIENTS ON THE ACRYLAMIDE LEVEL AND QUALITY OF EXTRUDATES

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Ingredients which are NaCl, $CaCl_2$, ascorbic acid, citric acid, glycine, cysteine and quercetin were blended at two different concentrations with whole wheat flour, mixed with D-glucose monohydrate (100:5, w/w), and fed to twin screw extruder. Effect of these ingredients on acrylamide level and quality characteristics of extrudates were investigated. Set zone temperatures were 80 ± 2, 90 ± 2, 115 ± 2 and 150 ± 2 ^{o}C with 180 rpm screw speed and feed flow rate of 30 g/min during extrusion. Feed moisture content was 18.24 ± 0.48%. Drying process was performed at 150 ^{o}C for 15 min after the extrusion process. Lower acrylamide level were observed with added ingredients with differences in degree of reduction. Increasing concentration led to further decrease in acrylamide level for all of the ingredients. Among the ingredients studied highest acrylamide reduction was obtained with 5 g/kg cysteine addition while lowest reduction was obtained with 7.26 g/kg citric acid and 13.36 g/kg ascorbic acid. Highest sectional expansion index (SEI) was obtained with 50 g/kg $CaCl_2$ while the lowest was obtained by 66.80 g/kg ascorbic acid when compared to control. Longitudinal expansion index

(LEI) reached the highest value at the addition of 66.80 g/kg ascorbic acid while the lowest value was obtained by 10 g/kg $CaCl_2$. 50 g/kg $CaCl_2$ or 36.30 g/kg citric acid addition did not cause any significant change in volume expansion index (VEI) compared to control. The highest bulk density found at the addition of 66.80 g/kg ascorbic acid. Addition of 1 g/kg glycine, 10 g/kg NaCl, 36.30 g/kg citric acid or 50 g/kg $CaCl_2$ did not affect the bulk density when compared to control and gave the lowest values of bulk density in extrudates. In terms of lightness, ascorbic acid addition at the level of 66.80 g/kg gave the highest L* value and lowest browning index in extrudates while the lowest L* value was observed with 5 g/kg glycine addition with the highest browning index. In conclusion, data indicated that added ingredients decreased the acylamide level at a certain level while affecting the quality parameters of the extrudates to some extend.

Keywords: Extrusion, Salts, Acids, Amino acids, Quercetin, Acrylamide

ÖZ

EKSTRÜDE ÜRÜNLERDE İLAVE İNGREDİYENLERİN AKRİLAMİD SEVİYESİ VE ÜRÜN KALİTESİ ÜZERİNE ETKİSİ

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NaCl, $CaCl_2$, askorbik asit, sitrik asit, glisin, sistein ve kuersetin ingrediyenleri iki farklı konsantrasyonda olmak üzere D-glukoz monohidrat içeren (100:5, w/w) tam buğday unu ile karıştırılmış ve ekstrüzyona beslenmiştir. Bu ingrediyenlerin akrilamid seviyesi ve ekstrüde ürün kalite özellikleri üzerine etkisi incelenmiştir. Ekstrüzyon işlemi sırasında, bölge sıcaklıkları 80 ± 2, 90 ± 2, 115 ± 2 ve 150 ± 2 ^oC; vida hızı 180 rpm, besleme akış hızı 30 g/dk'dır. Besleme nemi % 18,24 ± 0,48'dir. Ekstrüzyon işleminden sonra, kurutma işlemi 150 ^oC'de 15 dakika boyunca yapılmıştır. İlave edilen ingrediyenlerle farklı azalma derecelerinde daha düşük akrilamid seviyeleri elde edilmiştir. Tüm ingrediyenlerde artan konsantrasyon ile daha düşük akrilamid seviyesine ulaşılmıştır. Çalışılan ingrediyenler arasında en yüksek akrilamid azaltımı 5 g/kg sistein ilavesi ile sağlanırken, en düşük azaltım 7,26 g/kg sitrik asit ve 13,36 g/kg askorbik asit ile elde edilmiştir. Kontrole göre, en yüksek kesit genleşme indeksi (SEI) 50 g/kg $CaCl_2$ ilavesi ile sağlanırken en düşük kesit genleşme indeksi ise 66.80 g/kg

askorbik asit ilavesi ile elde edilmiştir. Boylamsal genleşme indeksi (LEI) en yüksek değerine 66.80 g/kg askorbik asit ilavesi ile ulaşırken, en düşük değer 10 g/kg $CaCl_2$ ilavesi ile elde edilmiştir. Hacimsel genleşme indeksinde (VEI) 50 g/kg $CaCl_2$ veya 36,30 g/kg sitrik asit ilavesi kontrole göre anlamlı bir değişikliğe neden olmamıştır. En yüksek yığın yoğunluğu 66.80 g/kg askorbik asit ilave edilen üründe görülmüştür. Kontrole göre, 1 g/kg glisin, 10 g/kg NaCl, 36,30 g/kg sitrik asit veya 50 g/kg $CaCl_2$ ilavesi yığın yoğunluğunu etkilememiş ve en düşük yığın yoğunluğu değerlerine neden olmuştur. Renk açıklığı ile ilgili olarak, ekstrüde ürünlerde 66,80 g/kg seviyesinde askorbik asit ilavesi ile en yüksek L* değeri ve en düşük esmerleşme indeksi sağlanırken, en yüksek esmerleşme indeksi ile birlikte en düşük L* değeri 5 g/kg glisin ilavesiyle elde edilmiştir. Sonuç olarak, elimizdeki veriler ilave ingrediyenlerin belirli bir düzeyde akrilamid seviyesini azaltırken kalite parametrelerini de etkilediğini göstermiştir.

Anahtar Kelimeler: Ekstrüzyon, Tuzlar, Asitler, Amino asitler, Kuersetin, Akrilamid

To my beloved family…

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INTRODUCTION

1.1 Extrusion

Food extrusion has been applied for more than sixty years to produce a wide variety of foods. There are three types of food extruders which are piston extruder, roller extruder and screw extruder. Piston and roller extruders have the purpose of forming and conveying (Gray & Chinnaswamy, 1995). Screw extruders which have the purpose of forming, expansion and cooking are able to manufacture breakfast cereals, snacks, pet foods etc. (Singh et al., 2007; Riaz, 2006). In the early years, extrusion involved mixing and forming manufacturing macaroni and ready-to-eat-cereals with little or no expansion (Gray & Chinnaswamy, 1995; Gonzalez et al., 2014). Today, extrusion is a high-temperature-short time, multi-functional and thermo-mechanical process which converts a moistened, expansive, starchy and/or proteinaceous raw material into an intermediate or finished products (Singh et al., 2007; Riaz, 2006).

A screw extruder consists of a feeder that has screws conveying the feed, single screw or twin screw which rotates in a metal cabinet called barrel and a small orifice called die (Gray & Chinnaswamy, 1995). Screw extrusion is based on the principle that the feed material is forced to flow through a die to puff-dry the material (Riaz, 2006). During the extrusion process, non-cohesive feed material is exposed to mixing, heating and shear stress and converted to a viscoelastic melt along the screw due to the structural transformations such as loss of crystalline structure of starch, destruction of granular structure, rupture of glycoside bonds and new molecular

interactions (Gonzalez et al., 2014). Finally, pressure drop at the die cause bubble nucleation and subsequently an expanded solid is obtained due to the vaporization of moisture (Altan & Maskan, 2011; Kaletunc & Breslauer, 2002). This definition is appropriate for high shear single screw or twin screw extruders which was explained below.

1.1.1 Extruder types

Screw extruders are generally divided in two major categories based on transport mechanism and material flow which are single screw and twin screw extruders. In single screw extruders, friction forces play the major role in material transport. Material adhesion to the barrel is important for transport because if the material sticks to screw, it can not reach to die. On the other hand, in twin screw extruders, one screw channel was cleaned up by the other screw crest, so material in high quantity can be feeded to extruder (Vauchel et al., 2011).

Single screw extruders have four groups based on shear or mechanical energy requirements which are low shear forming, low shear cooking, medium shear cooking and high shear cooking. Twin screw extruders have also several different groups based on mechanical design which are co-rotating intermeshing, co-rotating non-intermeshing, counter-rotating intermeshing, and counter-rotating non-intermeshing and conical intermeshing. Each type of extruders has its own operating principles and application area (Huber, 2001).

Twin screw extruders have the capability to accomplish a wide variety of operations which are mixing, kneading and cooking. In addition, venting of steam or addition of flavours into the extruder is possible in twin screw extruders which is an important characteristic for novel products (Heldman & Hartel, 1998). For example, snack products such as half products and third generation snacks with new and complex ingredients at different shape and color are manufactured using twin screw

extruders (Riaz, 2006). However, twin screw extruders are more expensive than single screw extruders. Single screw extruders are continued to use when the flexibility of a twin screw extruder was not required. Pasta production, forming applications (low shear), puffed snacks production and texturizing applications (high shear) can be performed by a single screw extruder (Heldman & Hartel, 1998).

1.1.2 Extrusion parameters

Independent variables of extrusion can be divided in two categories which are feed and operating parameters (Heldman & Hartel, 1998). Feed characteristics include:

Moisture level: Feed having low moisture content causes lower throughput and increased pressure drop due to the higher viscosity. Less moisture also causes lower gelatinization degree and accordingly reduced expansion rate (Heldman & Hartel, 1998). Moraru & Kokini (2003) reported that, moisture has a plasticizer role during extrusion for glass transition of feed which leads to deformation of matrix and expansion.

Type of feed material: Starch-rich raw materials are commonly used in extrusion such as wheat, maize, rice flour, potato, rye, barley, oat, sorghum, cassava, tapioca, buckwheat and pea flour (Guy, 2001). Starch, protein, lipid and fiber content is important for final product quality of extrudates. It was found that, amylopectin-rich starches expand more than amylose-based starches (Moraru & Kokini, 2003). Protein content is important for textural quality of extrudates (Harper, 1986).

pH of ingredients: pH of feed have an impact on protein state of feed which influences physical characteristics of final product. Acidic or alkaline conditions during extrusion causes reduction on tensile strength and also causes changes in color and nutritional value due to the Maillard reaction (Heldman & Hartel, 1998).

Particle size of feed materials: Starch-based feed material is generally in the form of powder (flour) or grit. Small particles in extrusion have the capability of higher hydration and accordingly higher gelatinization degree (Heldman & Hartel, 1998). Particle size distribution should be under control prior to extrusion in order to avoid some blockages during processing (Brent, 1999).

Other ingredients: Some ingredients such as oil and emulsifier may be added to decrease the viscosity of feed material passing through the extruder. It was reported that sucrose had a decreasing effect on expansion of maize flour extrudates (Fan et al., 1996). Salt was also determined to have a substantial effect on gelatinization properties of starch during extrusion (Huber, 2001).

Operating parameters affecting final product characteristics include:

Feed rate: Feed rate affects the flow of material being extruded and final products quality. It must be stable to prevent variations in bulk density of final product (Brent, 1999). It was reported that gelatinization was affected by feed rate during extrusion. Ilo et al. (1996) found an increase in gelatinization degree of maize grits by increasing feed rate. Ding et al. (2006) stated that hardness increased with increasing feed rate in wheat flour-based extrudates.

Screw geometry: It is important especially for twin screw extruders. By changing the screw configuration; degree of mixing, degree of shear, extent of mechanical energy input and residence time can be altered. Cooking and forming characteristics are affected by these variables. For example, adding kneading disks to screw increase mechanical energy dissipation and mixing and, alters residence time. In addition, venting of volatiles and moisture is possible by inserting reversed pitch screw elements that allows an incorporation of a depressurizing section into to the extruder (Heldman & Hartel, 1998). Low conveying efficiency of screw causes a low viscosity in melt which leads to decrease in expansion (Kirby et al., 1988).

Extruder length: Longer extruders cause higher pressure and longer residence time affecting both of chemical reactions and physical processes which occurs in

extruder (Heldman & Hartel, 1998).

Screw speed: Screw speed used in extrusion is generally between 100-500 rpm. It can be controlled during extrusion. Screw speed influences shear rate, degree of fill in the barrel and residence time distribution during extrusion which lead to changes in gelatinization (Kumar et al., 2010). It is important to adjust the screw speed according to desired cooking degree.

Die characteristics: Area of the die opening affects the die pressure which is a dependent variable in extrusion. Expansion ratio is directly influenced by die pressure (Heldman & Hartel, 1998). Sokhey et al. (1997) indicated that, a die with a small diameter and shorter length reduce the energy consumption and give rise to expansion of extrudates.

Barrel temperature: Most of food extruders design allows to control the temperature by heating or cooling. Barrel temperature affects degree of cooking and also nutritional characteristics of extrudate (Heldman & Hartel, 1998).

Interactions between dependent and independent variables and their effect on final product characteristics are complex in extrusion process. Four parameters are defined as critical which are moisture, thermal energy input, mechanical energy input and retention time (Huber, 2001).

1.1.3 Applications

Extrusion technology has many functions such as mixing, homogenization, shearing, thermal cooking, texturing, forming and shaping (Riaz, 2006). Therefore, a wide variety of foods or food constituents could have been processed using this technology. Vauchel et al. (2011) separated extrusion applications into three categories which are simple extrusion, extrusion cooking and reactive extrusion. Simple extrusion encompasses the shaping of food products. Pastas, candies or

chewing gums, textured meat-like materials and texturized vegetable protein etc. can be produced by means of simple extrusion. Extrusion cooking is used to produce value added products such as ready-to-eat cereals, breakfast cereals, snacks, co-extruded snacks, crouton for soups and salads, pet and aquatic foods etc. (Singh et al., 2007; Vauchel et al., 2011; Kaletunc & Breslauer, 2002). Process parameters allow us to produce a wide variety of foods. For instance, texturized vegetable protein or pasta is produced by a high moisture (up to 75%)-low temperature (as low as 50 ^{o}C)-low shear operation while breakfast cereal or snack production is required low moisture (as low as 11%)–high temperature (as high as 180 ^{o}C)–high shear (Kaletunc & Breslauer, 2002). In recent years, extrusion has also been used for high-yielded chemical reactions. Modified starch production and casein-caseinate conversion etc. are applications of reactive extrusion (Vauchel et al., 2011).

1.1.4 Extrudate characteristics

Extrudate quality can be specified in terms of physical, functional, textural and sensory properties. These parameters are affected from feed characteristics and operating parameters. Relationship between quality characteristics and extrusion parameters was shown in Figure 1.

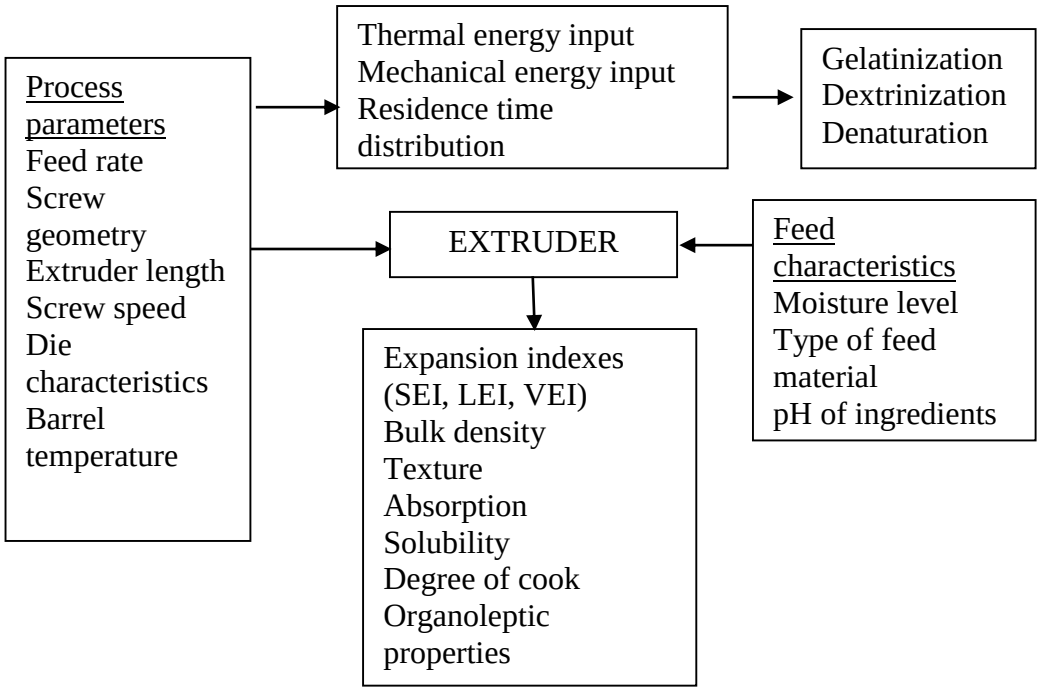

Figure 1. Relationship between quality characteristics and extrusion parameters (Adopted from Altan, 2008).

Physical properties encompass expansion indexes, bulk density and color. Expansion indexes characterize the degree of puffing after the melt exited the die of the extruder (Asare et al., 2004). Alvarez-Martinez et al. (1988) developed three different indexes to describe the expansion of extrudates which are volumetric expansion index (VEI), longitudinal expansion index (LEI) and sectional expansion index (SEI). Sectional expansion index describes the radial expansion index of the extrudates. Longitudinal expansion index measures the expansion in the axial direction. Volumetric expansion index is used to characterize the expansion on a volumetric basis (Neumann & Seib, 1993). They provide an accurate description of cell structure of extrudate.

The another important physical characteristic of an extrudate is bulk density. Bulk density is generally inversely proportional to expansion ratio and evaluates the

expansion in all directions (Wang et al., 2007a). Porosity is also used to describe the expansion characteristics of an extrudate (Yagci, 2008).

Color is very important factor influencing the acceptability of a food product. During extrusion, high temperature and low moisture conditions favor the nonenzymic browning reactions which are Maillard reactions, sugar caramelization and also pigment degradation (Ilo et al., 1996). Ilo et al. (1996) indicated that color of an extrudate is a visual indicator to evaluate process severity and nutritional loss.

Functional properties include water absorption and water solubility indexes. Water absorption refers to the dispersion of starch in excess water which increases with increasing damaged starch due to gelatinization (Rayas-Duarte et al., 1998). Therefore, it is used as gelatinization indicator (Sacchetti et al., 2004; Ding et al., 2006). Water solubility index is an indicator of dextrinization which causes the increase in soluble molecules (Kirby et al., 1988). Evaporation is applied to supernatant from water absorption determination and percentage of dry matter remainder gives the water solubility index (Anderson et al., 1969).

Texture is very important sensorial property for extruded products. Cell size distribution and cell wall thickness are two factors affecting the textural properties of extrudates. Large cells with thicker cell walls indicate a crunchy texture while small cells with thinner cell walls cause crispness. Hardness is another factor contributing to texture. Breaking strength have been used in some studies to characterize the hardness (Onwulata et al., 2001).

Physical and chemical modifications which take place during extrusion lead to alteration in sensorial properties such as appearance, aroma, flavor and texture. Descriptive sensory analysis and hedonic scale are the most used methods to evaluate the sensorial properties of extrudates. There are many studies including the effect of process variables (Chen et al., 1991; Jacoby & King, 2001; Liu et al., 2000) and feed material formulation on the sensory properties of extrudates in the literature (Rampersad et al., 2003; Obatolu Veronica et al., 2006).

Extrusion process has both of negative and positive effects on nutritional quality of foods. Since extrusion have been used to fortified, nutritionally balanced food, nutritional characteristics of an extrudate gain more importance. Firstly, formation of resistant starch and increase in dietary fiber during extrusion is one of the important points in terms of nutritional properties of extrudates. Extrusion was also determined to increase the solubility of dietary fiber in corn meal, oat meal and wheat (Bjorck et al., 1984; Camire et al., 1990). Extent of this changes depends on the process parameters as well as feed material formulation (Bjorck et al., 1984; Camire et al. 1990). Cheftel (1986) also indicated that the bacterial degradation of fiber in the human intestine may be altered in extruded products leading to changes in physiological effects.

Alteration in phenolic compounds by extrusion is another observation depending on the process parameters and feed formulation. However, there are contradictory results for the effect of extrusion on antioxidant activity. Sensoy et al. (2006) found no significant ($p < 0.05$) change in antioxidant capacity of dark buckwheat flour extruded at 170 ^{o}C. However, Altan et al. (2009) obtained a decrease in antioxidant capacity and phenolic content in barley extrudates. It was also observed in another study, change in phenolic content after extrusion depends on the cereal type (Zielinski et al., 2001). Apart from phenolics, extrusion was reported to have an effect on some other nutrient and phytochemicals such as inositol hexaphosphate, tocols, glutathione and melatonin depending on the type of grain used as feed and process temperature (Zielinski et al., 2001).

High temperature low moisture condition in extrusion process induce Maillard reaction in extrudates. Maillard reaction cause a decrease in available lysine at a range of 32-80% which can be due to hydrolysis of starch during extrusion and increasing the amount of sugars which can undergo Maillard reaction (Cheftel, 1986). Apart from that, some toxic compounds generating from Maillard reaction can also be formed such as acrylamide (Becalski et al., 2004). However, limited data is available in the literature about the effect of extrusion process on the acrylamide level.

1.2 Snack Food Production

Snack production includes different technologies and unit operations which provide a wide variety of products. Snack manufacturers categorized snacks as first generation, second generation and third generation snacks. First generation snacks include nuts, potato chips and popcorn which are manufactured from natural whole food materials. Second generation snacks contain the majority of snack foods. Corn tortilla chips, puffed corn curls and all directly expanded snacks are fallen into this category. Third generations snacks are half products or pellets containing multi-ingredients. Most of snack foods have been manufactured using extrusion technology. Majority of second generation and third generation snacks are extruded products (Riaz, 2006).

Extruded snacks are made from different sources such as wheat, barley, potato, tapioca and oats. Amylose content is important to provide expansion and good texture of extrudates. Starches used as feed of extrusion have 5-20% amylose content. Amylose content of wheat is in the range of 20-25% which gives a more expansion compared to the other grains. Protein content (8-15%) is also high in wheat flour which may prevent a good expansion, however it imparts a crispy texture to extrudates. Wheat flour also gives a mild flavor to extrudates (Riaz, 2006).

In snack and/or breakfast cereal production, extrusion barrel temperature is generally 150 ^{o}C and final moisture content at the die is approximately 15%. Moisture content of the extrudates decreases to 3-4% by drying. Extrudates are dried in rotary tumbling dryer or perforated conveyer dryers at a temperature of 150 ^{o}C for 5-6 min (Seker, 2011). Flow diagram of extruded snack was shown in Figure 2.

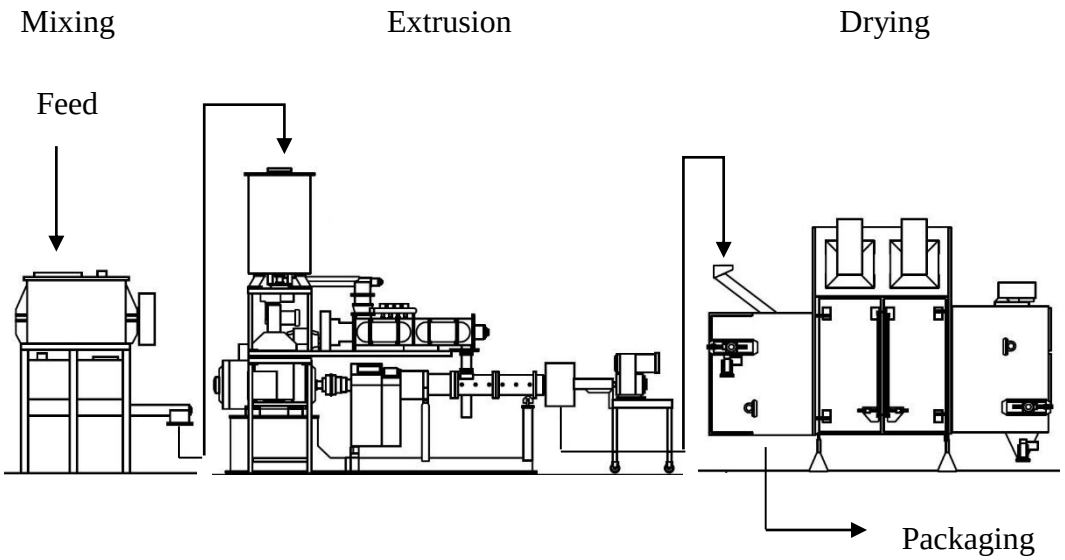

Figure 2. Flow diagram of extruded snack production (Adopted from Huber, 2001).

1.3 Acrylamide

Acrylamide (2-propenamide) is a chemical formed from hydration of acrylonitrile and has a colorless and odorless crystalline structure with a melting point of 84.5 ^{o}C. In the form of polyacrylamide, it has a wide application area in industry which are textile, paper, ore processing, waste-water treatment and cosmetics (Zhang & Zhang, 2007; Xu et al., 2014). It was reported that, polyacrylamide was depolymerized to acrylamide in the presence of heat, light and outdoor environmental conditions (Smith et al., 1996). Acrylamide was classified as a Group 2A carcinogen - 'probably carcinogenic to humans' by International Agency for Research on Cancer (IARC, 1994). Acrylamide has gained public attention after being discovered in heat-treated carbohydrate-rich foods by scienstists from the Swedish National Food Administration and the University of Stockholm in 2002. In the same year, European Union also classified acrylamide as Category-2 carcinogen and Category-2 mutagen. In 2010, European Chemical Agency put acrylamide into the list of chemicals of "very high concern". It was reported that

acrylamide is a multi-organ carcinogen in mice and rats and also cause reproductive, genotoxic and neurotoxic damage (Friedman et al., 1995; Tyl et al., 2000; Pedreschi et al., 2014).

Acrylamide forms in carbohydrate rich foods through heat processing at a temperature of higher than 120 ^{o}C. It mainly originates from baking, roasting and extruding of cereals, roasting of coffee and frying of potatoes (Studer et al., 2004). Acrylamide was found mostly in fried potatoes, bakery products, breakfast cereals, and coffee (Pedreschi et al., 2014).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) employed a margin of exposure (MOE) criterion for risk assessment of acrylamide (JECFA, 2011). MOE was defined as the benchmark dose divided by the exposure dose. Benchmark dose means lower 95% confidence limit of a specific increase in adverse response compared to that of unexposed subjects (Dakeishi et al., 2006). MOE level lower than 10000 is associated with health concern. MOE was found less than 200 in some cases for acrylamide which means there can be a relation between cancer and acrylamide exposure. From an epidemiological point of view, most studies showed that cancer is not associated with acrylamide exposure clearly (Pedreschi et al., 2014). However, determining an actual acrylamide exposure has difficulties because of large variations between different foods and between brands of the same food for acrylamide level (Amrein et al., 2004; EC., 2011). With all that, available information requires to limit acrylamide level as possible to lower the exposure. It is not possible to limit diet to reduce acrylamide exposure, because a wide variety of foods contain acrylamide. Moreover, foods having high acrylamide contribute to daily energy intake, daily iron intake and daily folate intake at a high level (Claus et al., 2008a). Acrylamide level in different foodstuff was shown in Table 1.

Food and Agriculture Organization/World Health Organization (FAO/WHO) determined long-term exposure estimate for acrylamide in the range of 0.3-0.8 µg/kg bodyweight per day for adults and 2-3 times more for children and

adolescents in the United States (FAO/WHO, 2002). The higher intake of acrylamide in children is probably due to the fact that they have higher caloric intake and consume the foods having higher level of acrylamide such as French fries and pototo crisps (Dybing et al., 2005). The contribution to acrylamide exposure shows differences between countries due to different dietary patterns and preparation methods (Claus et al., 2008a). While potato chips caused the highest exposure in USA, in European countries highest exposure to acrylamide was determined to be from bread (Xu et al., 2014). A recent study from China showed that higher exposure to acrylamide among Chinese people was from vegetable products, cereals, potatoes, meats, legumes and nuts (Zhou et al., 2013). In Turkey, Cengiz et al. (2013) studied the acrylamide exposure among toddlers from cereal-based products and found that the exposure come from bread, crackers, biscuits, baby biscuits, powdered cereal-based baby foods, baby bread-rusks and breakfast cereals which are from the highest to the lowest. Mean acrylamide exposure were determined to be 1.43 μg/kg body weight per day in the same study.

A maximum tolerable level has been established for drinking water as 0.1 μg/L by European Union (EC., 1998). However, there is no regulatory limits for acrylamide content in foods in any country (Pedreschi et al., 2014). However, European Commission has recommended monitoring the acrylamide level in foods considering the indicative values developed for 10 food categories and revised in 2011 which were shown in Table 1. These values are not safety level and are not mandatory (Xu et al., 2014).

Table 1. Indicative values for acrylamide contents in foods (Adopted from EC., 2013).

Food Groups	Indicative value (µg/kg)	2007			2010		
		n	Mean (µg/kg)	Maximum (µg/kg)	n	Mean (µg/kg)	Maximum (µg/kg)
French fries ready to eat	600	648	356	2668	256	338	2174
Potato crisps from fresh potatoes and from potato dough	1000	293	551	4180	173	758	4533
Potato based crackers	1000						
Pre-cooked French fries/potato products for home cooking		137	306	2175	117	331	3955
Soft bread		176	75	1778	150	30	425
Wheat based bread	80						
Soft bread other than wheat based bread	150						
Breakfast cereals (excl. porridge)		144	149	1600	174	138	1290
bran products and whole grain cereals, gun puffed grain	400						
wheat and rye based products	300						
maize, oat, spelt, barley, and rice based products	200						
Biscuit	500	222	309	4200	340	196	1940
Wafer	500	33	230	1378	37	389	1300
Crackers with the exception of potato based crackers	500	27	237	1526	64	178	1062
Crispbread	450	198	232	2430	54	249	1863
Gingerbread	1000	458	387	3615	207	415	3191

Table 1. (continued) Indicative values for acrylamide contents in foods (Adopted from EC., 2013).

Products similar to the other products in this category	500	222	309	4200	100	289	5849
Roast coffee	450	175	256	1158	103	256	1932
Instant (soluble) coffee	900	52	229	1047	15	1123	8044
Coffee substitutes		50	890	4700	24	1350	4200
coffee substitutes mainly based on cereals	2000						
other coffee substitutes	4000						
Baby foods other than processed cereal based foods		93	29	162	55	69	1107
not containing prunes	50						
containing prunes	80						
Biscuits and rusks for infants and young children	200	79	174	1215	46	86	470
Processed cereal based foods for imfants and young children excl. biscuits and rusks	50	65	69	353	128	51	578
Other foods							
Muesli and porridge		47	241	1315	14	80	420
Pastries and cakes		63	140	910	81	146	890
Non-potato savoury snacks		63	275	2110	80	192	1910
Unspecified other products		259	242	2529	161	293	3972

1.3.1 Acrylamide formation

Studies showed that there were various chemical pathways with different precursors leading to formation of acrylamide. The main chemical pathway is derived from Maillard reactions occuring between asparagine amino acid and carbonyl compounds such as reducing sugar (Tareke et al., 2002; Zyzak, 2003; Yaylayan et al., 2003). Asparagine is precursor and limiting compound for acrylamide formation via the Maillard reactions. It causes the formation of acrylamide at the temperatures above 120 ^{o}C in the presence of carbohydrates via the decarboxylation and deamination (Yaylayan et al., 2003).

Stadler et al. (2003) reported that α-hydroxy carbonyl which is found in such as fructose which was more effective than dicarbonyls in the formation of acrylamide. It was also reported that, decorboxylases from raw material may cause the formation of biogenic amine 3-APA from asparagine which can convert to the acrylamide via deamination without requiring the carbohydrates (Xu et al., 2014). There was another pathway suggested by Mottram et al. (2002) and Stadler et al. (2004) which leads to the acrylamide formation via the Maillard reactions. In this pathway, amino acids in raw material undergo Strecker degradation in the presence of dicarbonyl compounds and Strecker aldehyde route leads to formation of acrylamide.

Acrylic acid is another precursor of acrylamide formation. Acrylic acid generates from lipids at high temperature and converts to the acrylamide (Zhang et al., 2005). Moreover, small molecules liberated from monosaccharide heating also may form acrolein (Vattem & Shetty, 2003). Formation of acrylamide through acrylic acid or acrolein was found to be at minor level compared to pathways from asparagine precursor since asparagine and carbonyl compounds are more reactive than acrylic acid or acrolein (Zyzak et al., 2003). A summary of the acrylamide formation routes was represented in Figure 3.

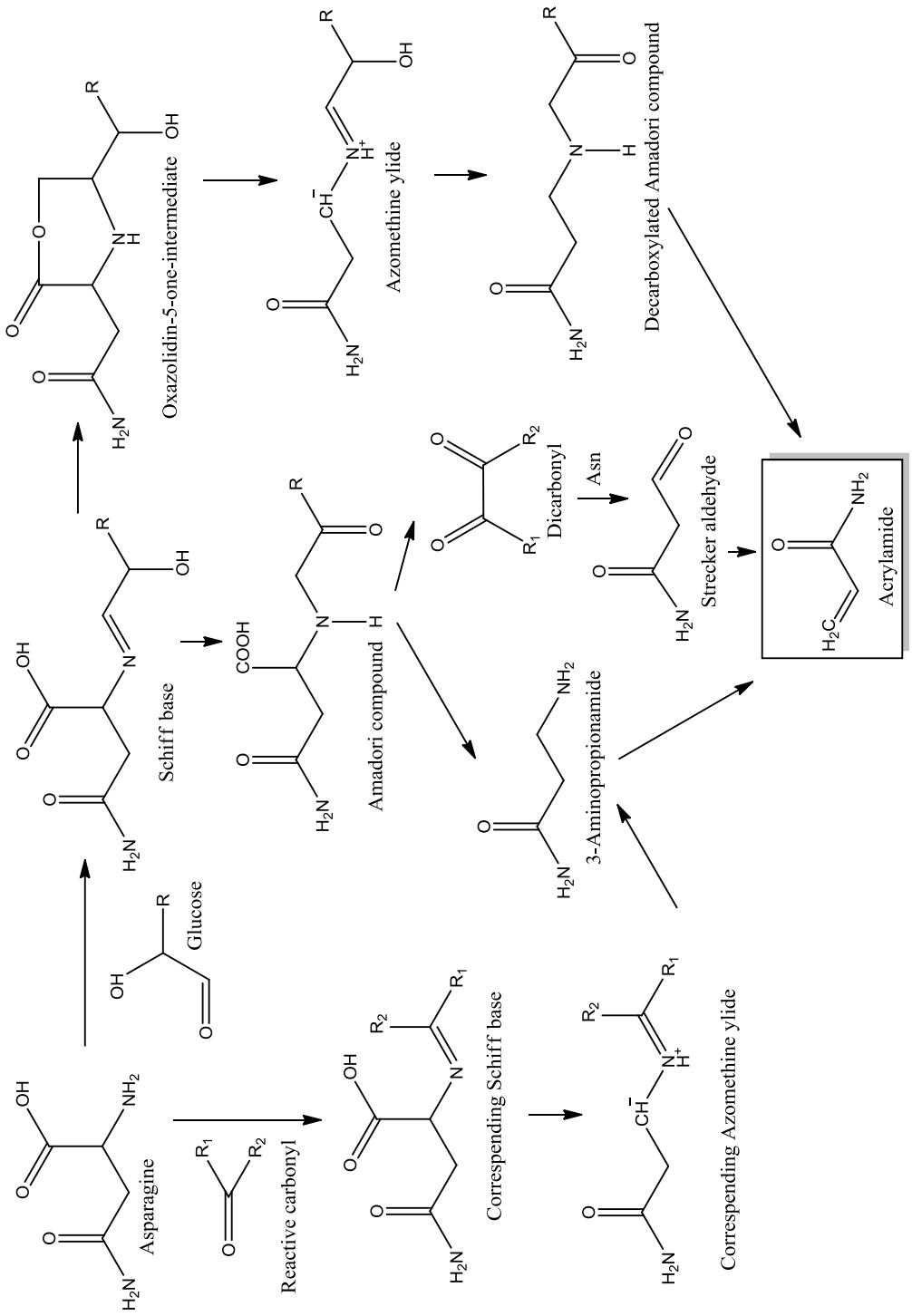

Figure 3. Summary of acrylamide formation pathways

1.3.2 Mitigation studies

In 2006, European Commission and the Confederation of European Union Food and Drink Industries (CIAA) organized a joint workshop and published a report exhibiting the knowledge and mitigation studies for acrylamide level in foods (Konings et al., 2007). In this report ALARA (As Low As Reasonably Achievable) principle was mentioned for acrylamide level in foods to encourage the minimization. It means a food processor should take all possible actions to minimize the acrylamide level. Acrylamide mitigation studies can be classified as modifications in raw material, processing and formulation.

1.3.2.1 Raw material factors

Decreasing the asparagine or reducing sugar content in the raw material leads to lower acrylamide formation. Asparagine level of raw material is important for the acrylamide formation in final product. Capuano et al. (2009) investigated the effect of flour type on acrylamide level and found that bread crisp model system made from rye and whole-wheat had higher acrylamide content compared with wheat flour due to their higher asparagine level.

Raw materials can be cultivated considering the asparagine or reducing sugar level by using genetic engineering (Pedreschi et al., 2014). Apart from genetic approach, Muttucumaru et al. (2006) determined that deficiency of sulfate in soil caused breeding of wheat with higher asparagine. In addition, breakfast cereal manufacturers observed that asparagine level in wheat was varied from season to season. Taeymans et al. (2004) reported that, influence of wheat variety have a high impact on the asparagine level. Claus et al. (2006) indicated that dry conditions and N-fertilizer caused accumulation of asparagine in cereal. Halford et al. (2012) indicated that, wheat and potatoes could be analyzed based on populations for

identification of quantitative trait loci (QTL) for low grain asparagine and/or reducing sugar. QTL means most probable location in DNA containing the genes which can promote the variation observed for a particular trait (Miles & Eayne, 2008).

Storage temperature is another factor that should be taken into consideration according to Biedermann-Brem et al. (2003) and Grob et al. (2003). Potato crisps produced from suitable cultivars of potatoes stored at temperatures not lower than 10 ^{o}C had 5-10 times less acrylamide content (Grob et al., 2003).

1.3.2.2 Processing factors

Time-temperature and moisture adjustments can control the acrylamide level. It was reported that, increasing temperature generally cause higher level of acrylamide (Friedman & Levin, 2008). Surdyk et al. (2004) found an increase in acrylamide level with increasing time and temperature. They also indicated that crust have the 99% of acrylamide in yeast-leavened wheat bread. Vacuum frying which reduce temperature and time, ensured lower acylamide level in potatoes (Granda et al., 2004). Microwave frying also decreased the acrylamide level in coating part of chicken which contains different kinds of flour (Barutcu et al., 2009). Confederation of European Union Food and Drink Industries (CIAA, 2011) reported that manufacturers should avoid to apply rework which increase the thermal input on food material leading to higher acrylamide level. However, Granby & Fagt (2004) found higher acrylamide level in medium-roasted coffee compared with in dark-roasted coffee. It was explained that, prolonged heating time decreased the acrylamide level under favour of evaporation and degradation.

Moisture is another important factor affecting the acrylamide level. Hedegaard et al. (2007) determined an increase in acrylamide level with decreasing water activity

in asparagine/glucose model system. However, in fat-rich foods lower initial moisture content ensured lower acrylamide level (Capuano et al., 2010).

It was reported that, pre-treatment methods had an important role in acrylamide level. Prolonged dough fermentation reduced acrylamide level by 50% in bread (Claus et al., 2008b). In crispbread, lactic acid fermentation also had an important decreasing effect on acrylamide level (Baardseth et al., 2004). Ciesarova et al. (2006) claimed that 50% reduction in acrylamide level was achieved by asparaginase pre-treatment in fried potatoes. Anese et al. (2011) applied asparaginase pre-treatment on short dough biscuits with changing asparaginase concentration, incubation time and temperature and found that acrylamide level was reduced at intermediate asparaginase concentration with an incubation at the lowest time and temperature in a high extent. Process equipment has also an effect on acrylamide level. In breakfast cereal production, Rufian-Henares et al. (2006) reported that direct expansion extrusion cooking (DEEC) process induced higher acrylamide level compared with pellet-to-flaking extrusion cooking (PFEC). Puffed breakfast cereals and also expanded snacks are produced by DEEC process. It was explained that, in DEEC process higher thermal input exerted on raw material provides a severe drying to a moisture content of 10% in a short time and this strong condition gives rise to acrylamide level in puffed extrudates.

1.3.2.3 Formulation

Addition of free amino acids into formulation was reported to have a strong decreasing effect on acrylamide formation probably due to the competition of added amino acids with asparagine for reactants and preventing the Maillard reaction between the asparagine and carbonyl compounds. Brathen et al. (2005) determined a 30% reduction in acrylamide level for potato crisps when glycine and glutamine were added during blanching and 50-90% reduction for bread crust when glycine was added. However, Claeys et al. (2005) indicated that glutamine had a strong

increasing effect on acrylamide level in asparagine-glucose model system. They also determined that, cysteine and lysine had a decreasing effect on acrylamide level in the range of 54-69% and 76-91%, respectively in different time-temperature profiles and alanine caused an increase in acrylamide level (Claeys et al., 2005). Amrein et al. (2004) indicated that glutamine and lysine had an increasing effect and glycine and cysteine had a decreasing effect on acrylamide level in gingerbread. Moreover, Levine & Smith (2005) found a decrease in acrylamide level in cracker model by addition of cysteine.

Acids are another ingredients added for mitigation purposes for acrylamide. Ascorbic acid and citric acid addition decreased the acrylamide level by 75% and 87%, respectively in cracker model. Kita et al. (2004) found a reduction by 90% in acrylamide level in potato crisps by soaking the potato strips in acetic acid solution and 50% reduction rate by soaking in citric acid solution. Rydberg et al. (2003) found an increase in acrylamide level around at a pH of 8 while lower pH value caused a decrease. Jung et al. (2003) hypothesized that, protonation of reactive nonprotonated amine ($-NH_2$) of asparagine generating the nonnucleophilic protonated amine ($-NH_3^+$) in the low pH blocks the Schiff base formulation which leads to reduce acrylamide formation.

Levine & Smith (2005) found a reduction by 65% on acrylamide formation by addition of NaCl at a level of ~1% in cracker model formulation. In addition, NaCl was found to decrease acrylamide content by 40% in asparagine/glucose model system (Kolek et al., 2006). Gokmen & Senyuva (2007a) found a complete prevention of formation of acrylamide by adding Ca^{+2} and a high reduction in acrylamide level by adding Na^+ to the asparagine-fructose model system. Soaking potato strips into NaCl or $CaCl_2$ solutions had a reduction effect on acrylamide formation (Lindsay & Jang, 2005; Pedreschi et al., 2007a).

Zhang et al. (2007) reported that, acrylamide reduction around by 74% was achieved in potato crisps and French fries by immersion in bamboo leave antioxidant solution before frying. Zhu et al. (2009) investigated effect of some

plant extracts and phenolic compounds on acrylamide level and found that extract of mint, cumin seed and star anise caused a reduction in the range of 69-75%. Phenolic compounds also reduced the acrylamide level by 9-53%. However, Acar & Gokmen (2009) determined grape seed extract had no effect on acrylamide level in crust-like model. Cheng et al. (2009) investigated the effect of six fruit extracts in acrylamide formation. They reported that, while apple extract decreased the acrylamide formation, dragon fruit extract enhanced the formation. Contradictory results for the effect of antioxidants may be due to their different structures and functional group which can react with acrylamide precursors or an intermediate in Maillard reaction which leads to inhibitory or promoting effects (Jin et al., 2013).

1.4 Objectives of the Study

The aim of the study was to determine the effect of added ingredients on acrylamide level of whole wheat flour extrudates. To accomplish that, NaCl, $CaCl_2$, ascorbic acid, citric acid, glycine, cysteine and quercetin were blended with whole wheat flour : D-glucose monohydrate (100:5, w/w) at two different concentrations. Drying process was applied to extrudates to imitiate the snack production. Effect of ingredients on expansion characteristics and color parameters of extrudates were also investigated.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Whole grain wheat flour was supplied by Katmer Un A.Ş. (Ankara, Turkey). All the reagents that were used in the analysis except the ones used in LC/MS/MS analysis were analytical grade where reagents used in LC/MS/MS were HPLC grade.

2.2 Methods

2.2.1 Sample preparation

Whole grain wheat flour and D-glucose monohydrate mixture (100:5 w/w) was moistened with distilled water and mixed in a mixer (Kitchen Aid, Ariston, USA) at medium speed. Moisture contents of the samples were determined by using a halogen moisture analyzer at 160 ^{o}C (MX-50, AND, Japan). Feed moisture contents were adjusted to 18.24±0.48%. After certain time of mixing, different agents which were sodium chloride, calcium chloride, ascorbic acid, citric acid, glycine, cysteine and quercetin were added to mixture at two different concentrations. Added concentrations of the ingredients were shown in Table 2. After the addition of the ingredients, mixing was continued for a certain time to ensure uniformity of the feed material. The control was prepared without chemicals to the same moisture

content. The samples were put in plastic bags and stored at room temperature overnight.

Table 2. Concentrations of the added ingredients.

	NaCl (g/kg)	$CaCl_2$ (g/kg)	Ascorbic acid (g/kg)	Citric acid (g/kg)	Glycine (g/kg)	Cysteine (g/kg)	Quercetin (g/kg)
Low	10	10	13.36 (pH=4.62)	7.26 (pH=4.59)	1	1	1.14
High	50	50	66.80 (pH=3.68)	36.30 (pH=3.30)	5	5	5.72

2.2.2 Extrusion and oven-drying

Extrusion was carried out using a laboratory scale co-rotating twin screw extruder (Feza Gıda Müh. Makine Nakliyat ve Demir Tic. Ltd. Şti., İstanbul, Turkey) with a computer control and data acquisition system. The extruder was fitted with a die of 3 mm diameter and had a 25:1 barrel length:diameter ratio. The extruder had four independent heating zones controlled by electrical heating and water cooling. Barrel zone temperatures and torque were monitored from the control panel during extrusion runs. A twin screw volumetric feeding device built into the extruder was used to feed the mixtures. Samples were taken from the extruder when temperature variations were no more than ±2 °C from the set barrel temperatures. Set zone temperatures were 80, 90, 115 and 150 °C. Samples were left to cool at room temperature before packaging in plastic bags. The final moisture content of the extrudates were 10.89±1.40%. Screw speed of 180 rpm was used, and flow rate was 30 g/min.

Oven-drying was carried out at 150 °C for 15 minutes. Extrudates were cut into 5 cm pieces and laid into a single layer in pre-heated oven. The final moisture content

of dried extrudates were 3.99±1.23%. Part of the dried extrudates were finely ground into powdered form with a blender (Waring Blender 8011ES, USA) and sieved through 800 μm test sieve. 5-cm cut samples and ground samples were kept in zip lock plastic bags at -20 ^{o}C until the analysis. Images of the extrudates were presented in Figure A.1.

2.2.3 pH determination

The pH was measured according to AACC 02-52.01 Hydrogen-Ion Activity (pH) - Electrometric Method with some modifications. 1 gr of ground sample was mixed with 10 mL of distilled water by using a homogenizer for a proper homogenization and pH was determined in the slurry under stirring.

2.2.4 Sectional expansion index

Diameters were measured using a digital caliper at random places of 5 cm cut samples. For each sample 20 measurements were taken and averaged. The sectional expansion index of the samples were calculated according to Pai et. al. (2009) by the following equation.

$$SEI = (D_e / D_d)^2 \quad (1)$$

Where;

D_d : Diameter of the die (mm)

D_e : Diameter of the extrudate (mm)

2.2.5 Longitudinal expansion index

The longitudinal expansion index was determined using the following equation (Alvarez-Martinez et al., 1988).

$$LEI = VEI / SEI \quad (2)$$

Where;

LEI : Longitudinal expansion index

SEI : Sectional expansion index

VEI : Volume expansion index

2.2.6 Volumetric expansion index

Volumetric expansion index was determined as described by Alvarez- Martinez et al. (1988).

$$VEI = \rho_s / \rho_b \quad (3)$$

Where ;

ρ_b : Bulk density (g/cm^3)

ρ_s : True density (g/cm^3)

VEI : Volumetric expansion index

2.2.7 True density

The true density of the extrudates were determined in triplicate using a helium picnometer (Quantachrome Ultrapycnometer 1000, Florida, USA). Archimides' principle of gas displacement and Boyle's law was employed in the method. Principle of gas pycnometry can be explained as follows: Valve which is fitted to sample chamber (V_c) is opened and the system is brought to ambient pressure, P_a, after being purged with helium gas (Chang, C. S., 1988). The initial condition is defined as

$$P_a V_c = nRT_a \quad (4)$$

Weighted extrudates with unknown volume are put into a sample chamber of known volume and chamber is sealed and the condition changes to

$$P_a(V_c - V_p) = n_1 RT_a \quad (5)$$

Then, system is pressurized to a pressure which is greater than the ambient pressure

$$P_2(V_c - V_p) = n_2 RT_a \quad (6)$$

Valve between the sample and reference chamber is opened and pressure decreases, the equation becomes

$$P_3(V_c - V_p + V_a) = n_2 RT_a + n_a RT_a \quad (7)$$

$P_a V_a$ can be used in place of $n_a RT_a$ in equation (7) and after equation (6) is substituted into equation (7), following equation is obtained

$$V_c - V_p = V_a(P_a - P_3)/(P_3 - P_2) \quad (8)$$

Because all pressure measurements are relative to P_a which is zeroed before pressurizing, it can be made to read zero and the working equation of ultrapycnometer is obtained

$$V_p = V_c + V_a / (1 - P_2 / P_3) \quad (9)$$

and the density is calculated as

$$\rho_{true} = m / V_p \quad (10)$$

Where ;

ρ_{true} : True density of the extrudate (g/cm^3)

m : Weight of the extruded sample (g)

n_a : Moles of gas in sample chamber at pressure P_a (mole)

n_1 : Moles of gas in sample chamber after placing the sample (mole)

n_2 : Moles of gas in sample chamber at pressure P_2 (mole)

P_a : Ambient pressure (Pa)

P_2 : Pressure in the sample chamber after pressurizing (Pa)

P_3 : Pressure of the whole system after the valve is opened (Pa)

R : The gas constant (J/(mol.K))

T_a : Ambient temperature (K)

V_a : Volume of the reference chamber (m^3)

V_c : Volume of the sample chamber (m^3)

V_p : Volume of the sample (m^3)

2.2.8 Bulk density

Liquid displacement method was used to determine bulk density. 5 cm cut samples were weighed and submerged into previously melted paraffin. After drying, the paraffin coated samples were weighed and submerged into the heptane in degree cylinder. The rise in the level was noted and the bulk density was determined using the following equations. Three measurements were taken and averaged.

$$V_p = (m_{s+p} - m_s) / \rho_p \quad (11)$$

$$V_b = V_d - V_p \quad (12)$$

$$\rho_b = m_s / V_b \quad (13)$$

Where ;

ρ_b : Bulk density of the extrudate (g/cm^3)

ρ_p : Density of the paraffin at room temperature (g/cm^3)

m_s : Weight of the extruded sample (g)

m_{s+p} : Weight of the extruded sample covered with paraffin (g)

V_b : Bulk volume of the extrudate (cm^3)

V_d : Displacement in cylinder after insertion of the sample (cm^3)

V_p : Volume of the paraffin (cm^3)

2.2.9 Porosity

The porosity was determined in triplicate using the true density of extrudates as determined by helium pycnometer and bulk density as determined by liquid displacement method.

$$\text{Porosity} = 1 - (\rho_b / \rho_t) \quad (14)$$

Where ;

ρ_b : Bulk density (g/cm^3)

ρ_t : True density (g/cm^3)

2.2.10 Colorimetric measurements

Sieved samples put onto a white paper and light projection tube was used to press gently the particles in order to provide a uniform surface. CIELAB color parameters of samples were measured in triplicate with a colorimeter (Koniko Minolta CR400, Japan). Measurements were expressed as tri-stimulus values (L*: lightness (0 = black, 100 = white), a* (−a = greenness, +a = redness) and b* (−b = blueness, +b = yellowness). Colorimeter was calibrated against a white plate (L* = 99.38, a* = 0.30, b* = 1.18). Browning index (BI) was calculated by the following equation (Buera et al., 1986).

$$BI = (x - 0.31) / 0.172*100 \quad (15)$$

where x is the chromaticity coordinate calculated from the XYZ tristimulus values according to the following formula;

$$x = X / (X + Y + Z) \quad (16)$$

2.2.11 Acrylamide analyis

Stock solution of acrylamide (1 mg/mL) were prepared by dissolving in distilled water. Working standards were prepared by diluting the stock solution of

acrylamide to concentrations of 0.25, 0.5 and 1 μg/mL.

Sample preparation for acrylamide analysis was conducted according to Mastovska & Lehotay (2006) with some modifications. 1 g of sample was weighed into 50 mL centrifuge tube. 10 mL of distilled water and 10 mL of acetonitrile was added for extraction. 4 g of anhydrous $MgSO_4$ and 0.5 g of NaCl was weighed as a salt mixture and then added to centrifuge tube. Immediately, tube was shaked by hand vigorously for 1 min to prevent crystalline agglomeration and to provide sufficient interaction of solvent and entire sample. Samples were centrifuged at 5000 rpm for 10 min (Sigma 2-16 PK, Shropshire, United Kingdom). 1 mL of acetonitrile supernatant was transferred into 2 mL minicentrifuge tube which 50 mg of primary secondary amine (PSA) and 150 mg $MgSO_4$ preweighed into. Extract and sorbent/desiccant in minicentrifuge tube mixed by vortex (VV3, VWR International) for 30 s. Centrifugation was performed at 14000 rpm for 5 min (Hettich Micro 120, Tuttlingen, Germany). Supernatant was filtered through 0.45 μm nylon syringe filter prior to LC/MS/MS analysis.

AB SCIEX QTRAP 4000 LC/MS/MS system was used for the determination of acrylamide. The analytical separation was performed on a Thermo Hypercarb (Thermo Electron Corporation, Waltham, MA, USA) graphitised carbon HPLC column (50 mm x 2 mm, 1.3 μm). Seperation was conducted isocratically with a mobile phase consisting 0.1 mL formic acid in 1 L of 98:2 water:methanol. The flow rate was 0.3 mL/min and the injection volume was 10 μL. LC/MS/MS was operated in positive electrospray ionization (ESI) and acquisition multiple reaction monitoring mode (MRM). Detection of acrylamide was achieved by monitoring the daughter ions formed by the collision-induced dissociation of the parent ions of acrylamide (m/z=72, M+1). Nitrogen was used for fragmentation of ions at pressures of 40 psi and 60 psi which is belonged the auxiliary gas. The curtain gas was nitrogen (40 psi), source temperature was set at 400 ^oC, the electrospray capillary voltage (IS) was set at 5500 V and the dwell time was 150 ms. The two daughter ions, (m/z=55) [CH_2=$CHCO^+$] which was used for quantification and

(m/z=44) [$O=C=NH_2^+$] which was used for confirmation, were monitored. The collision energy and declustering potential are given in brackets for each monitored daughter ions as follows m/z 72→55 (CE – 15 V, DP – 35 V) and m/z 72→44 (CE – 18 V, DP – 35 V). Identification of the tested compounds was achieved by using both retention time and mass spectra.

Analytical quality control was accomplished by the use of certified reference material (FAPAS 3044-biscuit, Sand Hutton York, United Kingdom). Assigned value for FAPAS 3044 was 523 µg/kg and the observed value was 543 µg/kg. Calculated z score (1.04) was in the given 95% confidence range ($-2 < z < 2$).

2.2.12 Statistical analyis

The results were analysed by analysis of variance (ANOVA) using Minitab statistical software (Minitab, Inc., State College, Pa., USA). Number of replications were indicated at the bottom of each table for all the analyses. Fisher Method was used to test significant differences which was accepted if *p* was less than 0.05.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Expansion Characteristics of Extrudates

The effect of salts on expansion characteristics of the extrudates was presented in Table 3 and Table 4.

Table 3. Effect of NaCl addition on expansion characteristics of extrudates.

Sample	Amount (g/kg)	SEI	LEI	VEI	Bulk Density (g/cm^3)	Porosity
Control	-	8.02 ± 0.71^{a}	1.58 ± 0.21^{a}	12.71 ± 1.22^{a}	0.14 ± 0.02^{c}	0.92 ± 0.09^{a}
NaCl	10	8.31 ± 0.78^{a}	1.30 ± 0.20^{ab}	10.77 ± 1.29^{ab}	0.16 ± 0.02^{b}	0.91 ± 0.11^{a}
NaCl	50	8.12 ± 0.33^{a}	1.13 ± 0.07^{b}	9.17 ± 0.38^{b}	0.18 ± 0.01^{a}	0.89 ± 0.04^{a}

SEI results are means ± SD (n = 20); LEI results are means ± SD (n = 3); VEI results are means ± SD (n = 3); BD results are means ± SD (n = 5); porosity results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

NaCl addition did not affect SEI and porosity in the studied range. Cut-view images of the extrudates were shown in Figure A.2. LEI decreased slightly, at high level of NaCl addition. VEI decreased and bulk density increased with increasing concentration. Pitts et al. (2014) found that, 2% NaCl addition increased the bulk density of wheat-corn extrudate and decreased SEI. They explained the reduction

in SEI that NaCl has a co-solute effect which competes for water with starch and accordingly limited gelatinization. Several researchers determined that volume of expansion decreases and bulk density increases with decreased gelatinization (Mercier & Feillet, 1975; Case et al., 1992). Accordingly, any ingredient that dilute the starch or interfere with starch melting or reassociation causes a decrease in expansion and increase in bulk density. Farahnaky et al. (2009a) indicated that salt have an important effect on the expansion characteristics of low-moisture (<20%, dry basis) starch-based extrudates. They explained the behavior of NaCl molecules in starch during extrusion as a plasticizer due to the remaining as a whole and not dissociating to its ions at low moisture. This behavior of NaCl causes a decrease in glass transition temperature (Tg) which has an important effect on expansion properties of extrudates. Gelatinized starch during extrusion acts as a viscous fluid and after emerging the die, it cools and the system goes below the Tg and expansion is maintained. If Tg decrease, system remain above the Tg and it collapse and contract (Blanshard, 1995). However, there are studies having contradictory results for the effect of NaCl on expansion of extrudates. Chinnaswamy & Hanna (1988) found an increase in expansion ratio in corn starch extrudates with NaCl addition at the level of 0-10 g/kg. Hsieh et al. (1990) determined that salt addition in corn meal at the level up to 3% caused an increase in SEI and LEI. This contradiction may be explained that salts have different effect on different feed material. Jyothi et al. (2005) mentioned that the starches from different sources show variable gelatinization parameters with NaCl addition.

Table 4. Effect of $CaCl_2$ addition on expansion characteristics of extrudates.

Sample	Amount (g/kg)	SEI	LEI	VEI	Bulk Density (g/cm^3)	Porosity
Control	-	8.02 ± 0.71^c	1.58 ± 0.21^a	12.71 ± 1.22^a	0.14 ± 0.02^b	0.92 ± 0.09^a
$CaCl_2$	10	9.00 ± 0.76^b	0.83 ± 0.09^b	7.44 ± 0.48^b	0.20 ± 0.02^a	0.87 ± 0.06^a
$CaCl_2$	50	10.02 ± 0.52^a	1.08 ± 0.13^b	10.82 ± 1.13^a	0.15 ± 0.01^b	0.91 ± 0.09^a

SEI results are means ± SD (n = 20); LEI results are means ± SD (n = 3); VEI results are means ± SD (n = 3); BD results are means ± SD (n = 5); porosity results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

As seen in Table 4 SEI increased with $CaCl_2$ addition. Bulk density increased and VEI decreased at low level of $CaCl_2$ addition. LEI showed a decrease when $CaCl_2$ was added. Porosity did not change in the studied range with salt addition. Effect of $CaCl_2$ differed from the effect of NaCl on extrudate expansion. Eliasson and Gudmundsson (2006) supported this finding by indicating that, effect of salts on gelatinization of starch widely depends on type of salt. There has been limited studies on the effect of $CaCl_2$ on extrudates. Studies are available investigating the effect of some other calcium sources on expansion characteristics of extrudates. The results of these studies differed from present result. Zazueta-Morales et al. (2002) found a decrease in SEI in blue maize extrudates with added $Ca(OH)_2$ at the level up to 0.2% at a screw speed of 100-180 rpm and at 150 ^oC barrel temperature. $Ca(OH)_2$ at a concentration of 0-0.35% caused a decrease in corn meal extrudates produced by single screw extruder at operating parameters of 130 rpm and 130-150 ^oC. $(C_6H_{10}O_6)Ca$ also caused a decrease in blue maize extrudates in a concentration range of 0.3-0.9 % in a study in which single screw extruder was used at 70-90 ^oC barrel temperature (Sanchez-Madrigal, 2014). However, operating conditions and type of extrusion, feed material and concentration of calcium sources were different from that of present study. Viturawong et al. (2008) indicated that Cl^- ions decreased the gelatinization enthalpy by breaking hydrogen bonds between starch

chains and ease the gelatinization. Extended gelatinization could lead to increase in expansion. Neverthless, effect of salt on gelatinization of starch have not been fully understood and also on the extrudate characteristics. Some authors have discussed effect of salts in consideration of remaining as an ion pair and acting like a sucrose (Farahnaky et al., 2009a) and others being ionizated during extrusion and affecting the starch chains (Sanchez-Madrigal et al., 2014; Zazueta-Morales et al., 2002).

Table 5. Effect of ascorbic acid addition on expansion characteristics of extrudates.

Sample	Amount (g/kg)	SEI	LEI	VEI	Bulk Density (g/cm^3)	Porosity
Control	-	8.02 ± 0.71^{a}	1.58 ± 0.21^{b}	12.71 ± 1.22^{a}	0.14 ± 0.02^{c}	0.92 ± 0.09^{a}
Ascorbic acid	13.36 (pH=4.62)	6.93 ± 0.66^{b}	1.15 ± 0.12^{b}	7.99 ± 0.27^{b}	0.20 ± 0.02^{b}	0.87 ± 0.03^{a}
Ascorbic acid	66.80 (pH=3.68)	1.96 ± 0.22^{c}	3.53 ± 0.49^{a}	6.93 ± 0.60^{b}	0.30 ± 0.03^{a}	0.86 ± 0.07^{a}

SEI results are means ± SD (n = 20); LEI results are means ± SD (n = 3); VEI results are means ± SD (n = 3); BD results are means ± SD (n = 5); porosity results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

SEI and VEI values decreased with ascorbic acid addition (Table 5), where VEI did not show significant difference at low or high level acid addition. LEI showed an increase at high level ascorbic acid addition. Bulk density increased with increasing concentration of ascorbic acid addition. Porosity did not change with ascorbic acid addition as shown in Table 5. Apart from starch structure, protein network may also be influenced by decreasing pH (Hickson, 1982) during extrusion which may lead to lower expansion. Sriburi & Hill (2000) conducted a study on cassava starch blended with several level of ascorbic acid and found an increase in expansion with increasing ascorbic acid addition at temperatures in the different barrel zones (40, 90, 110 and 75 °C). However, lower ascorbic acid level (0-1%, starch basis) were

used than that of present study and also extrusion was conducted at higher barrel temperatures in the present study which may cause higher degradation of granular structure leading to reduction of expansion. It was also possible to explain this contradiction by type of starch which may influence the expansion of extrudates (Chang & El-Dash, 2003).

Table 6. Effect of citric acid addition on expansion characteristics of extrudates.

Sample	Amount (g/kg)	SEI	LEI	VEI	Bulk Density (g/cm^3)	Porosity
Control	-	8.02 ± 0.71^a	1.58 ± 0.21^a	12.71 ± 1.22^a	0.14 ± 0.02^b	0.92 ± 0.09^a
Citric acid	7.26 (pH=4.59)	7.10 ± 0.49^b	1.09 ± 0.11^b	7.71 ± 0.61^b	0.20 ± 0.02^a	0.87 ± 0.07^a
Citric acid	36.30 (pH=3.30)	6.72 ± 0.68^b	1.72 ± 0.25^a	11.56 ± 1.19^a	0.16 ± 0.01^b	0.91 ± 0.09^a

SEI results are means ± SD (n = 20); LEI results are means ± SD (n = 3); VEI results are means ± SD (n = 3); BD results are means ± SD (n = 5); porosity results are means ± SD (n = 3). Values with a same letter are not significantly different from control level ($p < 0.05$).

The effect of citric acid on physical properties of extrudates was presented in Table 6. Citric acid addition at the level of 7.26 g/kg and 36.30 g/kg decreased ($p < 0.05$) SEI of extrudates compared to control. It may be due to the cross linking of starch granules via esterification between the carboxyl groups on citric acid and the hydroxyl groups on starch which cause contraction in extrudate. It was also reported that citric acid cause a reduction in cross-linking degree of protein network in wheat flour extrudates (Chabrat et al., 2012). That loosening of gluten structure can also lead the reduction in SEI. However, there was no significant ($p < 0.05$) difference between the SEI values of extrudates prepared by increasing level of citric acid. LEI and VEI showed a lower value from control sample at low concentration of citric acid, where bulk density increased. Hovewer, at high concentration LEI, VEI

and bulk density was not significantly ($p < 0.05$) different from control. Porosity values were not affected by citric acid additon. Reduction in expansion value of extrudates containing citric acid was supported by many studies in the literature. Wang et al. (2007b) found a decrease in SEI and increase in bulk density for starch-guar gum extrudates including citric acid in the range of 2-10%. They explained that, pattern of macromolecular degradation and gel structure properties may change by citric acid addition. It was contradictory with VEI and bulk density values we found in extrudates containing higher level of citric acid. However, feed of material was important for expansion properties of extruded products. Wang et al. (2007b) also mentioned that expansion ratios substantially dependent on starch types, amylose content and structure of amylopectin. In another study, SEI decreased in sorghum extrudates including citric acid at the level up to 7.1 g/kg and an increase were observed in extrudates prepared with 14.3-57.1 g/kg citric acid. It was attributed to increased fragmentation of starch by the combination of high barrel temperatures and high citric acid concentrations. Barrel temparatures applied in the study was similar to that of we used in the present study. They also observed higher bulk density values with higher citric acid concentrations which may be due to the alteration in macromolecular structure and conformation of extrudates and consequently the formation of starch-protein interactions leading to a higher density (Mendez-Albores et al., 2009).

Table 7. Effect of glycine addition on expansion characteristics of extrudates.

Sample	Amount (g/kg)	SEI	LEI	VEI	Bulk Density (g/cm^3)	Porosity
Control	-	8.02±0.71[a]	1.58±0.21[a]	12.71±1.22[a]	0.14±0.02[c]	0.92±0.09[a]
Glycine	1	7.39±0.74[b]	1.34±0.14[ab]	9.87±0.37[b]	0.17±0.01[b]	0.90±0.03[a]
Glycine	5	7.26±0.71[b]	1.10±0.12[b]	7.96±0.34[c]	0.20±0.01[a]	0.87±0.04[a]

SEI results are means ± SD (n = 20); LEI results are means ± SD (n = 3); VEI results are means ± SD (n = 3); BD results are means ± SD (n = 5); porosity results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

As shown in Table 7, glycine added extrudates had lowered SEI while increased level did not cause any significant ($p < 0.05$) further decrease. LEI was not significantly ($p < 0.05$) different at the low level of addition but increased concentration caused a slight decrease ($p < 0.05$). VEI decreased with increasing concentration where bulk density increased. Porosity values were not affected by glycine addition. There has been no discussion about the effect of glycine on physical properties of extrudates. Effect of protein addition have been investigated by some researchers (Kim & Maga, 1987; Onwulata et al., 2001; Allen et al., 2007). Whey protein concentrate-corn starch extrudates showed a decrease in SEI and increase in bulk density with increasing concentration of protein (Allen et al., 2007). They explained that, whey protein and amylose molecules may align linearly via a chemical interaction or an entraption or carrying of one by the other during extrusion and reduction in expansion results due to starch-protein complex. Onwulata et al. (2001) also observed the same result for corn-whey protein concentrate and rice-whey protein concentrate extrudates prepared at different concentrations.

Table 8. Effect of cysteine addition on the expansion characteristics of extrudates.

Sample	Amount (g/kg)	SEI	LEI	VEI	Bulk Density (g/cm^3)	Porosity
Control	-	8.02 ± 0.71^a	1.58 ± 0.21^a	12.71 ± 1.22^a	0.14 ± 0.02^c	0.92 ± 0.09^a
Cysteine	1	7.66 ± 0.51^a	1.13 ± 0.14^b	8.62 ± 0.86^b	0.19 ± 0.02^b	0.88 ± 0.09^a
Cysteine	5	6.51 ± 0.72^b	1.17 ± 0.18^b	7.59 ± 0.79^b	0.22 ± 0.02^a	0.87 ± 0.09^a

SEI results are means ± SD (n = 20); LEI results are means ± SD (n = 3); VEI results are means ± SD (n = 3); BD results are means ± SD (n = 5); porosity results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

SEI of extrudates prepared by cysteine at the level of 1g/kg whole wheat flour did not differ significantly ($p < 0.05$) from control, however it was decreased by 19% with increasing level of cysteine (Table 8). LEI and VEI were significantly ($p < 0.05$) different from control but did not present a significant ($p < 0.05$) difference between the extrudates having different level of cysteine. Bulk density increased with increasing concentration. Porosity of extrudates were not significantly ($p < 0.05$) affected. Reduction in SEI with the addition of cysteine was also determined by Li & Lee (1996) and Koh et al. (1996). However, Li & Lee (1996) determined an increase in volume of expansion and decrease in bulk density in extrudates containing cysteine at the level up to 0.75 g/kg. Bulk density slightly increased and volume of expansion decreased by cysteine addition in the range of 0.75-1.5%. It was explained by weakening of internal structure, thinner cell walls and less compact matrix caused by cysteine addition. That structure induced an increased volume of expansion due to lower resistance of structure to the steam emerging at the die and formation of much more air bubbles leading to higher expansion. However, addition of cysteine at higher level contributed to further weakining in structure resulted in densely packed cell walls and smaller cell size leading to reduced expansion. According to Li & Lee (1996), during extrusion, sulph-hydryl (SH) groups of cysteine are oxidized and disulfide (SS) bonds are formed between

cysteine and protein resulting a decrease in protein-protein interaction via the disulfide bonds which alters the structure.

Table 9. Effect of quercetin addition on the expansion characteristics of extrudates.

Sample	Amount (g/kg)	SEI	LEI	VEI	Bulk Density (g/cm^3)	Porosity
Control	-	8.02 ± 0.71^{a}	1.58 ± 0.21^{a}	12.71 ± 1.22^{a}	0.14 ± 0.02^{b}	0.92 ± 0.09^{a}
Quercetin	1.14	7.87 ± 0.50^{a}	1.06 ± 0.09^{b}	8.36 ± 0.43^{b}	0.19 ± 0.02^{a}	0.88 ± 0.05^{a}
Quercetin	5.72	6.94 ± 0.87^{b}	1.27 ± 0.18^{ab}	8.80 ± 0.64^{b}	0.20 ± 0.01^{a}	0.89 ± 0.06^{a}

SEI results are means ± SD (n = 20); LEI results are means ± SD (n = 3); VEI results are means ± SD (n = 3); BD results are means ± SD (n = 5); porosity results are means ± SD (n = 3). Values with same letter are not significantly different in the same column ($p < 0.05$).

Quercetin addition affected slightly the SEI at high level of quercetin but not at the low level of quercetin additon (Table 9). VEI and bulk density values were affected by addition of quercetin but increasing the level did not cause any additional effect. LEI decreased at the low level but did not significantly ($p < 0.05$) differ from control at the high level of addition. Porosity were not affected by quercetin addition. Camire et al. (2005) mixed cornmeal with quercetin at a level of 200 ppm and determined that quercetin addition gives more expanded extrudates having lower bulk density. However, the added quercetin concentration was lower than used in present study and also feed material was different. Camire & Dougherty (1998) used low level of phenolics (BHT, cinnamic acid and vanillin) and did not find any difference in expansion ratios of extrudates. According to another study, quercetin addition at the level of 0.5, 1 and 1.5 g/kg did not affect expansion characteristics of oat flour-sugar (9:1 w/w) extrudates (Viscidi et al., 2004).

3.2 Color

Color is an important parameter for acceptability of food products. During extrusion, high temperature and low moisture conditions affect color of extrudates via the chemical reactions and pigment degradation. L* value indicates the lightness, a* value redness/greenness and b* value yellowness/blueness.

Table 10. Effect of NaCl addition on the color parameters of extrudates.

Sample	Amount (g/kg)	L*	a*	b*	Browning Index
Control	-	62.58±0.29^{b}	8.78±0.37^{a}	29.38±1.26^{ab}	53.83±2.11^{a}
NaCl	10	65.63± 0.22^{a}	8.00±0.12^{b}	29.02±0.15^{b}	50.56±0.45^{b}
NaCl	50	62.68±0.30^{b}	9.33±0.29^{a}	30.65±0.26^{a}	56.14±0.88^{a}

Results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$)

NaCl addition at a level of 10 g/kg slightly increased the L* value while decreased the a* value and browning index which was shown in Table 10. However, higher concentration of NaCl did not cause any significant ($p < 0.05$) change on the L* value, a* value and browning index when compared to control samples. Addition of NaCl did not affect b* value in the studied range when compared to the control. There are contradictory results for the effect of NaCl on color development. Gokmen and Senyuva (2007a) determined no difference in colour of fried potatoes pre-treated with NaCl solution compared to the control. However, Pedreschi et al. (2007a) obtained lighter potato crisps after applying a pre-treatment with NaCl solution of 0.002 g/L. Chen et al. (2014) found a decrease in L* value for dried potato starch gels when prepared with 1-5 % NaCl. Farahnaky et al. (2009b)

determined that, lightness of extruded-baked cassava and potato starches decreased by added NaCl. They mentioned that caramelization reaction may be enhanced during extrusion in the presence of NaCl by means of two mechanisms. Firstly, NaCl increase the molecular mobility of starch-based products and secondly acts a catalyst during caramelization.

Table 11. Effect of $CaCl_2$ addition on the color parameters of extrudates.

Sample	Amount (g/kg)	L*	a*	b*	Browning Index
Control	-	62.58 ± 0.29^b	8.78 ± 0.37^a	29.38 ± 1.26^a	53.83 ± 2.11^a
$CaCl_2$	10	62.67 ± 0.42^b	8.59 ± 0.11^a	29.09 ± 0.42^a	53.14 ± 0.47^a
$CaCl_2$	50	67.51 ± 0.29^a	6.35 ± 0.24^b	26.96 ± 0.45^b	44.95 ± 0.88^b

Results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

$CaCl_2$ imparted a lighter color when added at a higher level while redness and yellowness of extrudates decreased slightly which was shown in Table 11. Browning index decreased significantly ($p < 0.05$) at the high level of $CaCl_2$ addition ($p < 0.05$). Ruiz-Gutierrez et al. (2012) obtained lighter and less yellow masa and tortillas by using $CaCl_2$ in nixtamalization process. Acar et al. (2012) determined that lightness increased and redness decreased while yellowness slightly increased by addition of $CaCl_2$ at the level of 0.1-1% in cookies. Severini et al. (2003) observed lighter potato slices by blanching in $CaCl_2$ solution prior to frying compared to that in NaCl solution. They indicated that $CaCl_2$ have an ability to scavenge metals such as iron and copper and cause discolouration. Moreover, Cl^- ions may inhibit the Maillard reactions.

Table 12. Effect of ascorbic acid addition on the color parameters of extrudates.

Sample	Amount (g/kg)	L*	a*	b*	Browning Index
Control	-	62.58±0.29^{c}	8.78±0.37^{a}	29.38±1.26^{a}	53.83±2.11^{a}
Ascorbic acid	13.36 (pH=4.62)	69.93±0.62^{b}	6.17±0.34^{b}	29.17±0.55^{ab}	46.36±1.37^{b}
Ascorbic acid	66.80 (pH=3.68)	77.45±0.38^{a}	3.69±0.06^{c}	27.77±0.18^{b}	38.69±0.05^{c}

Results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

Table 13. Effect of citric acid addition on the color parameters of extrudates.

Sample	Amount (g/kg)	L*	a*	b*	Browning Index
Control	-	62.58±0.29^{c}	8.78±0.37^{a}	29.38±1.26^{a}	53.83±2.11^{a}
Citric acid	7.26 (pH=4.59)	70.42±0.64^{b}	5.95±0.16^{b}	27.21±0.39^{b}	43.39±0.13^{b}
Citric acid	36.30 (pH=3.30)	74.70±0.20^{a}	4.30±0.07^{c}	26.70±0.34^{b}	39.13±0.46^{c}

Results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

It was found that, ascorbic acid and citric acid affected the color parameters of extrudates with a reduced browning index which was shown in Table 12 and Table 13. Results obtained by ascorbic acid addition was in an aggreement with the results of the study of Sriburi & Hill (2000). Wolfrom et al. (1974) reported that, ascorbic acid and citric acid reduce browning reactions in D-Glucose/glycine model system.

In another study, citric acid caused an increase in lightness of gingerbread (Amrein et al., 2004). Citric acid and ascorbic acid have been used as an antibrowning agents in a widespread manner (Chiabrando & Giacalone, 2012; Olivas et al., 2007; Jeong et al., 2008). Lightness value of extrudates with ascorbic acid were higher than that of extrudates with citric acid. However, pH of extrudates with added ascorbic acid at high level were lower compared to extrudates with added citric acid at high level. It can be concluded that, ascorbic acid have an ability to give lighter color because of its structure rather than its decreasing effect on pH. Sriburi & Hill (2000) supported this conclusion that, cassava starch extrudates had a lighter color with added ascorbic acid than that of pH adjusted samples.

Table 14. Effect of glycine addition on the color parameters of extrudates.

Sample	Amount (g/kg)	L*	a*	b*	Browning Index
Control	-	62.58±0.29^{a}	8.78±0.37^{c}	29.38±1.26^{b}	53.83±2.11^{c}
Glycine	1	62.67±0.08^{a}	9.72±0.25^{b}	31.66±0.23^{a}	57.96±0.47^{b}
Glycine	5	58.39±0.15^{b}	11.68±0.28^{a}	32.83±0.44^{a}	65.19±0.84^{a}

Results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

Lightness of extrudates prepared with low level glycine addition was not affected while decreased significantly ($p < 0.05$) at the high level. Redness and browning indexes increased with increasing concentration. Yellowness increased slightly at low level of addition while addition at the high level did not cause any significant ($p < 0.05$) further increase (Table 14). Mustafa et al. (2009) found that, redness value increased in bread crust with addition of glycine. Present results was also in agreement with the previous findings in cereal products (Brathen et al., 2005) and cereal and potato model systems (Low et al., 2006; Amrein et al., 2007; Capuano et al., 2009) which had a lower lightness by the addition of glycine. Capuano et al.

(2009) asserted that glycine could cause browning by acting as a precursor of Maillard reactions.

Table 15. Effect of cysteine addition on the color parameters of extrudates.

Sample	Amount (g/kg)	L*	a*	b*	Browning Index
Control	-	62.58 ± 0.29^{c}	8.78 ± 0.37^{a}	29.38 ± 1.26^{a}	53.83 ± 2.11^{a}
Cysteine	1	66.42 ± 0.30^{b}	7.45 ± 0.13^{b}	29.90 ± 0.31^{a}	50.61 ± 0.41^{b}
Cysteine	5	67.44 ± 0.38^{a}	5.28 ± 0.11^{c}	28.73 ± 0.22^{a}	46.06 ± 0.58^{c}

Results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

Extrudates prepared with cysteine at two different level, 1 and 5 g/kg caused an increase in L* and decrease in a* and browning index values which was shown in Table 15. Yellowness value was not affected by cysteine addition compared to control. It was agreed well with the findings of Li & Lee (1996) who explained that Maillard browning which takes place during extrusion could be reduced by SH-containing compounds. They indicated that, cysteine could interact with intermediate products of Maillard and supress free radicals which are formed during extrusion. Zeng et al. (2009) also obtained lighter potato strips which went through a cysteine pre-treatment compared to the control.

Table 16. Effect of quercetin addition on the color parameters of extrudates.

Sample	Amount (g/kg)	L*	a*	b*	Browning Index
Control	-	62.58±0.29^{c}	8.78±0.37^{a}	29.38±1.26^{b}	53.83±2.11^{b}
Quercetin	1.14	65.48±0.42^{a}	7.14±0.06^{b}	30.71±0.46^{b}	51.86±0.48^{b}
Quercetin	5.72	64.31±0.24^{b}	6.53±0.24^{c}	34.31±0.27^{a}	56.52±0.48^{a}

Results are means ± SD (n = 3). Values with a same letter are not significantly different in the same column ($p < 0.05$).

Quercetin addition affected ($p < 0.05$) CIELAB color parameters and browning index of samples which was shown in Table 16. Lightness increased with quercetin addition. Redness showed a decrease with increasing concentration. Yellowness and browning index increased at the high level of quercetin addition. Viscidi et al. (2004) determined an increase in yellowness and decrease in redness in oat cereal extrudates with added quercetin at a level of 1.5 g/kg.

3.3 Acrylamide

The effect of added ingredients on the acrylamide level of the extrudates was presented in Figure 4. All of the added ingredients caused a significant ($p < 0.05$) reduction in acrylamide formation for both of low and high level of addition. Addition of cysteine at the high level led to highest decrease in acrylamide level while ascorbic acid and citric acid caused the lowest reduction when added at the low level.

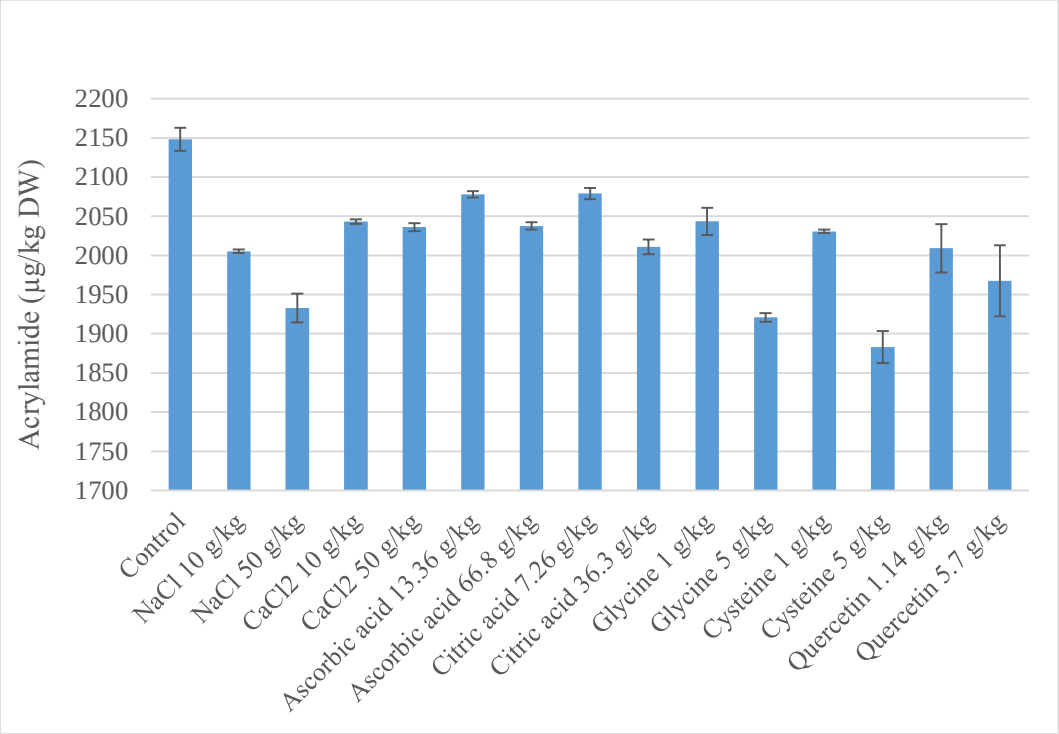

Figure 4. Effect of added ingredients on the acrylamide level of extrudates.

Table 17. Effect of added salts on acrylamide level in extrudates.

Sample	Amount (g/kg)	Acrylamide level (µg/kg DW)	Reduction (%)
Control	-	2148±15[a]	-
NaCl	10	2005±2[c]	6.64
NaCl	50	1933±18[d]	10.02
$CaCl_2$	10	2043±3[b]	4.88
$CaCl_2$	50	2036±5[b]	5.22

Results are means ± SD (n = 4). Values with a same letter are not significantly different in the same column ($p < 0.05$).

Effect of salts on acrylamide level was shown in Table 17. NaCl decreased the acrylamide level with increasing concentration. Effect of NaCl on acrylamide level in potatoes (Pedreschi et al., 2007a; Pedreschi et al., 2010), bakery products (Claus et al., 2008b; Levine & Smith, 2005) and model systems (Kolek et al., 2006; Gokmen & Senyuva, 2007b) were investigated by different researchers. A higher ability for acrylamide reduction was found for NaCl in these studies than that of current study. Kolek et al. (2006) found 32%, 36% and 40% reduction in acrylamide level with preparation of model food matrix with NaCl at the levels of 1, 5 and 10%, respectively. Levine & Smith (2005) determined a 42 and 65% reduction rate for acrylamide level in a cracker model system which was baked at 180 ^{o}C for 15 min with NaCl addition at the level of ~13 and ~33 g/kg, respectively. However, Mestdagh et al. (2008) did not find any significant ($p < 0.05$) effect of NaCl at the concentration of 1% on the acrylamide level of baked potato model system. Gokmen & Senyuva (2007a) attributed the decreasing effect of NaCl to preventing of cation the formation of Schiff base of the asparagine which is called pyrolyzates which was determined by mass spectrometric analyses of pyrolyzates. However, behavior of NaCl molecules in starch during extrusion may be different as mentioned by Farahnaky et al. (2009a) who explained that NaCl behaves as a plasticizer due to remaining as a whole and not dissociating to its ions at low moisture while Sanchez-Madrigal et al. (2014) and Zazueta-Morales et al. (2002) indicated that salts are ionizated during extrusion. Kolek et al. (2006) noted that, reduction in acrylamide level was not linear with the level of the addition of NaCl and 1% addition of NaCl provided the highest decrement in acrylamide level. They explained the decreasing effect of NaCl on acrylamide level by DSC thermograms of acrylamide and acrylamide/NaCl 1:1 mixture which an increase in polymerization of acrylamide with NaCl addition was demonstrated. Moreover, 0-2% addition of NaCl to wheat bread provided a decrease in acrylamide content while increasing concentration caused an increase (Claus et al., 2008b). However, Moreau et al. (2009) found that, increasing level of NaCl addition caused a proportional decrease in acrylamide level in breakfast cereal model sytems which

is accordance with present study. Breakfast cereals were produced by steam cooking and toasting in the afaromentioned study.

As shown in Table 17, $CaCl_2$ caused a moderate reduction in respect to effect of other added ingredients and increasing concentration did not cause any significant ($p < 0.05$) effect on acrylamide level. The decreasing effect of $CaCl_2$ on acrylamide level has resulted in many studies which are conducted on fried potatoes (Lindsay & Jang, 2005), potato chips (Elder et al., 2004; Ou et al., 2008), extruded product (Mulla et al., 2011) and model systems (Gokmen & Senyuva, 2007b). However, the effect of $CaCl_2$ in these studies is much higher than that of current study. Mulla et al. (2011) found 65% reduction in acrylamide level by the addition of $CaCl_2$ at the level of ~5.6 g/kg in potato flour/semolina 70:30 extrudates. Extrusion was performed at 120 rpm and at a temperature of 170 ^oC with a single screw extruder. It was reported that, at lower temperatures and in dry systems acrylamide formation is higher than acrylamide elimination (Becalski et al., 2003). Moreover, Gokmen & Senyuva (2007b) did not observe an apperant acrylamide reduction at temperatures below 150 ^oC while it increases at higher temperatures. Decreasing effect of $CaCl_2$ was attributed to inhibition of Schiff base formation by Ca^{2+} addition by Gokmen & Senyuva (2007b). Ou et al. (2008) explained calcium inhibition of acrylamide formation by complex formation of calcium with amines and some intermediates of Maillard reaction especially for acrylic acid which might be the cause of reduction in products prepared with fat.

Table 18. Effect of added acids on acrylamide level of extrudates.

Sample	Amount (g/kg)	Acrylamide level (μg/kg DW)	Reduction (%)
Control	-	2148±15[a]	-
Ascorbic acid	13.36 (pH=4.62)	2078±4[b]	3.27
Ascorbic acid	66.80 (pH=3.68)	2038±5[c]	5.14
Citric acid	7.26 (pH=4.59)	2079±7[b]	3.21
Citric acid	36.30 (pH=3.30)	2011±9[d]	6.39

Results are means ± SD (n = 4). Values with a same letter are not significantly different in the same column ($p < 0.05$).

Acids had a less decreasing effect on acrylamide level with respect to effect of other added ingredients (Table 18). Reduction effect of ascorbic acid on acrylamide level were studied in potato products (Rydberg et al., 2003; Zeng et al., 2009; Adams et al., 2010) and model systems (Levine & Smith, 2005; Adams et al., 2010). Biedermann et al. (2002) found a slight decrease in 1% ascorbic acid added potato model system. Accordingly, reduction rate of 10% was achieved in fried potatotes treated with 1% ascorbic acid solution prior to frying in the study of Zeng et al. (2009). However, 50% and 42% reductions were found for chemical and food model systems when ascorbic acid was added in the same study and they mentioned that it is not possible to regard ascorbic acid as a potential acrylamide formation inhibitor due to these different results. Levine & Smith (2005) reported a high reduction in model crackers baked at 180 ^{o}C for 5-45 min at the addition of ascorbic acid at 2.2 and 6.7% (w/w). pH of dough was attained a value of 4.2 at high addition of ascorbic acid in the study. They noted that, ascorbic acid decomposed thermally to many products and it is a compound having antioxidative effect involving in free radical reactions. Therefore, it was concluded that there is no exact mechanism of inhibition of acrylamide formation for ascorbic acid.

Inhibition effect of citric acid on acrylamide formation have been reported by some researchers especially for potato products (Jung et al., 2003; Kita et al., 2004; Low et al., 2006; Pedreschi et al., 2007b). Reduction potential of citric acid for acrylamide formation in extrusion products was investigated by Mulla et al. (2011). In the study, 9.6 g/kg citric acid was added to potato flour/semolina 70:30 feed and found 87% reduction in extrudates which were extruded at 120 rpm and at temperature of 170 ^{o}C and dried at 50 ^{o}C for 2 h. Moreover, addition of citric acid at the levels of 5 g/kg caused a three fourths reduction and 10 g/kg provided an almost complete inhibition of acrylamide formation in gingerbread with a pH of 5.0 (Amrein et al., 2004). Jung et al. (2003) reported 58.2 and 72.8% inhibition of acrylamide formation for baked corn chips treated with 0.1 and 0.2 % citric acid solution prior to baking at 255 ^{o}C for 100 s. In contrast, potato flake blended with 0.3% w/w citric acid solution at a rate of 1:1.3 had only an average reduction at approximately 20% which was cooked at 180 ^{o}C for 10-60 min (Low et al., 2006). In these studies, citric acid was added to feed in a solution while direct addition was applied in the current study. Jung et al. (2003) and Amrein et al. (2004) hypothesized that, lowering the pH in the food system prevents formation of Schiff base which is the key intermediate of acrylamide formation via the Maillard reaction. During Schiff base formation, nucleophilic amino group ($-NH_2$) of asparagine is added to the carbonyl group and N-substituted glycosylamine is formed. Low pH converts this free non-protonated amine ($-NH_2$) to nonnucleophilic protonated amine ($-NH^{3+}$) and the reaction is blocked. Another mechanism of inhibition was suggested by De Vleeschouwer (2006) which was the sterical or spatial hindrance effect of ions which means preventing the reaction between acrylamide precursors due to occupying of ions of high amount of space in food system. In contrast, Mestdagh et al. (2008) did not find any significant ($p < 0.05$) effect of citric acid on acrylamide elimination at constant pH which was in line with present study. Same acrylamide levels were resulted at constant pH (approximately 4.6) for ascorbic acid and citric acid at low addition. High level of addition of citric acid caused a lower pH than ascorbic acid did and led to a higher reduction ratio for

acrylamide formation which may lead to conclusion that pH is the leading factor for reduction. However, low reduction rates in acrylamide level were observed for acids added extrudates which supports the other mechanism of inhibition that acids may not be dissociated to their ions in low-moisture conditions and their reduction effect may be due to spatial hindrance of molecules.

Table 19. Effect of added amino acids on the acrylamide formation.

Sample	Amount (g/kg)	Acrylamide level (µg/kg DW)	Reduction (%)
Control	-	2148±15[a]	-
Glycine	1	2043±17[b]	4.87
Glycine	5	1921±5[c]	10.58
Cysteine	1	2031±2[b]	5.47
Cysteine	5	1883±20[d]	12.34

Results are means ± SD (n = 4). Values with a same letter are not significantly different in the same column ($p < 0.05$).

Increasing concentration of glycine addition caused an increasing reduction on the acrylamide level as shown in Table 19. Glycine was used in the purpose of acrylamide elimination in potato products (Rydberg et al., 2003; Brathen et al., 2005; Low et al., 2006; Mestdagh et al., 2008), baked products (Amrein et al., 2004; Brathen et al., 2005; Capuano et al., 2009) and model systems (Claeys et al., 2005) in a range of reduction at 14-95%. Almost a complete reduction was achieved by glycine addition at the level of 6.075 g/kg flour in flat bread which were baked at 148 ^{o}C for 10.1 and 24.9 min and ~93% reduction was obtained at 232 ^{o}C for 10.1 and 24.9 min. Addition at the same level of glycine reduced acrylamide content by 82% in bread crust baked at 200 ^{o}C for 21 min (Brathen et al., 2005). However, Mulla et al. (2011) found a lower ability of glycine for acrylamide elimination in

extruded products. They prepared potato flour-semolina 70:30 extrusion feed with 3.75 and 7.5 g/kg flour and found 22 and 33% reduction in extrudates using a single-screw extruder performed at 120 rpm and at a temperature of 170 ^{o}C. Moreover, Amrein et al. (2004) reported a 14% reduction in gingerbread prepared with 2 g/kg glycine. Increased concentration of glycine (10 g/kg) led to 70% reduction in acrylamide level in the same study. Brathen et al. (2005) and Fink et al. (2006) suggested that, when glycine is added to food, glycine and asparagine competes for the consumption of reactive carbonyls and reaction between asparagine and carbonyl groups is partly inhibited leading to the acrylamide elimination. Another mechanism of inhibition was suggested by Friedman (2003) who determined that acrylamide formed may alkylate NH_2 group of glycine which leads to acrylamide elimination. It was indicated in the study of Claeys et al. (2005) that acrylamide has conjugated double bond and the amide group which can react with other food constituents.

Cysteine also had an increasing reduction effect at increasing concentration. Effect of cysteine on acrylamide elimination was proved by different researchers who are studied on baked products (Amrein et al., 2004; Claus et al., 2008b), in potato products (Ou et al., 2008) and in model systems (Claeys et al., 2005; Mestdagh et al., 2008; Koutsidis et al., 2009; Adams et al., 2010). Amrein et al. (2004) found 26 and 24% reduction in gingerbread baked, at a temperature of 180 ^{o}C for 3 min and 190 ^{o}C for a 7 min in a two-step programmed oven, with addition of 0.5 and 2 g/kg, respectively. Levine & Smith (2005) lowered the acrylamide level in model crackers baked at 180 ^{o}C for 20 min by 26 and 32% with 0.4 and 0.2% addition of cysteine, respectively. Different process conditions may be the cause of different reduction ratios from that of found in present study. It should be noted in here that cysteine had an increasing reduction effect with increasing heating time according to Claeys et al. (2005). As reported by Koutsidis et al. (2009) the little larger mitigation effect of cysteine than that of glycine for addition at the high level is most probably due to the reaction of thiol (SH) groups of cysteine with α-dicarbonyls which blocks the reaction of α-dicarbonyls with asparagine. Secondly,

it was reported that, thiol (SH) groups of cysteine give an alkylation reaction with acrylamide formed (Koutsidis et al., 2009). Claeys et al. (2005) indicated that cysteine has lower reactivity in Maillard reaction which also may be the cause of lower browning index in extrudates prepared with cysteine than those with glycine (Table 15 and 15). However, thiol groups of cysteine are 100-300 times more reactive for conjugated vinyl compounds than amino groups which take place in Maillard reaction.

Table 20. Effect of added quercetin on acrylamide formation.

Sample	Amount	Acrylamide (μg/kg DW)	Reduction (%)
Control	-	2148±15[a]	-
Quercetin	1.14	2009±31[b]	6.47
Quercetin	5.72	1968±46[c]	8.40

Results are means ± SD (n = 4). Values with a same letter are not significantly different in the same column ($p < 0.05$).

Quercetin is a flavonoid belonging to group of flavonol, it has five hydroxyl and one carbonyl group in its structure (Materska, 2008). Quercetin addition also led to a decrease in acrylamide formation, however higher level of quercetin had only a little additional effect on acrylamide elimination (Table 20). There is little information in the literature about the effect of quercetin on acrylamide formation. There are contradictory results in literature about the effect of antioxidative compounds on acrylamide level whether these compounds inhibit or enhance acrylamide formation. Oregano phenolics containing quercetin was investigated for its acrylamide mitigation effect and it was determined that oregano extracts lowered in a high extent the acrylamide level in potato products (Koutsiou et al., 2010). Zhu et al. (2009) determined a reduction by 38% in asparagine-glucose model system

with addition of quercitrin which is very similar compound to quercetin. They found an inhibition effect for acrylamide by 13-53% of nine different phenolic compounds while an increasing effect was seen for two phenolics investigated. However, different products were used in these studies having undergone different processes. Effect of quercetin could be explained by its reactive groups. Koutsiou et al. (2010) acknowledged that hydroxyl groups are acting as a hydrogen donors between 3-amino-propionamide and phenols inhibiting the pathway leading to acrylamide formation. However, carbonyl compound in quercetin could give rise to acrylamide (Zyzak et al., 2003). Therefore, quercetin may give rise to acrylamide formation and inhibition at the same time.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

All the added ingredients led to a decrease in acrylamide level in extrudates at certain amount. Cysteine at a ratio of 5g/kg was the most efficient inhibitor, followed by glycine and NaCl when added at levels of 5g/kg and 50 g/kg, respectively. The most expanded extrudates were obtained by the addition of $CaCl_2$ at the high concentration. However, only a moderate reduction of acrylamide was achieved in respect to other added ingredients with $CaCl_2$ when added at a high level. Citric acid and ascorbic acid showed a lower potential for acrylamide reduction than that of other added ingredients in the studied range. Citric and ascorbic acid highly reduced the browning index of extrudates especially when added at a high level. Ascorbic acid gave the lowest expansion to extrudates among all the ingredients studied.

Extrusion is a complex process where heat and mechanical forces exerted on food material. Therefore, it is important to investigate the effect of extrusion on the structure of ingredients, Maillard reactions or other reactions involved, seperately to determine the functionalities of the added ingredients. In conclusion, acrylamide level can be reduced by added ingredients to some extend while affecting quality parameters. Optimum processing conditions and added ingredient levels should be determined to lower the acyrlamide level while keeping the quality parameters the same.

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APPENDIX

IMAGES OF EXTRUDATES

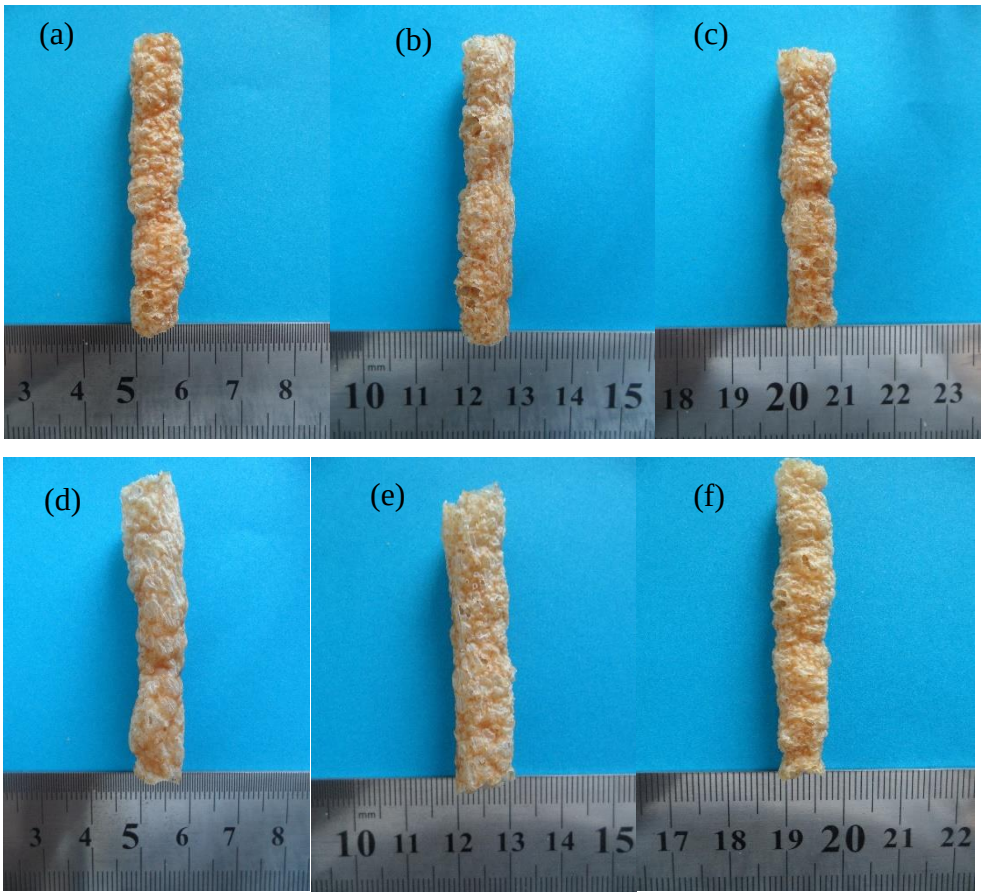

Figure A.1. Surface images of extrudates: (a) Control, (b) 10 g/kg NaCl addition, (c) 50 g/kg NaCl addition, (d) 10 g/kg $CaCl_2$ addition, (e) 50 g/kg $CaCl_2$ addition, (f) 13.36 g/kg ascorbic acid addition.

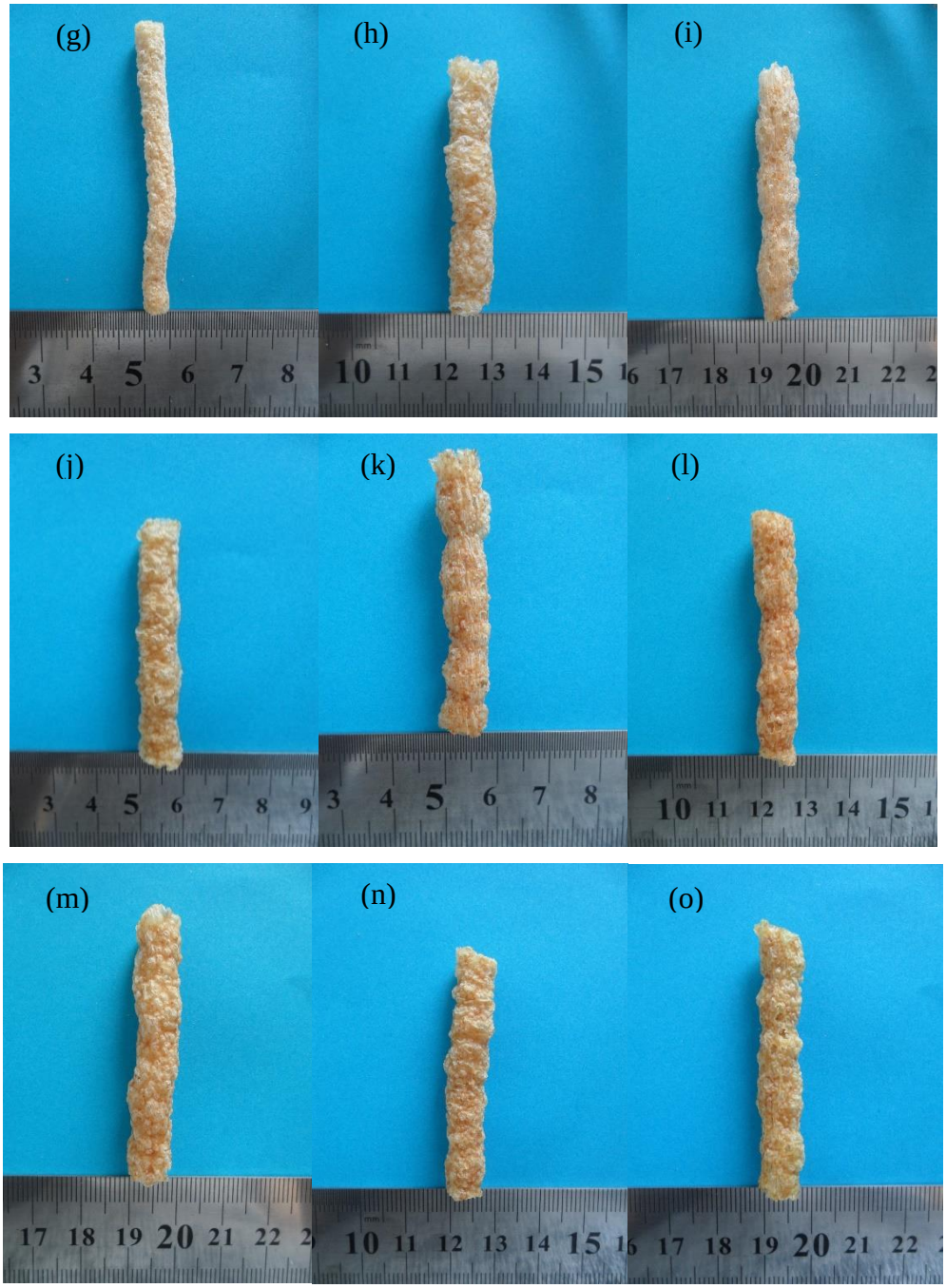

Figure A.1. (continued) Surface images of extrudates: (g) 66.80 g/kg ascorbic acid addition, (h) 7.26 g/kg citric acid addition, (i) 36.30 g/kg citric acid addition, (j) 1 g/kg glycine addition, (k) 5 g/kg glycine addition, (l) 1 g/kg cysteine addition, (m) 5 g/kg cysteine addition, (n) 1.14 g/kg quercetin addition, (o) 5.72 g/kg quercetin addition.

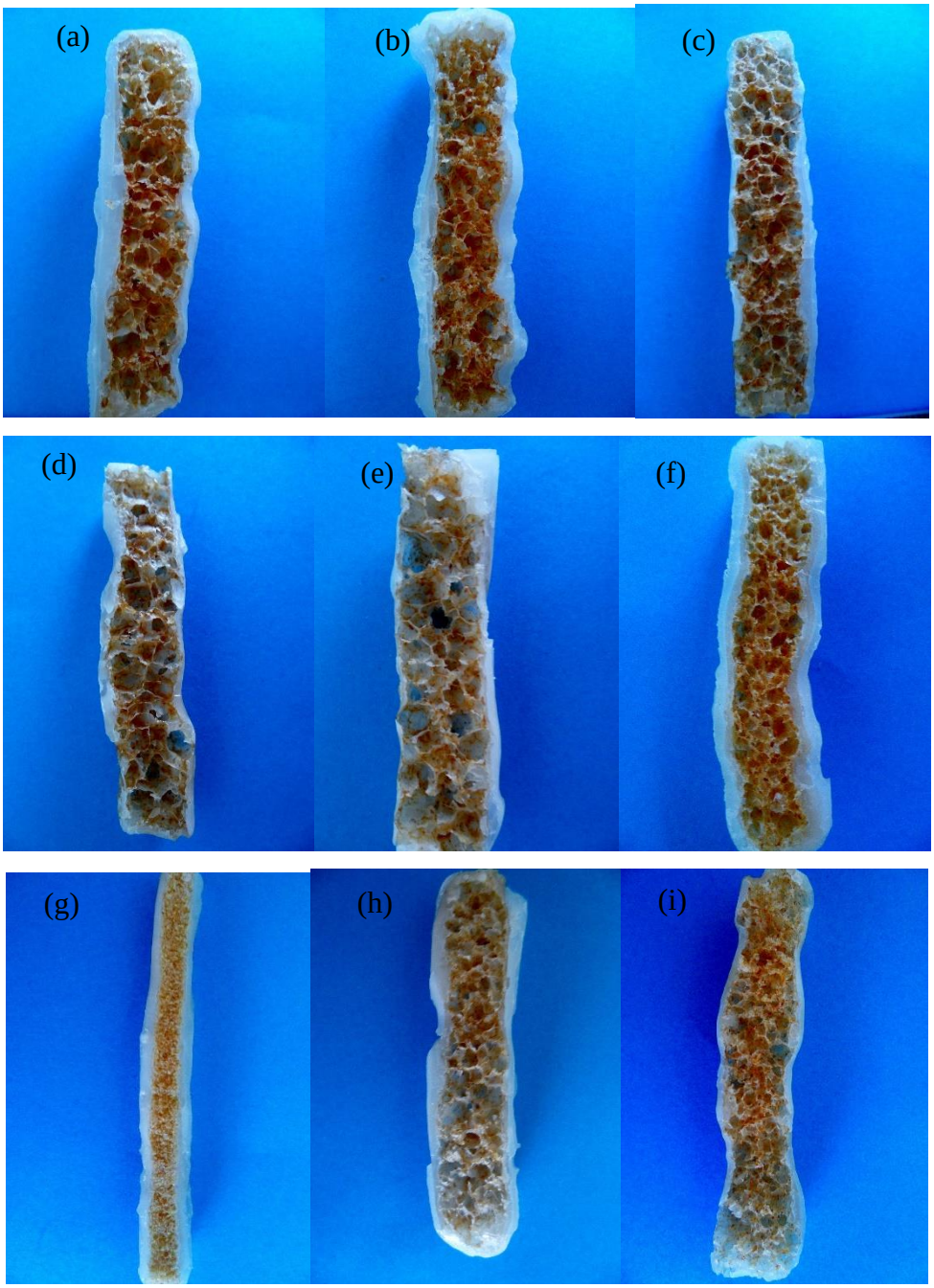

Figure A.2. Cut-view images of paraffin-coated extrudates: (a) Control, (b) 10 g/kg NaCl addition, (c) 50 g/kg NaCl addition, (d) 10 g/kg $CaCl_2$ addition, (e) 50 g/kg $CaCl_2$ addition (f) 13.36 g/kg ascorbic acid addition, (g) 66.80 g/kg ascorbic acid addition, (h) 7.26 g/kg citric acid addition, (i) 36.30 g/kg citric acid addition.

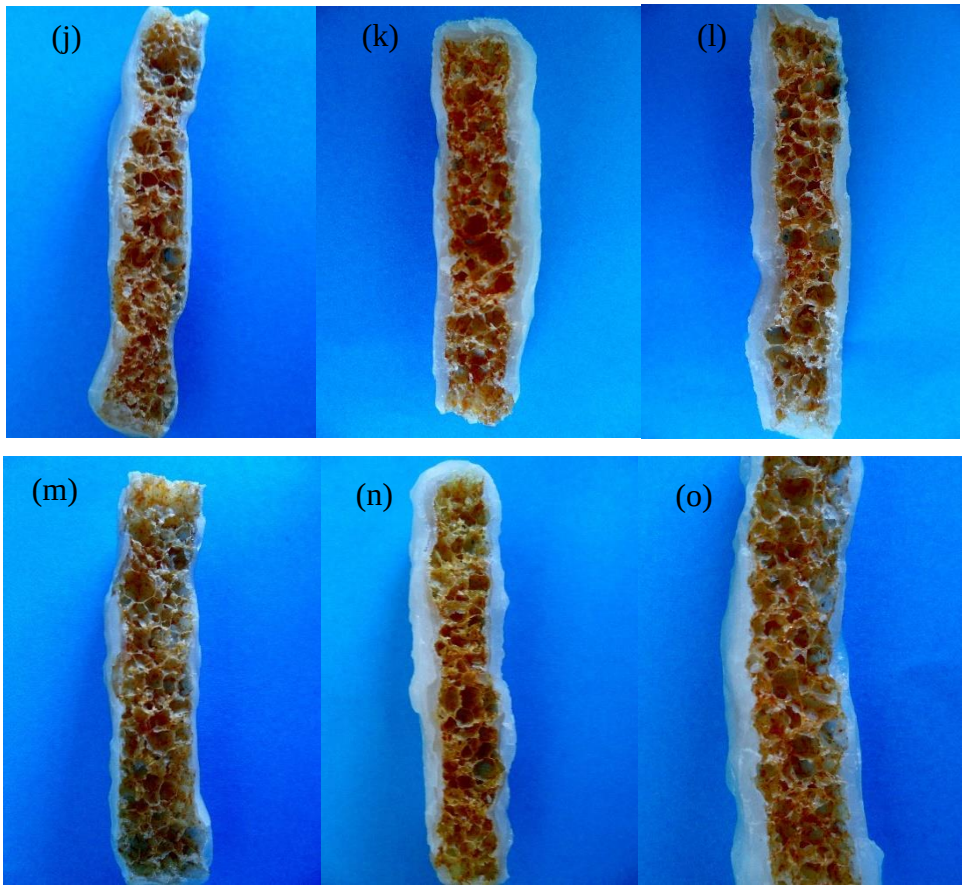

Figure A.2. (continued) Cut-view images of paraffin-coated extrudates: (j) 1 g/kg glycine addition, (k) 5 g/kg glycine addition, (l) 1 g/kg cysteine addition, (m) 5 g/kg cysteine addition, (n) 1.14 g/kg quercetin addition, (o) 5.72 g/kg quercetin addition.

CALIBRATION CURVE

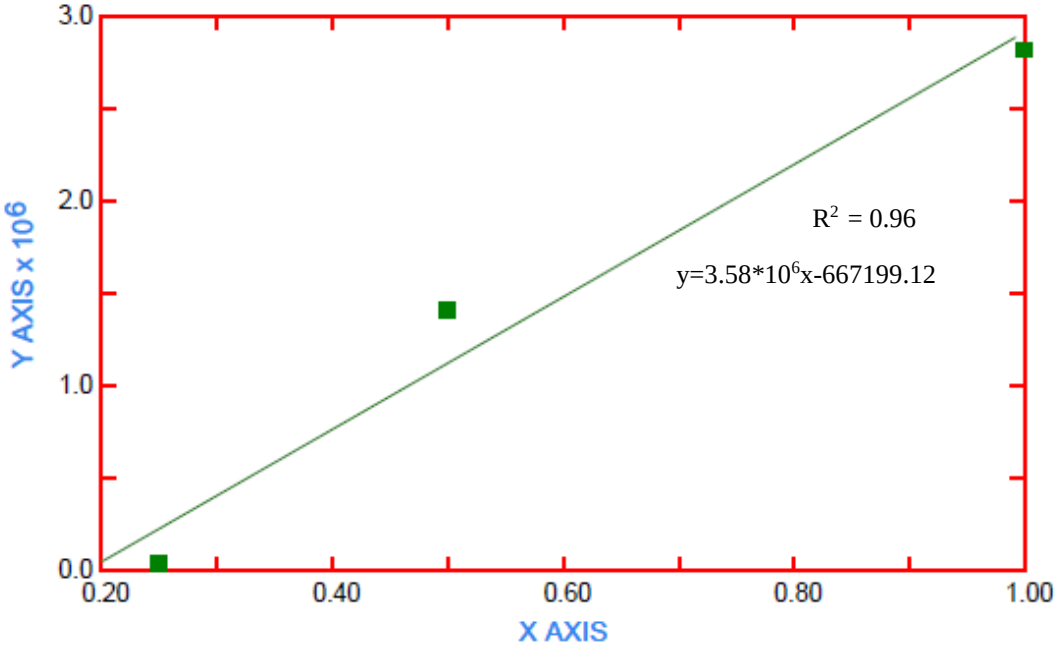

Figure A.3. Calibration curve for acrylamide analysis **(**y axis : intensity (cps), x axis : acrylamide level (mg/kg DW)**)**.

CHROMATOGRAMS

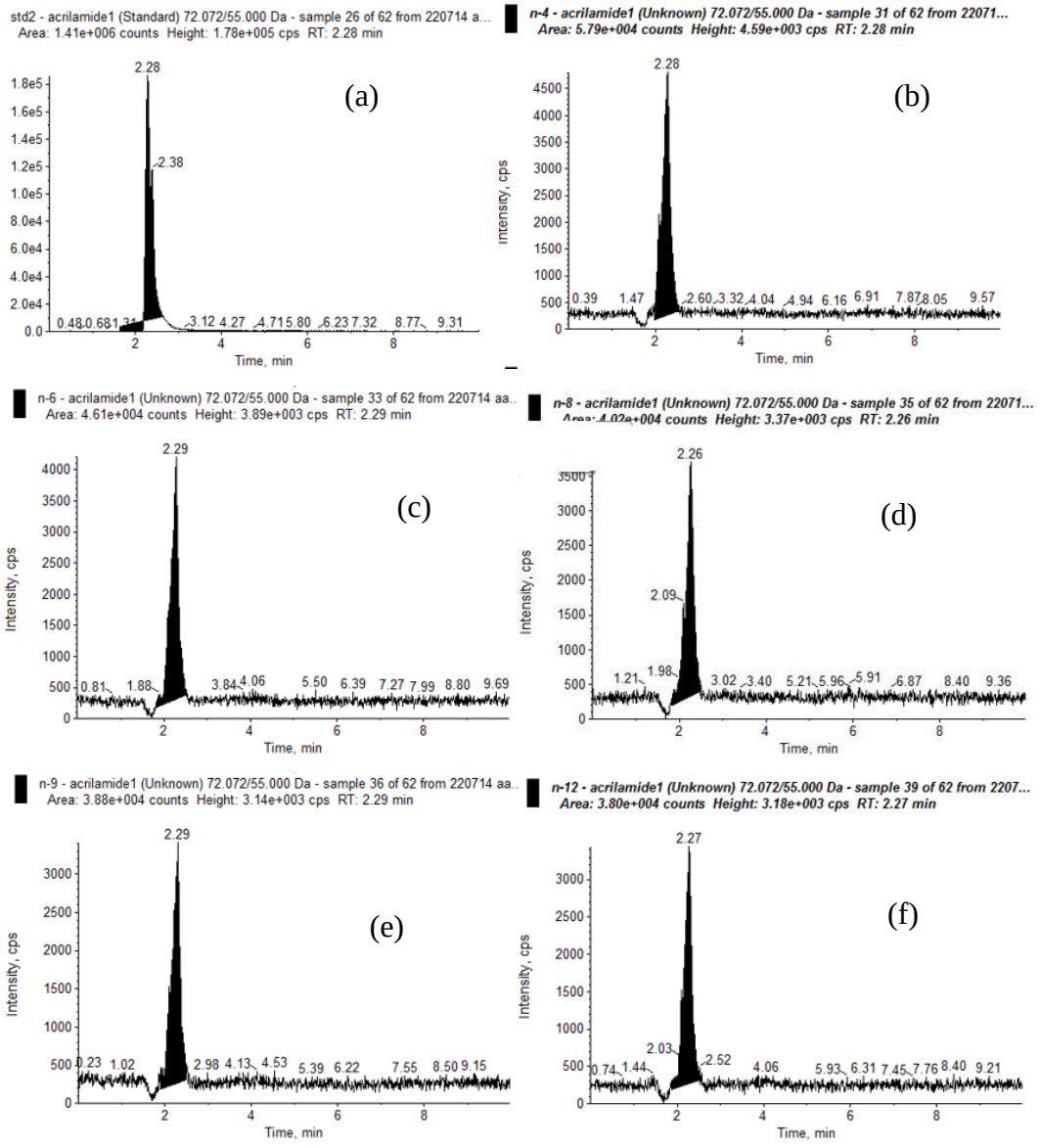

Figure A.4. Chromatograms of extrudates: (a) Acrylamide standard of 500 µg/L (b) Control, (c) 10 g/kg NaCl addition, (d) 50 g/kg NaCl addition, (e) 10 g/kg $CaCl_2$ addition, (f) 50 g/kg $CaCl_2$ addition.

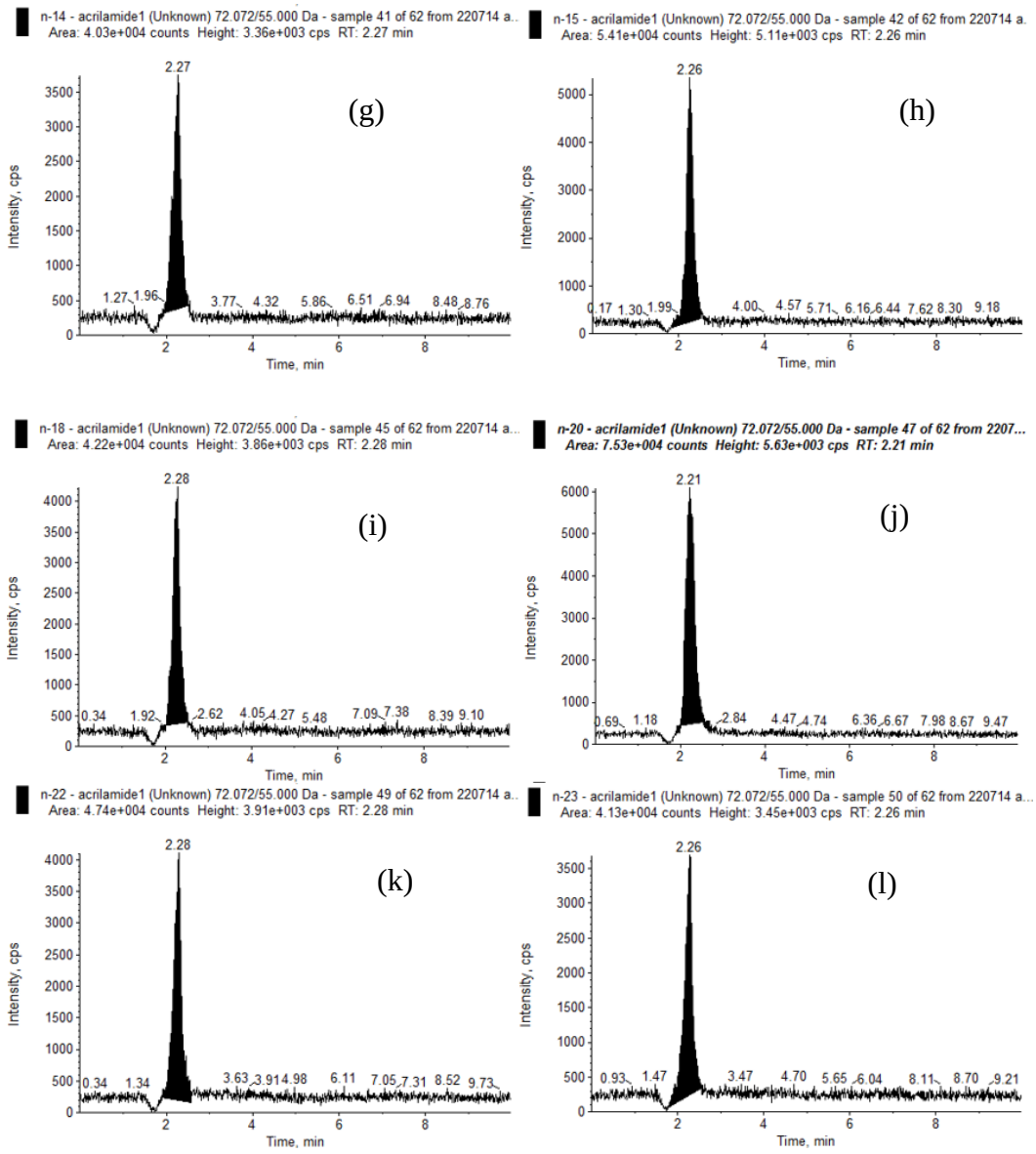

Figure A.4. (continued) Chromatograms of extrudates: (g) 13.36 g/kg ascorbic acid addition, (h) 66.80 g/kg ascorbic acid addition, (i) 7.26 g/kg citric acid addition, (j) 36.30 g/kg citric acid addition, (k) 1 g/kg glycine addition, (l) 5 g/kg glycine addition.

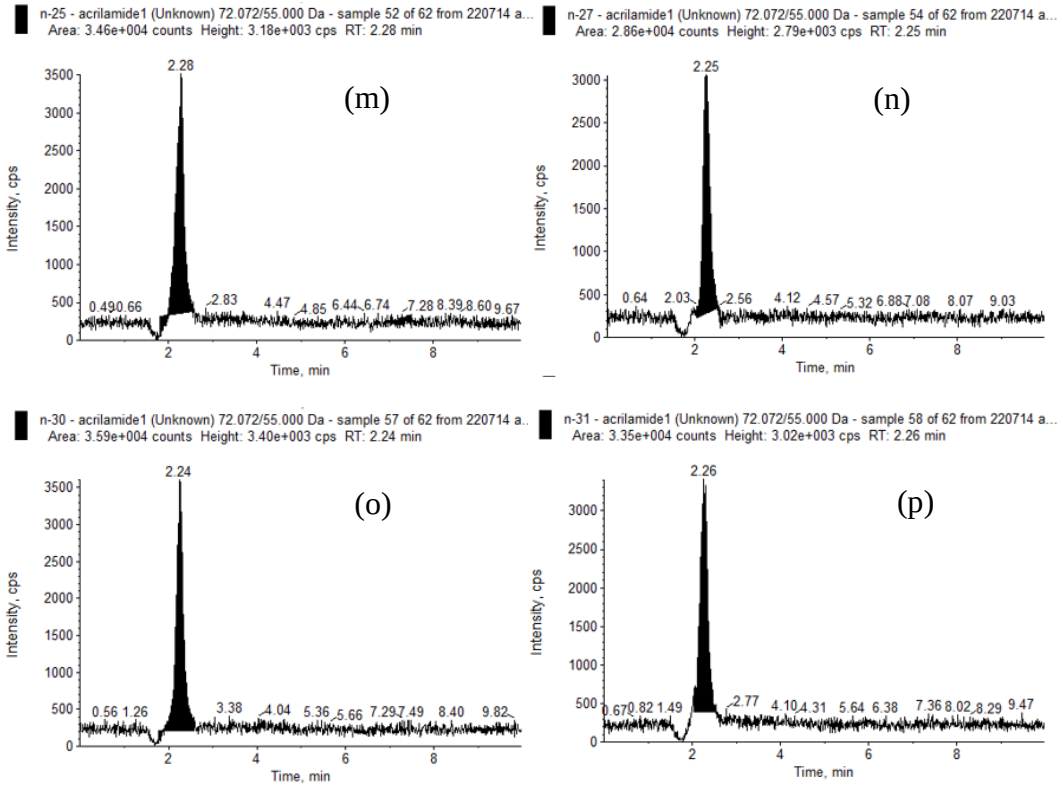

Figure A.4. (continued) Chromatograms of extrudates: (m) 1 g/kg cysteine addition, (n) 5 g/kg cysteine addition, (o) 1.14 g/kg quercetin addition, (p) 5.72 g/kg quercetin addition.

ACRYLAMIDE ANALYSIS

Table A.1. Acrylamide level and some parameters of extrudates.

Sample	Amount (g/kg)	Acrylamide Level (μg/kg DW)	Browning Index	Final pH	Feed pH	Final moisture
Control	-	2199±15[a]	53.83±2.11[a]	6.30		5.22
NaCl	10	2053±2[b]	50.56±0.45[b]	6.12		2.80
NaCl	50	1978±19[c]	56.14±0.88[c]	6.02		3.31
$CaCl_2$	10	2091±3[d]	53.14±0.47[ad]	5.36		3.79
$CaCl_2$	50	2084±5[d]	44.95±0.88[e]	5.25		4.76
Ascorbic acid	13.36	2126±4[e]	46.36±1.37[f]	4.82	4.62	4.69
Ascorbic acid	66.80	2086±5[d]	38.69±0.05[g]	3.78	3.68	3.10
Citric acid	7.26	2128±7[e]	43.39±0.13[h]	4.59	4.59	4.84
Citric acid	36.30	2058±9[fb]	39.13±0.46[g]	3.08	3.30	3.59
Glycine	1	2091±18[d]	57.96±0.47[i]	5.99		3.03
Glycine	5	1966±6[c]	65.19±0.84[j]	5.86		3.30
Cysteine	1	2078±2[df]	50.61±0.41[k]	6.02		3.63
Cysteine	5	1927±21[g]	46.06±0.58[fl]	5.82		4.49
Quercetin	1.14	2056±31[fb]	51.86±0.48[dk]	6.16		5.01
Quercetin	5.72	2014±46[h]	56.52±0.48[c]	6.16		4.26

Values with a same letter are not significantly different in the same column ($p < 0.05$).