An investigation into the nutritional and physicochemical properties of extruded products containing tomatoes

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Abstract

Extruded expanded products are becoming an important part of the diet in today's fast-paced life, however due to the presence of high amounts of fully gelatinised starch and low amounts of other nutrients, regular consumption of these products can result in health issues such as obesity and cardiovascular disease. Limited information is available on the addition of tomato derivatives that contain fibre and lycopene, the red pigment of tomatoes, to extruded products. Furthermore, the effect of extrusion processing on lycopene, especially how this process may affect lycopene bioavailability is not known. The aim of the present study was to evaluate the possibility of adding tomato derivatives, mainly tomato waste skin, to improve the nutritional value of extruded snacks without detracting from their organoleptic properties.

Varying the formulation of the extruded products showed that ingredients that have higher starch contents such as corn and rice as compared with wheat, and also lycopene sources that are resistant to shear such as tomato skin as compared with tomato paste, result in higher lycopene retention values in the final products. Although, the utilization of tomato skin alone resulted in hard and dense products, the addition of limited amounts of tomato paste to the tomato skin resulted in consumer acceptable products.

In-vitro digestion of the extruded products containing tomato derivatives showed that a large portion of the lycopene in the extruded products was released into micelles, thus it was potentially bioavailable. The uptake rate by Caco-2 cells (a human carcinoma cell line) from the extruded product was similar to the unextruded control. The utilization of tomato paste powder in the extruded snacks significantly reduced the starch digestibility, while tomato skin was less effective.

Finally, the majority of lycopene present in the extruded products containing tomatoes was shown to be inaccessible to solvent extraction and only after digestion was it able to be extracted. Enzymatic hydrolysis of the extruded product confirmed that lycopene was associated with the starch component of the food matrix and an amylolytic digestion procedure was required to break the bonds with starch and release the lycopene.

The findings from the present study confirm that it is possible to produce consumer acceptable extruded tomato products that contain bioavailable lycopene and fibre. The results obtained improve our understanding on the fate of heat-labile molecules such as lycopene during extrusion cooking and can have potential applications for the industry.

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- Dehghan-Shoar, Z., Hardacre, A.K., Meerdink, G., Brennan, C.S. (2011). Lycopene extraction from extruded snacks containing tomato skin. *International Journal of Food Science & Technology*. 46 (2), 365-371
- Dehghan-Shoar, Z., Hardacre, A.K., Reynolds, G.W. (2010). Effect of bile and pancreatin concentration on the in-vitro bioavailability of lycopene and starch in extruded snacks containing tomato paste powder. *Food Digestion*, 1(1), 40-46.
- Dehghan-Shoar, Z., Hardacre, A.K., Brennan, C.S. (2010). The physicochemical characteristics of extruded snacks enriched with tomato lycopene. *Food Chemistry*, 123(4): p. 1117-1122.

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- Dehghan-Shoar, Z., Hardacre, A.K., Reynolds, G.W. (2010). The development of functional extruded products containing bioavailable lycopene and fibre. ISNFF Annual Conference on Nutraceuticals, Functional Foods, and Dietary Supplements: Science, Methodologies, and Applications, 11-15 Oct, Bali, Indonesia, p96.
- Dehghan-Shoar, Z., Hardacre, A.K., Meerdink, G., Brennan, C.S. (2010). An investigation on the effect of ingredients and extrusion temperature on physico-chemical characteristics of tomato enriched snacks. Proceedings of Total Food: Sustainability of the Agri-Food chain International Conference, K. W. Waldron, G. K. Moates, C. B. Faulds (eds). 22-24 Apr, Norwich, UK, 244-249.
- Dehghan-Shoar, Z., Hardacre, A.K., Reynolds, G. W., Brennan, C.S. (2009). Effect of varying tomato paste and peel concentration on physico-chemical and nutritional properties of tomato enriched extruded products, NZIFST annual Conference, 23-24 June, Christchurch.
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Chapter One Introduction

The fast pace of life of today and the desire to freely choose what and when to eat, has resulted in an increase in the consumption of snacks. Snack foods are defined as "a light meal" or "food eaten between regular meals" by the Webster's New Ninth Collegiate Dictionary (*http://www.merriam-webster.com*). The high demand for snack foods by consumers has led to a rapid growth in the market for these products and more than US$334 billion worth of these foods have been predicted to be sold by the year 2015 (*Global Industry Analysts, 2012*). Within snack foods, the market for expanded extruded foods is one of the fastest growing.

Extrusion cooking is commonly used to produce expanded snack and breakfast foods. Extrusion involves applying high temperature and pressure conditions to the raw ingredients inside an enclosed barrel. The ingredients are pushed forwards by the rotation of screws towards the end of the barrel where a small opening exists which is called the die (*Camire et al., 1990*). During this process, the chemical composition and physical state of the ingredients change. Starch is gelatinised resulting in the formation of slurry of ingredients, known as the melt. This melt has a foam-like structure where materials such as water vapour, proteins and fibre are trapped within the viscuos gelatinised starch matrix. At the die exit, the sudden drop in pressure and temperature results in the instant release of water vapour, leaving bubbles in the product. As the product cools, the gelatinised starch sets around the bubbles and the expanded structure of the extruded product is formed.

Cereal grains, namely maize, rice and wheat, form the principle base ingredient for the extruded products as they generally contain more than 60% starch (*Alldrick &*

Hajselova, 2002). Apart from starch, cereal grains also contain protein. However, cereal protein is deficient in essential amino acids especially lysine, thus it is not nutritionally balanced. Furthermore, considerable amounts of fibre can be found in the husk or bran section of cereal grains, but it is usually removed during mechanical processing in order to make the grains more palatable. Further, grains may be considered as important sources for vitamins, especially from the B family, however thermal processing required to improve the edibility of the grain destroys the majority of these vitamins (*Alldrick & Hajselova, 2002; Riahi & Ramaswamy, 2003*). In addition, flavoured and coloured coating oil and salt are applied to the extruded products to obtain the desired taste and appearance for the food application. While extruded snacks often have high levels of added fat and salt, extruded breakfast cereals often contain high levels of sugar.

Despite the high consumer demand for extruded snacks, these foods consist of high proportions of fully gelatinized starch, which is rapidly digested when the snack is consumed, and less than 2% protein and 1% of fat before coatings are added. Excessive consumption of extruded foods can subject the consumers to potential health hazards, such as obesity, high blood pressure and cardiovascular disease (*Brennan et al., 2008a*).

By improving the nutritional value of extruded snack foods without detracting from the flavour and taste of these indulgence and convenience foods, diets can be improved. The addition of other ingredients can introduce more nutritionally balanced products to the market and with careful product design, taste and flavour can be enhanced. The current literature in this area suggests that bioactive components, mainly dietary fibre and protein, can be used to enrich the extruded products. More recently, food industry waste streams, mainly fruit and vegetable by-products, have been shown to provide a low cost source of valuable nutrients which may be used to enhance the nutritional

value of extruded products. The utilization of by-products from the food industry has the additional benefit of being cost effective and returning waste streams to the food chain (*Altan et al., 2008a*).

Tomato (*Solanum lycopersicum)* is extensively cultivated and consumed worldwide, with an annual production mass of more than 140 million tons (*FAOSTAT, 2009*). Tomatoes contain nutrients including lycopene and fibre both of which are beneficial to health when consumed in the diet (*Canene-Adams et al., 2005*). Lycopene is a pigment from the carotenoid family. More than 85% of human consumption of dietary lycopene comes from the tomato, which can thus potentially be considered a valuable dietary supplement. Lycopene has recently received much attention from nutritionists due to reports of its anti-inflammatory, antimutagenic and anticarcinogen properties (*Boon et al., 2010*). Lycopene has also been shown to reduce the risk of adenoma and promote immune system functionality (*Giovannucci, 1999; Kun et al., 2006; Omoni & Aluko, 2005; Shi & Maguer, 2000; Stahl & Sies, 1996*). Most of these health benefits are related to the singlet oxygen and free radical scavenging properties of lycopene (*Canene-Adams et al., 2005; Di Mascio et al., 1989*). Daily consumption of 6-15 mg lycopene is recommended for improved health (*Kun et al., 2006*).

Tomatoes contain soluble and insoluble fibre. Soluble fibre has been shown to help modulate blood glucose and cholesterol levels (Weickert & Pfeiffer, 2008). Insoluble fibre promotes laxation and may assist in preventing certain cancers such as colon cancer. It has been shown in rats that the fibre present in tomato pomace, the processing waste consisting of tomato skin and seeds, significantly increases the faecal mass without negatively affecting growth rate (*Alvarado et al., 2001*). Incorporation of 20-40 g fibre in the diet on a daily basis is recommended (*Brennan, 2005; Wiseman,*

2002).

Tomatoes can be added to an extruded product, however, production of well-expanded extruded products that contain bioavailable lycopene and fibre is challenging. The addition of fibre at high concentrations (> 5%) may have deleterious effects on the organoleptic properties of the extruded snacks. Expansion of the extruded products enriched with fibre can be reduced by up to 4 times, the density increased by up to 3 times and hardness up to 5 times. Optimizing the raw ingredients and the extrusion process has been shown to improve the organoleptic defects of fibre-enriched extruded products (*Ainsworth et al., 2007; Altan et al., 2008a & b; Camire & Dougherty, 1998; Yanniotis et al., 2007*). However, this optimisation approach has not been implemented for extruded products made from cereals and tomato.

The available knowledge on the stability of pigments during extrusion cooking is limited and inadequate, so currently we are unable to make comprehensive conclusions. The studies available from conventional food processing techniques suggest that the high shear and temperatures, which are applied during extrusion, can destroy labile molecules such as lycopene (*Chinnaswamy, 1993; Lee & Chen, 2002; Moraru & Lee, 2000; Shi et al., 2008*). Further, complex reactions take place during extrusion that may adversely affect the bioavailability of both starch and lycopene and thus the nutritional value of snacks. The bioavailability of nutrients in snacks needs to be investigated if functional extruded snacks with improved nutritional value are to be produced.

The present study seeks to understand the relation between the processing parameters and raw ingredients with the lycopene content and the organoleptic and physical characteristics of extruded corn-tomato products, including expansion, density, hardness and colour. In addition, the changes in the nutritional properties of the snacks,

namely, lycopene bioaccessibility and starch digestibility in response to extrusion processing and formulation is investigated. The findings from the current study could significantly improve our understanding of effect of the extrusion cooking process on raw ingredients containing bioactive compounds, provide knowledge to produce functional extruded products that contain bioavailable lycopene and fibre that are acceptable to consumers and also suggest a means to utilize fruit and vegetable waste streams.

The thesis is divided into eight chapters. The first chapter discusses the need to develop functional extruded snacks that contain tomato derivatives and the requirement to reach this goal. The current knowledge in this area is summarized in the second chapter. In Chapter 3, apart from a general methodology section, two methodologies are described which were developed during the course of the study. These were lycopene extraction from extruded products and the simultaneous determination of lycopene bioaccessibility and starch digestibility in extruded products made with corn and tomato. Chapter 4 describes the effect of the raw ingredients on the physicochemical characteristics of extruded products made with corn and tomato. In Chapter 5, the effect of changes in the formulation on the lycopene bioaccessibility and starch digestibility of extruded products made with corn and tomato is examined. In Chapter 6, the effect of extrusion processing conditions on the physicochemical characteristics of extruded products made with corn and tomato is presented. This is followed by an examination of the effect of extrusion cooking parameters on lycopene bioaccessibility and starch digestibility from the extruded products made from corn and tomato in Chapter 7. In Chapter 8, an enzymatic digestion system is employed to determine the possible interaction between lycopene with starch during extrusion cooking. A general

discussion outlining the main findings of the experiments and the conclusions obtained from the study are described in Chapter 9.

Chapter Two Review of Literature

2.1. Extrusion cooking

2.1.1. Introduction

Extrusion is a processing technology that has been borrowed from the polymer industry where it is used to form a wide variety of films and regular solid shapes. In the food industry, it has been developed to be a versatile and cost effective procedure that is used to produce a variety of attractive food products such as spaghetti, expanded snacks and breakfast cereals. Extruders are designed to continuously convert uncooked ingredient mixes into palatable food products. Processing typically is achieved in the order of seconds to minutes and, apart from the manufacture of pasta, generally involves the use of high shear and temperatures above 120 °C and even as high as 190 °C. Pressure in the barrel may reach 200 bars, therefore extrusion cooking is considered as a high temperature, short time, high shear (HTST) process (*Guy, 2001; Guy & Benjamin, 2003; Riaz, 2006*).

The design of the extruder assists in the efficient transfer of energy from friction generated by the processing of the screws and heat added from the barrel elements to the ingredient. The extruder can be used to manufacture products, including spaghetti, that contain more than 30% moisture at processing temperatures below 70 °C and with low shear. Breakfast cereals and expanded snacks on the other hand, are produced using much higher shear and pressure at temperatures usually between 120 and 140 °C (*Camire et al., 2002; Guy, 2001; Riaz, 2000*).

2.1.2. Extrusion process

The schematic design of the extruder and the expansion process is shown in Figure 2.1. The extrusion starts when the ingredients enter the barrel from the feeder. The feed hopper is an open-ended bin fitted with a mixing and metering device to control the rate of delivery of ingredients into the barrel. The ingredients enter the barrel and the rotating screws force the ingredients forward down the barrel towards the die. Friction between the walls of the barrel, the screws and the ingredients add heat and shear which results in the formation of a viscous melt. If necessary, the temperature of the barrel walls can be increased manually to add more energy to the melt. As the screws knead and compress the ingredients, cell walls are disrupted and proteins denatured. Starch granules are also torn apart and the pseudo-crystalline organization of the amylose and amylopectin in the granules is lost and the starch forms an amorphous continuous phase in the extruded material in which protein and fibre are dispersed in (*Camire, 2002; Campanella et al., 2002; Johnson & Benjamin, 2003*).

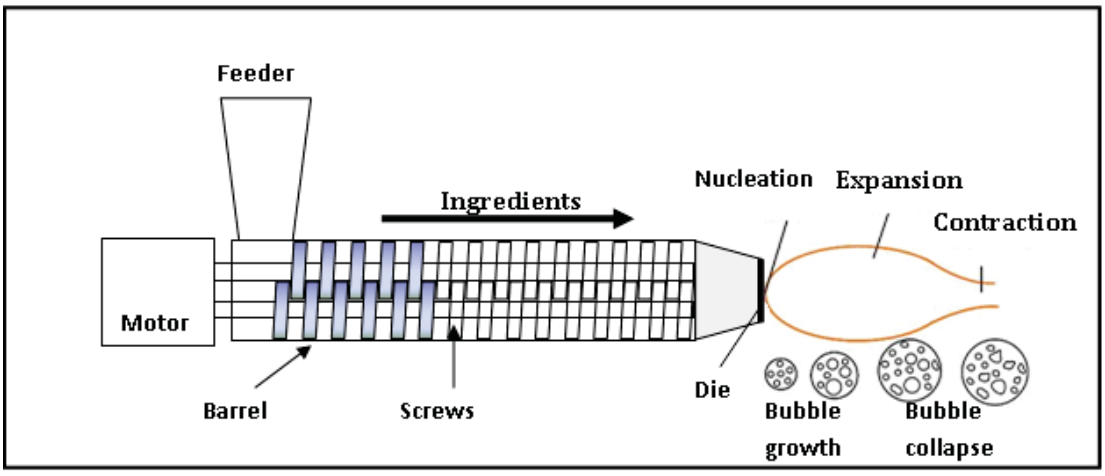

Figure 2-1 Schematic design of the extruder

The melt is then discharged through the restriction in the die. Dies are shaped to provide the profile required for the final product and also restrict the flow of material through the extruder so the pressure and shear can be developed in the barrel. As the melt is forced from the die at temperatures above 100 °C, rapid reduction in pressure causes the residual water in the melt, typically less than 12%, to vaporize instantly forming bubbles, around which the starch matrix hardens to form rigid foam. As the product cools, some collapse may occur as the pressure within the bubbles falls before they have reached a glassy state. These complex changes take place in only 1-20 seconds.

The extruder parameters, such as screw speed, ingredient feed rate, feed moisture and temperature of the barrel, can be controlled to change the characteristics of the molten mass inside the extruder and thus the product properties.

2.1.3. Physical characteristics of extruded products

The physical characteristics of extruded products can be measured and are a good estimate of many of the subsequent organoleptic characteristics of the final products, and therefore can predict consumer acceptability. Expansion, texture and density are the main physical parameters determined for extruded snacks.

2.1.3.1. Expansion ratio

Expansion is one of the main characteristics of extruded products which is necessary to obtain the desired crispy texture. The final expansion of the product can be measured as

a combination of longitudinal or radial expansion. This parameter is calculated from the longitudinal or sectional diameter of the product.

The melt viscosity is the determining factor in the expansion of the extruded products. Extrusion parameters, especially an increase in the temperature of the barrel and decrease in the moisture content, increase the viscosity of the melt, thus improve melt extensibility, enabling bubble growth and expansion (*Lillford, 2008*).

The chemical composition of the melt plays an important role in the expansion of the products. Starch is mainly responsible for expansion while the presence of other components such as fibre and protein reduce expansion (*Chinnaswamy, 1993; Moraru & Kokini, 2003*).

2.1.3.2. Textural parameters

Crispness, hardness, and brittleness are parameters used to determine the textural properties of the extruded products. It has been shown that the instrumental determination of these parameters correlates well with human perception of texture of extruded products. In this work, an Instron Universal testing machine or texture analyser was used to measure the textural characteristics of extruded products including hardness.

Hardness is manipulated by variation in the raw ingredients and the extrusion process. For example, increases in the fibre and moisture content of the raw ingredients, also increases the hardness of the final product. High screw speeds, high temperatures and low levels of glycerol mono-stearate on the other hand may all decrease hardness (*Huang et al., 2006; Mendonça et al., 2000*).

2.1.3.3. True density and bulk density

When the melt at high temperature and pressure leaves the die, much of the present water flashes off into steam. Bubbles develop within the melt forming a low density foam that sets rigid as it cools. The density of this product can be defined as the mass of a given volume of packed pellets. Bulk density is an important physical attribute from a commercial point of view, as products with lower true density values are more acceptable to consumers (*Brennan et al., 2008a*).

The density of extruded products usually increases with the addition of fibre, although the texture may be finer due to greater number of smaller bubbles when fibre is added to the ingredients. Further, fibre may weaken the bubble walls causing them to collapse before the foam sets (*Altan et al., 2008b; Jin et al., 1995; Lue et al., 1991*). In addition, soluble fibres and sugars absorb water which increases the net weight of the extruded product resulting in an increase in true and bulk density values (*Brennan et al., 2008a & b*).

2.2. Chemical changes during extrusion

2.2.1. Carbohydrates and starch

2.2.1.1. Chemical structure

Starch is a polysaccharide consisting of long chains of the simple sugar, D- glucose. The glucose units may be linked in either of two patterns: (1) a non-branched chain of between 500 to 6000 units of α-(1→4) linked glucose molecules called amylose; or (2) a highly branched molecule consisting of α-(1→4) linked glucose molecules with α-(1→6)

linked branches approximately every 15 α-(1→4) linked glucose molecules. This form of starch is called amylopectin (Figure 2.2) (*Alldrick & Hajselova, 2002; Matz, 1991; Monro & Melcion, 1997; Murray et al., 2007; Quezada-Calvillo & Nichols, 2009; Zobel & Alistair and Alistair, 2006*).

The proportion of amylose and amylopectin in starch granules depends on the source of starch, but it is generally about 25% amylose and 75% amylopectin for most of the cereal grains (*Alldrick & Hajselova, 2002; Monro & Melcion, 1997; Murray et al., 2007; Zobel & Alistair, 2006*).

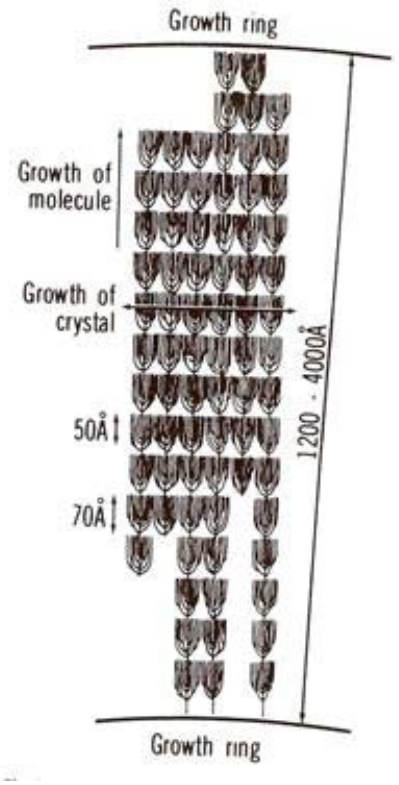

Figure 2-2 Chemical structure of amylopectin adapted from Thomas and Atwell (1999)

2.2.1.2. Effect of extrusion on starch

Starch is the dominant carbohydrate present in extruded products. After processing, the starch is usually gelatinized and forms the solid structure of the product.

Gelatinization is a change of state in the starch granule, from an ordered and partially crystalline structure into an amorphous material. The gelatinization of starch at temperatures below 90 °C requires moisture contents of more than 63%. In these conditions the starch granule absorbs water and swells. Gelatinisation begins when the swollen granules are heated above about 60 °C in excess water. During this process, amylose and some amylopectin leach out into the water surrounding the granule.

During extrusion conditions where the moisture content is typically below 12-22% of the total weight of the ingredients, gelatinization is defined as a loss of granular structure caused by temperatures above 120 °C and high shear within the extruder. With increases in the shear forces resulting from higher screw speeds, the apparent viscosity of the melt may decrease. This phenomenon is called shear thinning, and occurs in non-Newtonian materials under stress. The viscosity of the molten mass as it leaves the die determines the characteristics of the final product.

Under very high shear, large starch molecules may be broken down to smaller sugars, a process known as dextrinisation. The extent of dextrinisation depends on the temperature and shear developed with the extruder (*Eliasson & Gudmundsson, 2006*).

2.2.2. Carotenoids and lycopene

2.2.2.1. Chemical structure

Carotenoids are a group of pigments produced by plants that effectively assist the absorption of light by chlorophyll during photosynthesis. They may also protect cells against photo-oxidation (*Castenmiller & West, 1998*). Carotenoids have the general formula $C_{40}H_{56}O_n$, where n can be 0-6. Some carotenoid molecules do not contain an oxygen molecule in their formula and are called carotene, while others that contain oxygen are known as xanthophylls. The building blocks for the carotenoids are isoprenoids which are formed by polymerization of the basic five-carbon structure of isoprene (Figure 2.3).

Carotenoids are known to possess antioxidant properties due to the presence of conjugated double bonds. These bonds are highly unstable and active, thus, they can react with singlet oxygen and free peroxyl radicals. Furthermore, these bonds can undergo isomerization to produce different *cis*- and *trans*-isomers. The *trans*-isomers are thermodynamically more stable and therefore carotenoids are predominantly found in this form in nature. The conversion to *cis*-isomers is induced by parameters such as light and heat. Some carotenoids, such as β-carotene, have a β-ionone ring structure (Figure 2.4), which enables them to be converted into vitamin A (*Castenmiller & West, 1998; Clinton, 1998; Delgado-Vargas et al., 2000*), while others, such as lycopene, the red/orange coloured pigment from tomatoes, are linear (Figure 2.5), with the β-ionone ring absent. Compared to α and β carotene, lycopene reacts more rapidly with singlet oxygen and free peroxyl radicals, thus it is one of the most effective antioxidants (*Anguelova & Warthesen, 2000; Stahl & Sies, 1996*).

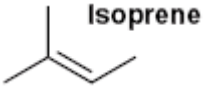

Figure 2-3 Isoprene, the building block for carotenoids

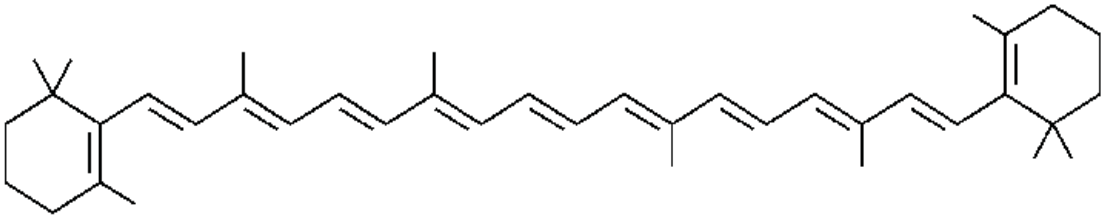

Figure 2-4 Chemical structure of β-carotene with two β-ionone rings

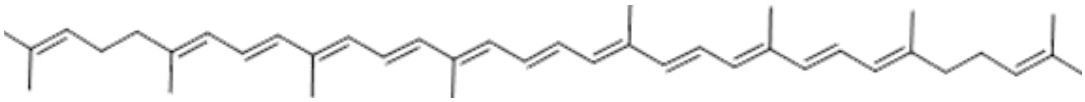

Figure 2-5 Chemical structure of lycopene

2.2.2.2. Lycopene in tomato

In their natural form, carotenoids are found intact within the plant cells. In tomato, lycopene is found crystalized and is associated with the thylakoid membranes in specialized chloroplasts-like structures called chromoplasts. Lycopene concentration is higher in the pericarp or skin of the tomato fruit, compared to the tomato pulp. The tomato cell walls mainly comprise cellulose fibrils combined with pectic substances and other non-starch polysaccharides. During processing of tomato paste, the majority of the cell walls are ruptured and thus the cell constituents including lycopene are released (*Nguyen et al., 2001; Shi & Maguer, 2000*). The cell walls of the pericarp are resistant to rupture and more likely to retain lycopene

2.2.2.3. Effect of extrusion on carotenoids

Little is known of the stability of carotenoids during extrusion cooking and what information that does exist has been mainly derived from stability studies on β-carotene and annatto (Table 2-1). Annatto is the yellow-coloured carotenoid extracted from *Bixa orellana* L., the seeds of a type of tropical tree. These two carotenoids have been studied in most detail as they are extensively used in commercial preparations (*Berset, 1989*).

Generally, carotenoids are heat-sensitive molecules that degrade at temperatures above 100°C. Thermal degradation takes place due to the instability of the conjugated bonds resulting in the isomerization and, to a lesser extent, oxidation of the bonds (*Lee & Chen, 2002; Takyi, 2001*).

The extent of degradation of carotenoids during processing depends on the time and temperature they are exposed to within the extruder. In the barrel, temperatures of over 180°C may occur, although typically for durations of less than a minute. According to previous reports (Table 2.1), up to 92% of carotenoids can be lost during extrusion. Apart from thermal degradation, loss of carotenoids can be also mechanically induced (*Guzman-Tello & Cheftel, 1990; Yajnik et al., 2010*). Carotenoids in the final product can also be lost due to oxidation during storage, unless the product is stored under low oxygen conditions (*Athar et al., 2006; Berset, 1989; Guzman-Tello & Cheftel, 1990; Yajnik et al., 2010*).

Carotenoid loss during extrusion cooking may be minimized by optimizing the ingredients and process conditions used to make the products. For example, the addition of β-carotene in a Gelatine and maltodextrin matrix resulted in 7 times higher

pigment concentration compared to the control. Also antioxidants such as rosemary oleoresin have been reported to improve stability of β-carotene (*Berset, 1989; Berset et al., 1989; Grela et al., 1999; Guzman-Tello & Cheftel, 1990; Maga & Kim, 1990; Marty & Berset, 1988; Moraru & Lee, 2000*). Increasing the level of oil has been shown to reduce carotenoid degradation and oxidation during processing (*Delgado-Vargas et al., 2000*). Increases in moisture content have also been reported to improve β-carotene retention (*Guzman-Tello & Cheftel, 1990; Knoblich et al. et al., 2005*). However, this may simply be as a result of the reduced effect of shear and temperature in the extruder.

Choudhari *et al.* (2011) added different concentrations of encapsulated and free lycopene to the ingredients used for extrusion. Encapsulated lycopene was more stable and less was lost during extrusion processing. In the study, the red colour or a-value from the Lab system of measurement of the extruded snacks was determined as an indicator of retained lycopene. However, a correlation between the redness of the snacks and the lycopene content was not established.

Costa *et al*. (2010) also added lycopene preparations at various concentrations to ingredients used for extrusion. The characteristics of the extruded products including colour parameters were reported. However, no data was reported on the final lycopene concentration. The highest a-value occurred in ingredients containing 30% moisture extruded at 100 °C while the highest acceptability was achieved for samples extruded at 125 °C.

Table 2-1 Stability studies on carotenoids during extrusion cooking

Ingredients	Extruder conditions			Carotenoid type and retention	Reference
	T (°C)	SS (rpm)	M%		
Corn starch	180	150	NA	Pure β-carotene, 8%	*Marty & Berset, 1988*
Corn starch	170	150	10	Pure β-carotene, 42% +rosemary, 79-81% +BHT, 83%	*Berset et al., 1989*
Rice flour	125-155	100	NA	Annatto, 95%	*Maga & Kim, 1990*
Wheat flour	133-173	126-174	13.7-23.7	β-carotene, 38-73%	*Guzman-Tello & Cheftel, 1990*
Corn, curry powder, carrots	120	NA	10	β-carotene, 80-85%	*Bhavani & Kamini, 1998*
Grass pea	100-200	NA	NA	β-carotene, 43 to 33%	*Grela et al., 1999*
Tomato puree + rice flour	NA	NA	NA	Lycopene, 60-75%	*Moraru & Lee, 2000*
Rice/sweet potato flour	170, 180	70-120	10, 13	Total carotenoids, 40-97%	*Fonseca et al., 2008*
Wheat flour	110, 130	NA	NA	β-carotene, 58-97%	*Yajnik et al., 2010*

T=Temperature, SS=Screw speed, M%= Percentage of Moisture, NA= Not available

2.3. Effect of extrusion on starch digestibility and lycopene bioaccessibility

2.3.1. Introduction

The presence of a nutrient in a food does not prove that it can provide health benefits. It must be absorbed and be bioavailable to provide any health benefits (*Brennan et al., 2011*). Thus, starch and lycopene bioavailability should be determined in extruded products containing tomatoes if these snacks are to be shown to deliver nutritional benefits.

2.3.2. Effect of extrusion cooking on starch digestibility

The availability of starch to digestion in an extruded product can be categorized as rapidly digestible (RDS), slowly digestible (SDS) or non-digestible starch (NDS). RDS is usually α-linked glucose units that are broken down by salivary and pancreatic amylase to glucose, maltose, and maltotriose. Glucose is absorbed by the brush border cells in the small intestine. When gelatinized starch is digested, the absorption of glucose is rapid, thus blood glucose levels rise (*Wong & Jenkins, 2007*)**.** During extrusion cooking, most of the starch is fully gelatinized and becomes rapidly digestible (*Alldrick & Hajselova, 2002; Bjorck & Asp, 1983; Camire, 1998; Camire, 2002; Cheftel, 1986; Johnson & Benjamin, 2003; Riaz, 2006; Singh et al., 2007*).

During extrusion, SDS can be produced by the reaction of reducing sugars, formed during dextrinisation of the starch, with the other starch molecules to produce anhydro linkages. Furthermore, interaction of starch with lipids and proteins that are added or

naturally present in the raw ingredients during extrusion processing may result in the formation of amylose-lipid complexes that are digested slowly (*Camire, 1998*). Some of these reactions can also result in the formation of non-digestible (NDS) also called resistant starch (RS).

Furthermore, the presence of soluble fibre at high concentrations in extruded snacks can reduce the rate of starch digestion and glucose absorption. Soluble fibre has been suggested to directly interfere with the amylolysis by inhibiting the action and limiting the accessibility of digestive enzymes to starch, thus limiting the starch digestibility (*Altan et al., 2009a; Brennan, 2005; Brennan & Samyue, 2004; Jenkins et al., 2004*).

2.3.2.1. *In-vitro* measurement of starch digestibility

Starch bioavailability can be measured using human or animal models. However, due to the high cost of *in-vivo* trials, *in-vitro* digestion models that simulate starch digestion in the gastrointestinal tract are commonly used to estimate the rate and extent of starch digestion from different food materials in the small intestine (*Englyst & Englyst, 2005*). These systems have been used to estimate the amount of RDS, SDS or RS (*Englyst et al., 2003*). An outline of the steps involved for the digestion model to determine the RDS and SDS is shown in the Figure 2.6.

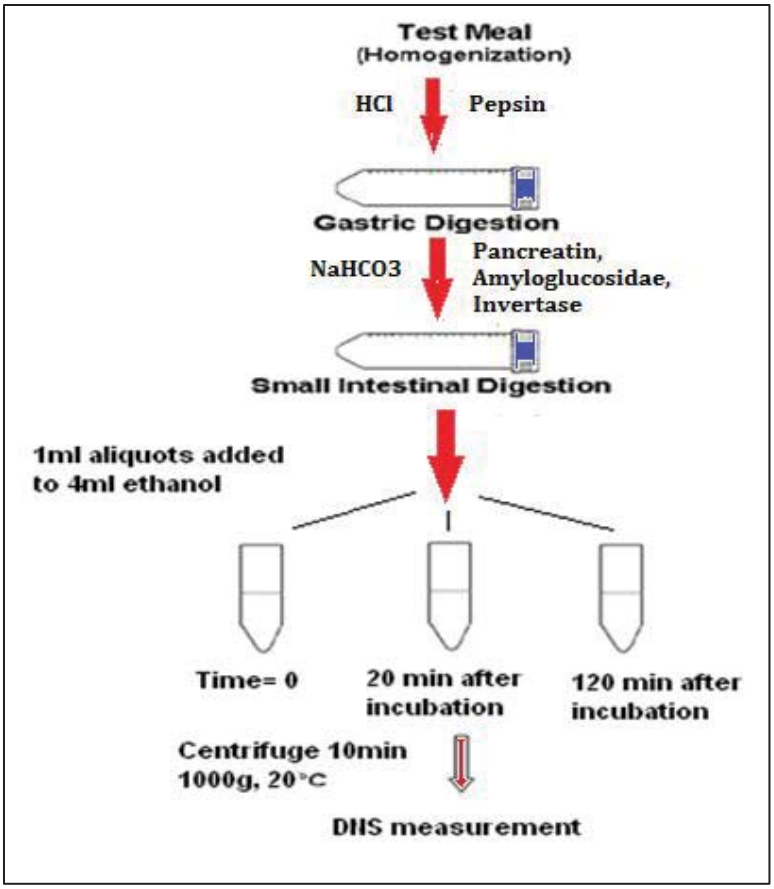

Figure 2-6 Schematic outline for the determination of the in-vitro bioavailability of starch [adapted from Mishra et al. (2008)]

2.3.3. The effect of extrusion cooking on carotenoid bioavailability

During digestion, carotenoids are initially released from the food matrix by the action of the digestive enzymes. Once released in the gut, the lipophilic carotenoids in the water-based digesta are moved towards the absorption sites by entering the mixed micellar phase of the digesta with the aid of bile salts. At the border of the epithelial cells located in the mucosa of the small intestine, the pigments are absorbed by passive diffusion, where they are incorporated into chylomicrons, and secreted into the lymphatic system for delivery to the blood (*Failla et al., 2008a; Faulks & Southon, 2005; Yeum & Russell, 2002*).

Many parameters affect carotenoids bioavailability. For example, tomato cell walls, especially in the tomato skin, are tough and resistant to digestion, preventing lycopene release. Heat treatment and mechanical processing weaken and break down the cell walls, improving the bioavailability of lycopene (*Clinton, 1998; Gartner et al., 1997; Southon & Faulks, 2003*)(Figure 2.7).

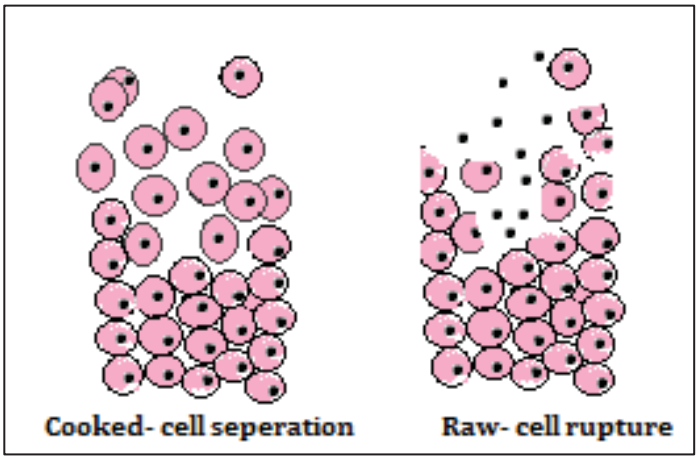

Figure 2-7 Effect of thermal and mechanical processing on plant cells (Southon and Faulks, 2003)

The incorporation of the crystalline lycopene into lipid droplets, thus the micellar phase of the digesta is less likely to occur when compared to other vegetables such as pumpkin and papayas, which have β-carotene already present in oil droplets (*Castenmiller & West, 1998*).

Furthermore, lycopene can be found in *cis*- and *trans*-isomers. *Cis*-isomers of lycopene are reported to be more easily absorbed compared to *trans*-isomers, possibly due to their higher solubility in oil, and therefore greater tendency to form micelles (*Boileau et al., 1999; Castenmiller & West, 1998; Failla et al., 2008b; Stahl & Sies, 1996*). Some evidence exists that extrusion can increase the proportion of *cis*-isomers of carotenoids (*Marty & Berset, 1986*).

The presence of fibre may reduce the amount of bioavailable lycopene as it can bind with the digestive enzymes or bile and limit their bioavailability (*Castenmiller et al., 1999; Parada & Aguilera, 2007; Riedl et al., 1999*).

2.3.3.1. *In-vitro* measurement of carotenoid bioavailability

Human nutrition trials are the best method of determining carotenoid bioavailability. However, there are limitations associated with this approach, particularly cost and obtaining ethic approval. Using animal models has advantages, but no animal model can truly mimic the absorption and metabolism of lycopene in humans (*Failla et al., 2008a*).

In-vitro models are time and cost efficient (*Woolnough et al., 2008*). Using these methods, the apparent bioaccessibility of the pigment or amount of lycopene released from the food matrix and incorporated into the micellar phase of digesta is estimated. Further, absorption and/or uptake of carotenoids can be determined using model cell culture systems, such as Caco-2 cells, derived from human carcinoma colon cells (*Failla et al., 2008a; Faulks & Southon, 2005*). These *in-vitro* techniques have been reported to closely mimic absorption/uptake in humans (*Failla et al., 2008a; Reboul et al., 2006*).

The outline of a scheme to measure the *in-vitro* carotenoid bioavailability with Caco-2 cells is shown in Figure 2.8.

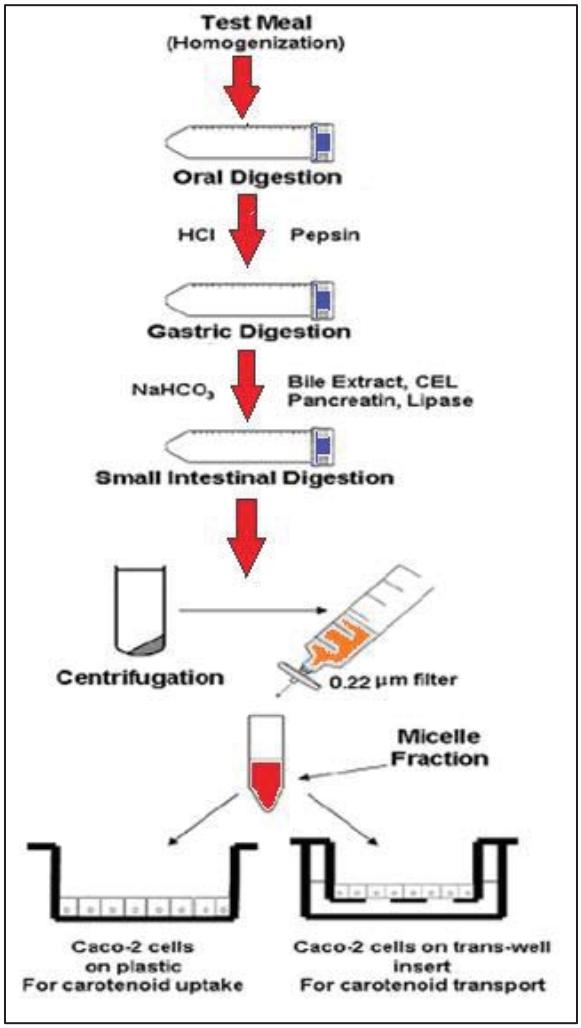

Figure 2-8 Schematic outline of the determination of the in-vitro bioavailability of carotenoid [Adapted from Failla et al. (2008a)]

2.4. Production of extruded products derived from tomatoes

The addition of fruit and vegetables to extruded products can improve their nutritional properties by adding phytochemicals such as fibre and pigments. The enrichment of extruded snacks with a range of fruit and vegetable derivatives has been achieved by many researchers. Carrot powder (*Ozer et al., 2004; Vainionpaa et al., 1989*), cactus pear (*El-Samahy et al., 2007*), grape and blueberry concentrate (*Aldaous, 2005; Camire et al.,*

2002), and fruit powders (*Camire et al., 2007*) have all been used to produce extruded snacks or breakfast cereals.

Fruit and vegetable waste products potentially can be used in the preparation of other food materials after appropriate processing methods. Utilization of these waste streams not only provides extra income to the food industry, but also reduces the environmental impact of their disposal (*Altan & Maskan, 2012; Ilo & Berghofer, 1999*). A wide range of fruit and vegetable by-products have been used by researchers to enrich extruded products (Table 2.2).

Tomato processing is a major food industry for which 2 to 5% of the product weight appears in the waste stream and is called pomace (*Heinz Watties, Hastings, New Zealand, Personal communication*). Pomace contains 44% seeds and 56% tomato skin (*Kaur et al., 2008*). Tomato skin is a rich source of lycopene (> 20 ppm) and fibre (> 70%) (*Alvarado et al., 2001; Canene-Adams et al., 2005; Del Valle et al., 2003; Del Valle et al., 2006; Kaur et al., 2008*). The lycopene content of the tomato skin has been reported to be up to 5 times greater than the pulp (*Alvarado et al., 2001; Sharma & Le Maguer, 1996*). Further, the concentration of fibre in the tomato skin is also higher compared to the flesh. The dominant type of fibre in the tomato skin is insoluble fibre while the pulp contains about 20% of soluble fibre mostly in the form of pectin (*Alvarado et al., 2001; Campbell, 2004; Del Valle et al., 2006*).

Table 2-2 Studies on the utilization of fruit and vegetable by-products in extruded products

Vegetable Derivative	Reference
Blueberry pomace	*Khanal et al., 2010*
Brewer's spent by-products	*Ainsworth et al., 2007; Stojceska et al., 2008*
Cauliflower by-products	*Stojceska et al., 2008*
Corn bran	*Mendonça et al., 2000*
Defatted hazelnut flour, orange peel, grape seed and tomato pomace	*Yagci & Gogus, 2008*
Pea hulls	*Rzedzicki et al., 2004*
Potato peel	*Camire et al., 1997*
Orange pulp	*Larrea et al., 2005*
Sugar beet pulp	*Lue et al., 1994; Rouilly et al., 2006*
Tomato pomace	*Altan et al., 2008a*
Wheat bran	*Al-Wandawi et al., 1985; Brennan et al., 2008a*

2.5. Concluding remarks

Expanded extruded products are regarded as having limited nutritional value, and in the worst scenario are described as "junk foods", due to the presence of a high proportion of fully gelatinized starch and limited amounts of other nutrients. Although the market for extruded snacks contributes to less than 20% of the total snack market *(Global Industry Analysts, 2012),* it's rapid growth, the importance of it's target

consumers, i.e. children, and consumer awareness of food composition and its impact on health, makes it essential to improve the nutritional value of these food products (*Brennan et al., 2008a*).

Attempts to enrich the extruded snacks by adding protein, vitamins and particularly fibre, are on-going. Previous studies have shown that improving the nutritional value of expanded snacks is usually at the expense of their organoleptic characteristics (*Altan et al., 2008a & b*), therefore reducing consumer acceptability (*Moraru & Kokini, 2003*). In the case of breakfast cereals enriched with fibre, some evidence exists that optimising the raw ingredients, i.e. fibre type, can provide manufacturers with extruded products that are accepted by the consumers in addition to improving products' health benefits (*Brennan et al., 2008a & b*).

Pigments such as lycopene can be used to replace colouring additives and may also provide additional nutritional benefits to extruded foods. The available literature investigating the fate of pigments, such as lycopene, during extrusion cooking is scarce, thus the knowledge in this area is limited. This is mainly as a result of the fact that heat-labile molecules such as pigment are thought to degrade during extrusion cooking. Most of the studies carried out on the enrichment of extruded products with pigments have concentrated on the addition of purified pigments to the ingredients. Knowledge on the effect of extrusion cooking on the pigments present within the natural fruit or vegetable matrix, such as lycopene in the tomatoes, is lacking. The incorporation of pigments in their native environment may improve their stability due to the protective effect of layers of fibre in the cell walls encompassing the cell constituents.

Furthermore, the presence of a given nutrient in food does not ensure that it can provide health benefits or be bioavailable. Although the effect of extrusion cooking on

the bioavailability of nutrients such as starch and protein has been examined extensively, the effect of extrusion processing on the bioavailability of pigments present in fruit and vegetable is still not known. This is partially due to the complexity of the reactions taking place during extrusion cooking. For example, during extrusion cooking, starch is gelatinized and cell walls rupture, thus pigments that are bound within the cells are released and become more susceptible to digestive enzymes, increasing their availability for absorption (*Gartner et al., 1997; Riaz, 2000*). Conversely, extrusion cooking can reduce the concentration of the nutrients by thermal and mechanical effects. Also, during this process, the bioavailability of some nutrients is reduced, for instance by the formation of starch-lipid complexes (*Strange & Schaich, 2000*). Furthermore, the presence of other food components within the extruded food, such as fibre, can reduce the bioavailability of the nutrient (*Altan et al., 2009a; Riedl et al., 1999; Slaughter et al., 2002*).

A comprehensive study is needed to determine if addition of tomato, as a model for fruit and vegetable enrichment, can result in consumer acceptable extruded products which contain nutritionally significant amounts of fibre and bioavailable lycopene. In order to reach this goal, the following were the aims of this study:

1- To develop a method to measure lycopene in the extruded products;

2- To develop a method to simultaneously measure *in-vitro* lycopene bioavailability and starch digestibility in the extruded products;

3- To investigate the effect of ingredient and extrusion process parameters on the physicochemical characteristics of extruded products derived from tomatoes;

4- To investigate the effect of ingredient and extrusion process parameters on the *in-*

vitro lycopene bioaccessibility and starch digestibility in extruded products derived from tomatoes.

5- To optimise the formulation and processing conditions to produce extruded tomato products that are consumer acceptable and contain nutritionally significant amounts of bioavailable lycopene and dietary fibre.

Each of these points will be investigated in the following chapters.

Chapter Three Materials and Methods

3.1. Introduction

In this section of the thesis, the methodologies used for the core analyses in the study are described. Materials and methods that are specific to some experiments carried out during this work are described in the related chapters. Methodology to determine the lycopene content of snack foods was a core requirement for the study. As the continuous matrix that formed these products was starch, the *in-vitro* measurement of digestion of starch was also required. These methods were developed through this study and are described in Section 3.3.

3.2. General materials and methods

3.2.1. Materials

3.2.1.1. Starch based ingredients

Sunrice brand medium rice flour and Champion brand wheat semolina were supplied by Davis Trading, Palmerston North, New Zealand. Medium grind 320 spec corn grits were supplied by Corson Grain Ltd (Gisborne, New Zealand).

3.2.1.2. Tomato based ingredients

Tomato pomace (tomato skin and seed) and paste were donated by Heinz Watties (Hastings, New Zealand). Tomato skin was separated from tomato seeds by flotation on

water, freeze dried (model FD18LT "ISLA", Cuddon, Blenheim, New Zealand) at -35 °C for 36 hours and milled to particle size of less than 710 microns (ZM200, Retsch, Haan, Germany).

3.2.1.3. Chemicals

All chemicals including pepsin (from porcine stomach mucosa, Sigma product code P7125, 56 units/mg solids), pancreatin (from porcine pancreas, Sigma product code P7525, 8×USP), porcine bile extract (Sigma product code B-8631), invertase concentrate (Sigma product code E.C. 3.2.1.26), analytical standard for lycopene (L9879) along with solvents including hexane, acetone, ethanol and other reagents were purchased from Sigma Chemicals Co. (St. Louise, MO, USA). Amyloglucosidase (AMG-300L, BrewQ) was obtained from Novozymes Ltd. (New Castle, USA).

3.2.2. General methods

3.2.2.1. Extrusion cooking

All products were made using a Clextral BC21 twin-screw laboratory scale extruder (Clextral, Firminy, Cedex, France) (Figure 3.1). The total working barrel length was 700mm which was divided into 7 temperature controlled barrel sections each 100mm in length. The screw diameter was 24.7 mm and the screw configuration used was 200 mm forward, 13 mm pitch; 200 mm forward, 10 mm pitch; 25 mm forward, 7 mm pitch; 50 mm forward, 10 mm pitch; 225 mm forward, 7 mm pitch. A single die with 3 mm diameter was used as the die aperture. Process variables including temperature of the

barrel sections, screw speed, feed rate, and water feed rate were varied depending on the experiment and are described in their related sections.

Figure 3-1 Clextral BC 21 twin-screw extruder

3.2.2.1.1. *Extrusion parameters*

Specific mechanical energy (SME) is an extrusion process parameter used to relate the energy used to extrude the product with the product characteristics (*Guy, 2001; Lillford, 2008*). It was calculated by dividing the net power input by the mass flow rate of the extruded product (*Altan et al., 2008b*). This parameter is useful for describing aspects of the thermal and shear modification of the products being processed. It can be calculated from the following equation (Equation 3.1):

Equation 3-1 Calculation of specific mechanical energy (SME)

$$SME\ (kWh/kg) = \frac{\text{Total energy}}{\text{Flow rate}} = \frac{\text{Motor power used}}{\text{Output}} = \frac{N * T * kW}{Q}$$

Where N= Screw speed (rpm), T= Torque (N.m), kW= Motor power and Q= Output (kg/h).

Torque is an extrusion parameter used to describe the turning force being applied by the motor which is used to shear the material in the barrel. The power used to rotate the screws during extrusion processing is measured in kilowatts (kW) and is used to determine SME and correlates with the physical properties of the extruded products.

Pressure at the die (bar) is another process variable that is used to measure the conditions under which the extruder is being operated (*Guy, 2001*). The thrust pressure (bar) is also measured and directly proportional to the die pressure.

These parameters are used to describe the processing conditions used to transform the raw ingredients into the melt within the extruder and ultimately into the final extruded snack product (*Moraru & Kokini, 2003*). These parameters were recorded at least 3 times during the processing of each product type for each experiment. Average values are reported for each product.

3.2.2.2. Chemical analysis

3.2.2.2.1. *Moisture determination:*

Samples of all products for which a moisture determination was required were ground to a fine powder (< 200 μ). Approximately 3.0 grams of the finely ground, raw ingredients and extruded products were sub-sampled and placed in a thin layer on a shallow aluminium pan. The moisture content of the test material was measured by drying to constant weight at 105 °C in the oven overnight and moisture content determined according to Equation 3.2 (*AACC, 2005*). Moisture loss was calculated as the proportion of the moisture in the extruded product compared to the moisture content of the raw ingredients according to the method of Brennan *et al.* (*2008a*).

Equation 3-2 Calculation for percentage of moisture

$$\%\text{Moisture} = \left(\frac{W_2 - W_1}{W_1} \right) * 100$$

Where W_1= Initial weight of sample (g), W_2= Final weight of sample (g)

3.2.2.2.2. *Ash content:*

The AOAC method (No. 945.18) was used for this measurement. Approximately 2.5 g of the finely ground sub-samples (< 200 μ) of the raw ingredients and extruded samples were weighed into crucibles. The crucibles containing the samples were charred over a Bunsen burner before being placed in a furnace at 550 °C. Ashing was continued for 5

hours until the residue became grey-white in appearance. The weight of ash was used to calculate the proportion of uncombusted material according to Equation 3.3.

Equation 3-3 Calculation for percentage of ash

$$\%\text{Ash in food} = \left(\frac{W_2 - W_1}{W_1}\right) * 100$$

Where W_1= Initial weight of sample (g), W_2= Final weight of sample (g)

3.2.2.2.3. *Fat content (Soxhlet method):*

According to the AOAC method (No. 945.16) (*1998*), the sample was dried. Two grams of the finely ground and dried samples were weighed in filter papers and placed inside thimbles. Round-bottomed flasks (250 ml) were placed in the oven (180 °C) for 1 hour to reach a constant weight. Approximately 170 ml of petroleum ether was placed in the soxhelet apparatus and thimbles were placed inside. The flasks were placed on the heating pans. The distillation rate was adjusted to 4 drops/s. The soxhlet extraction was carried out for four hours. The weight of the recovered fat collected in the flask was determined and used to calculate the proportion of fat in the sample according to the following Equation:

Equation 3-4 Calculation for percentage of fat

$$\%\text{Fat in food} = \left(\frac{W_2 - W_1}{W_1}\right) * 100$$

Where W_1= Initial weight of sample (g), W_2= Final weight of sample (g)

3.2.2.2.4. *Protein content*

The standard Kjeldahl method was used to determine the nitrogen content of the material. The appropriate conversion factors were derived from the AOAC method (No. 920.53; AOAC, 1998). Samples were dissolved by boiling in sulphuric acid to oxidise the organic material and liberate nitrogen as ammonium sulphate. The solution was then neutralised by sodium hydroxide to convert the ammonium salt to ammonia (NH_3). The ammonia was then reacted with boric acid and the remaining acid titrated with sodium carbonate solution. Using this method, the amount of crude nitrogen present in the sample was obtained. As many plants contain more than 50% non-protein nitrogen, the percentage of N was multiplied by 5.7 for wheat flour or 6.25 for the other ingredients to give the percentage of protein (*AOAC, 1998*).

3.2.2.2.5. *Fibre*

Total dietary fibre was determined using Megazyme Total Dietary Fibre assay kit (Megazyme International, Wicklow, Leinster, Ireland) according to AOAC (*1998*) method No. 991.43. Briefly, the sample was digested using amylolytic and proteolytic enzymes to convert all degradable polysaccharides to sugar. The soluble fibre was then precipitated by ethanol, filtered, then dried and weighed. The amount of ash and protein was also measured in the solution and subtracted from the weight of the filtrate as these components interfere with the calculations for fibre.

3.2.2.2.6. Starch

Total starch was determined using the Total Starch assay kit (Megazyme International, Wicklow, Leinster, Ireland). The starch present in the sample was incubated with dimethyl sulphoxide (DMSO) to disrupt the granule structure, and amyloglucosidase to hydrolyse the starch to glucose. The amount of glucose released was quantitatively determined by spectrophotometric method at 510 nm.

3.2.2.3. Physical analysis

3.2.2.3.1. Expansion

The diameters of at least 30 extruded pellets were measured using Vernier calipers (Mitutoyo, Tokyo, Japan). Expansion ratios of the samples were calculated by dividing the average diameter of the extruded products by the diameter of the die and multiplying the result by 100 to express the result as a percentage (Equation 3.5) (*Huang et al., 2006*).

Equation 3-5 Calculation for expansion ratio

$$\text{Expansion ratio} = \left(\frac{\text{Average diameter of the extruded product (mm)}}{\text{Diameter of the die (mm)}} \right) * 100$$

3.2.2.3.2. Density

The true density of the extruded snacks was determined by the poppy seed displacement method applied to approximately one litre of bulk of extruded products

(*Brennan et al., 2008a*). Variation in the true density of the product is largely dependent on the proportion of voids or air bubbles that are trapped in the extruded products as they solidified from the extruder. The true density correlates with the void fraction of the products, their hardness measured instrumentally and by consumer testing. All measurements are reported as a mean of between three and five replicates (*Brennan et al., 2008a*). True density was determined according to Equation 3.6.

Equation 3-6 Calculation for density

$$\text{Density (kg/m}^3\text{)} = \left(\frac{\text{Weight of sample (kg)}}{\text{Displacement volume (l)}} \right) * 1000 \text{ (l/m}^3\text{)}$$

3.2.2.3.3. <u>*Hardness*</u>

The hardness of samples was measured using Instron Universal testing machine (4502) (Canton, Massachusetts, USA) fitted with a 1000 N load cell (Figure 3.2). Samples were prepared according to the method of Hardacre *et al.* (*2006*) and gently packed into a Kramer shear cell to 60% of the cell height (about 200 to 300 grams of product). The Kramer probe (3 blades, 3 mm thick, 125 mm high, 70 mm wide, 20 mm apart) was set to move at a test speed of 180 mm/min for a distance of 50 mm. The maximum force needed to penetrate the aggregated sample was recorded and analysed by the software associated with the Instron. Measurements are reported as an average of 4 to 6 replicates.

Figure 3-2 Instron universal testing machine 4502

3.2.2.3.4. *Colour evaluation*

The raw ingredients and extruded products were milled using a Breville laboratory grinder (Botany, New South Whales, Australia) to obtain a fine powder (< 200 μm) which was packed into a shallow dish. Colour readings were taken from five separate points on the surface of the powders using a Minolta Chroma meter (CR200, Minolta, Osaka, Japan). Before readings were made, the colorimeter was calibrated against a standard white tile. The values of luminance (L), red-blue saturation index (a) and yellow-green (b) saturation index were recorded (*Altan et al., 2008b*). The difference in colour (ΔE) was determined according to Equation 3.7.

Equation 3-7 Calculation for change in colour

$$\Delta E = \sqrt{(L-L_0)^2 + (b-b_0)^2 + (a-a_0)^2}$$

Where the 0 subscript is related to the raw ingredients.

3.3. Analytical methods Developed

3.3.1. Lycopene extraction and quantification from extruded snacks containing tomato skin[1]

3.3.1.1. Abstract

To determine the lycopene content of extruded products containing 10% tomato skin, the conditions of extraction by solvents were optimised. After three extraction cycles at 50 ˚C each for 15 minutes at a solvent-to-meal ratio of 40:1, a maximum of 6.6 ppm lycopene was extracted. However the extraction was considered incomplete as pigment was remaining in the product. Thus, a digestion process using pancreatin at various incubation times and concentrations was carried out prior to extraction. The extracted lycopene content was increased to 23.5 ppm using after 20 minutes of digestion with 10 mg/ml pancreatin. These conditions were considered the optimum incubation conditions. To validate the extraction efficiency, a set of products were produced at various extrusion conditions to obtain a range of intensities in red colour. Digestion of the products increased the extracted lycopene content by more than 2.5 times between the products. Furthermore, including a digestion process prior to extraction improved the correlation coefficient between the red colour and the extracted lycopene content. The digestion of the products will therefore be included for all subsequent lycopene analyses.

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[1] Part of the material presented in this section has been previously published as a peer-reviewed journal article: Dehghan-Shoar, Z., Hardacre, A.K., Meerdink, G., Brennan, C.S. (2011). Lycopene extraction from extruded snacks containing tomato skin. *International Journal of Food Science & Technology*. 46 (2), 365-371.

3.3.1.2. Introduction

Although colorimetric a-values (a measure of redness) have been shown to correlate with the lycopene content of food products, such as tomatoes and extruded snacks containing tomato pomace (*Altan et al., 2008b; Arias et al., 2000; Schieber et al., 2001*), accurate measurements of lycopene concentration are required if nutritional claims are to be made. These measurements are carried out by extracting the pigment from the food matrix by lipophilic solvents such as hexane, ethanol or acetone, and quantifying it using the spectrophotometric or HPLC methods (*Kaur et al., 2008; Nunes & Mercadante, 2004; Olives Barba et al., 2006; Sadler et al., 1990*). An efficient extraction procedure improves the accuracy of the measurements which can be achieved by optimizing the extraction conditions such as time, temperature, number of extraction cycles and the amount of extracting solvent. The optimum extraction conditions depend on the processing technique used to manufacture the food product. For example, longer extraction times and more extraction cycles are required to efficiently extract lycopene from raw tomato and tomato skin (*Kaur et al., 2008*) compared to tomato paste (*Sadler et al., 1990*). In the unprocessed tomato, the lycopene is bound within the cellular structures, while the cells in the tomato paste have been weakened by processing, resulting in the higher extraction efficiency of lycopene from the tomato paste (*Gartner et al., 1997*).

During the extrusion cooking, severe thermal and mechanical processing is applied to the food materials that breakdown the cell walls, thus improving the vulnerability of the cell constituents including lycopene, to solvents. On the other hand, extrusion processing reduces the extractability of compounds such as lipids by solvents (*Bhatnagar & Hanna, 1995; Singh et al., 2007; Strange & Schaich, 2000; Wicklund &*

Magnus, 1997). The extractability of lipids has been shown to increase with the digestion of the extruded product prior to extraction by solvents (*Eliasson, 1994; Guzman-Tello & Cheftel, 1990; Strange & Schaich, 2000*).

While considerable work has been carried out on the extractability of lipids from the extruded products (*Bhatnagar & Hanna, 1995; Singh et al., 2007; Strange & Schaich, 2000; Wicklund & Magnus, 1997*), studies on lipophilic compounds, especially lycopene, are scarce. The objective of this study was to determine the conditions that would result in the maximum lycopene extraction from extruded products containing tomato skin in order to obtain an accurate measurement of the lycopene content of these products. To do this, the solvent extraction conditions were first optimized for one product type. Then, the product was digested with various pancreatin concentrations and incubation times to determine the optimum conditions that extracted the highest amount of lycopene. Finally, to examine the ability of the developed method to extract lycopene, a range of products were prepared using various extrusion conditions and their lycopene contents determined using the optimum conditions obtained from previous experiments, either including or excluding the digestion step. Furthermore, as a confirmation for the adequacy of extraction, the correlation coefficients between the a-value of the snacks and the amount of the extracted lycopene using both extraction methods were determined. The method resulting in the highest correlation coefficient was chosen as the most efficient extraction technique.

3.3.1.3. Materials and methods

3.3.1.3.1. Ingredients

Tomato skin powder was prepared as described in Section 3.2.1.20. Rice flour specifications are described in section 3.2.1.1. For all the experiments, a mixture comprising of 90% rice flour and 10% freeze dried tomato skin powder was extruded. This formulation was based on the preliminary trials which produced well expanded extruded snacks.

3.3.1.3.2. Extrusion cooking conditions

The extruder specifications are described in Section 3.2.2.1. Throughout the present study, the die temperature was set at 140 °C and the feed rate of ingredients into the extruder was kept constant at 6.5 kg/h. One sample was extruded at a constant screw speed of 300 rpm at feed moisture content of 11% and used to optimize the solvent extraction and digestion conditions. To validate the optimized conditions, another set of extruded samples were prepared by varying the feed moisture content (11, 15, 19 or 23%) and screw speed (250 or 350 rpm), to obtain a range of lycopene concentrations within the snacks.

3.3.1.3.3. Optimization of extraction by solvents

The experimental conditions to optimize solvent extraction were determined according to the report of Kaur *et al.* (*2008*). The effect of four independent variables, i.e. time, temperature, number of extraction cycles and solvent-to-meal ratio, at three levels, was

investigated on the lycopene extraction yield using a central composite design (Table 3.1). Thirty combinations of the independent variables were chosen and along with their response (extracted lycopene content) are presented in Table 3.2. Response surface graphs plotted between two independent variables at a time while keeping the other two variables constant at zero level were used to investigate the effect of different levels of the independent variables on the extracted lycopene content.

Lycopene extraction was carried out according to the method of Sadler *et al*. (*1990*) after some modifications. In brief, 1.0 gram of the ground sample along with 1.0 gram of diatomaceous earth was weighed into amber coloured glass jars to exclude light. A mixture of solvents, i.e. hexane: acetone: ethanol (2:1:1 v/v), containing 0.05% (w/v) butylated hydroxytoluene (BHT) to minimize oxidation, was then added in different volumes according to the experimental design (Table 3.2). The suspension was agitated constantly at 75 rpm in a shaking water bath at various temperatures and duration times (Table 3.1). The top layer, solvent phase, was separated and depending on the experimental design the extraction process was repeated once or twice more. The solvent phases were combined, 15 ml of cold distilled water containing 10% NaCl (w/v) was added and the mixture shaken vigorously for one minute so the phases would mix completely. The suspension was then allowed to stand for 15 minutes for the separation of non-polar (solvent) and polar (water) phases. The top polar phase containing lycopene was collected and then the absorbance was measured at 472 nm using a UV-visible spectrophotometer (Helios Epsilon spectrophotometer, Thermo Electron Corporation, Pittsford, New York, USA).

A set of standards were prepared by dissolving pure lycopene in hexane at various concentrations ranging from 1 to 20 mg/ml and a calibration curve of concentration

against absorbance was plotted (R^2= 0.96). The lycopene concentration of the samples was calculated from this curve by measuring the absorbance. The results are reported as mean of two replicates.

Table 3-1 Independent variables and their levels used for central composite design

Independent variables	Symbol	Coded variable levels		
		-1	0	1
Time (min)	X_1	5	10	15
Temperature (°C)	X_2	20	35	50
Number of extractions	X_3	1	2	3
Solvent/meal ratio (v/w)	X_4	20:1	30:1	40:1

3.3.1.3.4. *Optimization of digestion procedure*

One gram of the ground extruded material was weighed into amber coloured glass jars and 5 ml of distilled water or pancreatin solution at concentrations of 2.5, 5, 10, 15, 30 or 45 mg/ml (pH= 6.5) was added. The jars were placed in a shaking water bath set at 37 °C at shaking speed of 75 rpm. Samples were removed 20, 60 or 120 minutes from the start of incubation and lycopene was extracted by solvents. The extraction was carried out using the optimum conditions obtained from Section 3.3.1.3.3. All measurements are reported as a mean of three replicates.

Table 3-2 Central composite design for time, temperature and number of extractions and solvent/meal ratio on the lycopene extraction yield from an extruded snack containing 10% tomato skin powder *

Run	Coded and actual values				Extracted lycopene (ppm, dwb)
	Time, X_1 (min)	Temperature, X_2 (˚C)	No. of extraction, X_3	Solv/meal ratio, X_4 (v/w)	
1	-1 (5)	-1 (20)	-1 (1)	-1 (20:1)	3.0
2	1 (15)	-1 (20)	-1 (1)	-1 (20:1)	3.2
3	-1 (5)	1 (50)	-1 (1)	-1 (20:1)	3.4
4	1 (15)	1 (50)	-1 (1)	-1 (20:1)	4.0
5	-1 (5)	-1 (20)	1 (3)	-1 (20:1)	4.6
6	1 (15)	-1 (20)	1 (3)	-1 (20:1)	4.8
7	-1 (5)	1 (50)	1 (3)	-1 (20:1)	5.2
8	1 (15)	1 (50)	1 (3)	-1 (20:1)	6.7
9	-1 (5)	-1 (20)	-1 (1)	1 (40:1)	3.0
10	1 (15)	-1 (20)	-1 (1)	1 (40:1)	3.7
11	-1 (5)	1 (50)	-1 (1)	1 (40:1)	4.0
12	1 (15)	1 (50)	-1 (1)	1 (40:1)	4.3
13	-1 (5)	-1 (20)	1 (3)	1 (40:1)	4.8
14	1 (15)	-1 (20)	1 (3)	1 (40:1)	5.7
15	-1 (5)	1 (50)	1 (3)	1 (40:1)	6.1
16	1 (15)	1 (50)	1 (3)	1 (40:1)	6.6
17	-1 (5)	0 (35)	0 (2)	0 (30:1)	4.5
18	1 (15)	0 (35)	0 (2)	0 (30:1)	5.1
19	0 (10)	-1 (20)	0 (2)	0 (30:1)	4.0
20	0 (10)	1 (50)	0 (2)	0 (30:1)	5.8
21	0 (10)	0 (35)	-1 (1)	0 (30:1)	4.4
22	0 (10)	0 (35)	1 (3)	0 (30:1)	5.1
23	0 (10)	0 (35)	0 (2)	-1 (20:1)	4.8
24	0 (10)	0 (35)	0 (2)	1 (40:1)	5.4
25	0 (10)	0 (35)	0 (2)	0 (30:1)	4.8
26	0 (10)	0 (35)	0 (2)	0 (30:1)	4.9
27	0 (10)	0 (35)	0 (2)	0 (30:1)	5.4
28	0 (10)	0 (35)	0 (2)	0 (30:1)	4.7
29	0 (10)	0 (35)	0 (2)	0 (30:1)	4.8
30	0 (10)	0 (35)	0 (2)	0 (30:1)	5.4

* Extrusion conditions: Feed rate, 6.5 kg/h; Die temperature, 140 ˚C, Feed moisture content, 11%; Screw speed, 300 rpm.

3.3.1.3.5. Validation of the efficiency of the extraction

By changing the extrusion parameters namely, moisture content (11, 15, 19 or 23%) and screw speed (250 or 350 rpm), eight types of snacks were produced that were expected to have different lycopene contents. Their lycopene content was determined using both methods, i.e. only solvent extraction or pre-digestion with pancreatin and then solvent extraction. For both methods, optimum conditions reported in Sections 3.3.1.3.3 and 3.3.1.3.4 were used, i.e. solvent extraction was carried out three times for 15 minutes each at 50 °C and at a solvent-to-meal ratio of 40:1 and digestion was carried out using 10 mg/ml pancreatin for a 20 minutes incubation time.

To determine the redness or the a-colorimetric value, the products were finely ground (< 200 μm) and packed into shallow dishes. Colour readings (CIE Lab) were taken from five separate points on the surface of the powders using a Minolta Chroma meter (CR200, Minolta, Isaka, Japan). The light source was D_{65}. Before readings were made, the colorimeter was calibrated against a standard white tile (*Altan et al., 2008b*). All measurements are reported as a mean of three replicates.

3.3.1.3.6. Recovery assay

Recovery of lycopene was determined to investigate the efficiency of the extraction procedure. It was assessed according to the method of Sadler *et al.* (*1990*). Pure lycopene dissolved in hexane was added to the ground extruded product at a concentration of 5 ppm before extraction, and the amount of lycopene recovered after the extraction was determined. Lycopene recovery was calculated from the total amount of lycopene content obtained after the extraction divided by the sum of the

lycopene content of sample plus the added pure lycopene. All measurements are reported as a mean of three replicates.

3.3.1.3.7. Experimental design and statistical analysis

A series of studies were carried out to optimize the lycopene extraction conditions from extruded products containing tomato skin.

Optimization of extraction by solvents was carried out according to the method of Kaur *et al.* (*2008*) using a central composite design at three levels. The generation of response surface plots and their associated statistical analysis was performed using Design Expert software version 6.0.2 (Statease Inc, Minneapolis, MN, USA). Data was modelled by multiple regression analysis adopting stepwise analysis. Only the variables significant at $p < 0.05$ levels were selected. Statistical significance of the variables in the regression equation was examined by analysis of variance (ANOVA) for each response. The optimization of the extraction was carried out by considering two independent variables at a time. The response surface graphs gave values for the independent variables where the extraction yield reached its maximum point.

The digestion step using pancreatin was optimized using a full factorial design comprising 7 pancreatin concentrations (0, 2.5, 5, 10, 15, 30 and 45 mg/ml) × 3 incubation times (20, 60 and 120 min). A completely randomized design was used to evaluate the results and analysis of variance (ANOVA) was carried out to compare the mean values. The optimum digestion conditions were determined based on the point where maximum amount of the extracted lycopene was obtained. All significant

differences are reported at $p < 0.05$ level. The statistics were calculated using SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA).

To validate the extraction method, the lycopene content of the eight snack types were determined with or without the digestion step. Student paired t-test was used to indicate the difference in the extracted lycopene obtained from both methods ($p < 0.05$). The correlation coefficient between the lycopene contents obtained using both of the extraction methods with the a-values of the snacks were determined using the Pearson's correlation coefficient (R^2). The statistics were calculated using SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA).

3.3.1.4. Results and Discussion

3.3.1.4.1. Optimization of extraction by solvents

The extraction of lycopene from the extruded product at various conditions resulted in a regression equation (Equation 3.8) with linear coefficients as follows in terms of coded factors:

Equation 3-8 Linear regression effect for lycopene extraction conditions from an extruded rice product containing 10% tomato skin

$$\text{Extracted lycopene yield} = 4.73 + 0.30 * X_1 + 0.51 * X_2 + 0.92 * X_3 + 0.22 * X_4$$

Where X_1 is time (min), X_2 is temperature (°C), X_3 is number of extractions and X_4 is solvent-to-meal ratio (v/v).

Increases in all of the independent variables, enhanced lycopene extraction yield significantly, although the extent of their effect varied depending on the parameter. For example, the increases in the number of extraction cycles enhanced the amount of the extracted lycopene more than the time of extraction. With increases in the number of extractions from 1 to 3 times, the extracted lycopene increased by more than 150%, after 5 minutes extraction, while increases in the time of extraction from 5 to 15 minutes increased the extracted lycopene content by less than 14% (Figure 3.3).

Also increases in the temperature of extraction improved extraction yield more than the time of extraction. For example, increases in temperature from 20 to 50 °C enhanced the amount of the extracted lycopene by 29% after 5 minutes extraction while increases in the time of extraction from 5 to 15 minutes increased the extraction yield by 7% (Figure 3.4).

Furthermore, the increases in the solvent-to-meal ratio were less effective on the extraction yield compared to the other parameters. For example, when more solvent was added, lycopene extraction yield slightly increased (15%), while by increasing the number of extractions from 1 to 3 times, the extracted lycopene was 160% higher (Figure 3.5).

The positive effect of time and number of extraction on lycopene extractability has been reported previously by Kaur *et al.* (*2008*) who extracted lycopene from dehydrated tomato skin waste. Increases in the extraction duration time and volume of the extracting solvent increases the amount of lycopene to be extracted by the solvent. Also, increases in temperature had a positive effect on the lycopene extraction due to the reduction in the integrity of the cell comprising the lycopene and therefore higher

accessibility of the lycopene entrapped within the cells to solvents (*Baysal et al., 2000; Kaur et al., 2008*).

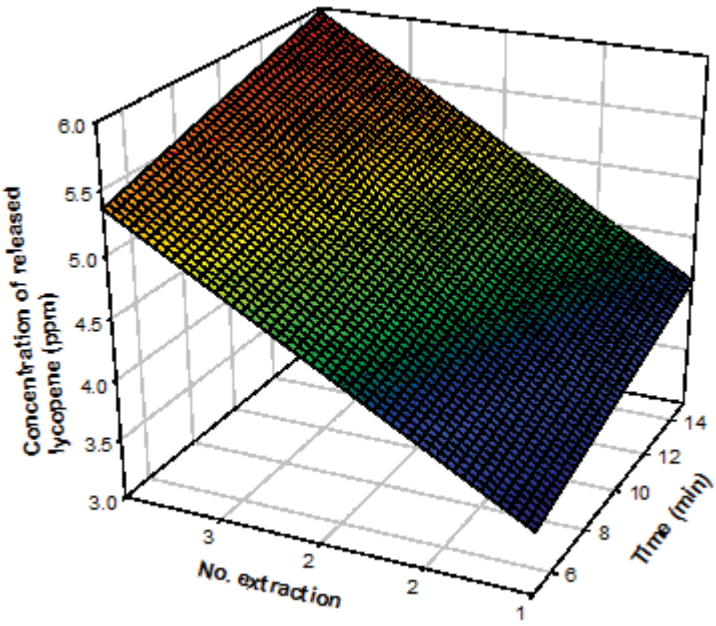

Figure 3-3 Predicted effect of number and time of extraction on lycopene extraction yield from an extruded rice product containing 10% tomato skin powder using a solvent to meal ratio of 30:1 v/w at 35 ˚C

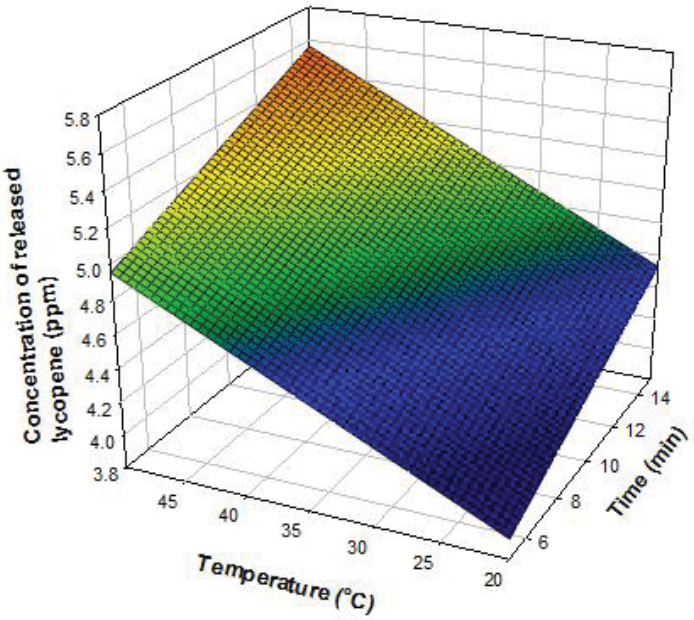

Figure 3-4 Predicted effect of temperature and time of extraction on lycopene extraction yield from an extruded rice product containing 10% tomato skin powder using a solvent to meal ratio of 30:1 v/w after 2 extraction runs

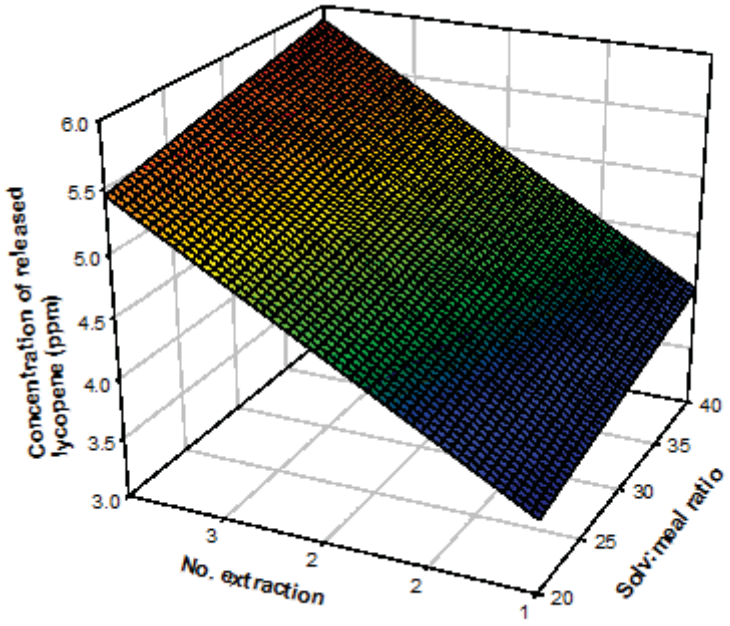

Figure 3-5 Predicted effect of solvent to meal ratio and number of extractions on lycopene extraction yield from an extruded rice product containing 10% tomato skin powder after 10 minutes extraction at 35 ˚C

A maximum amount of 6.6 ppm (dry weight basis, dwb) lycopene was extracted from the extruded snack after 3 times extraction at 50 °C each for 15 minutes using a solvent-to-meal ratio of 40:1 (v/w).

Recovery of pure lycopene ranged between 95-103% with a mean recovery of 99.5% suggesting that free lycopene was effectively extracted. Sadler *et al*. (1990) also reported a mean recovery of 99.6% for tomato paste ranging from 98.6 to 101.5%. Olives-Barba *et al.* (*2006*) reported a recovery of 98.7% with higher standard deviation values of 18.3%.

Although the acceptable range of lycopene recovery (*AOAC, 1998*) confirmed the efficiency of the solvent extraction procedure, the resulting meal obtained after

extraction still contained red colour suggesting that the lycopene was not extracted completely by solvents. It was concluded that some of the pigment was unavailable to solvents for extraction.

3.3.1.4.2. *Optimization of the digestion procedure*

By wetting the snack prior to extraction, the extracted lycopene concentration was increased to 9.4 ppm after 20 minutes incubation (Figure 3.6), which was 140% higher compared to the value obtained after the extraction from the dry snack under optimum conditions (Table 3.2). This is in line with the findings of Strange & Schaich (*2000*) on the extractability of lipids from extruded corn-soy blends. They suggested that hydrating the food matrix of the extruded product enhanced the lipid extraction yield to some extent, while digesting the product prior to extraction, improved the extractability significantly. This was also observed in our results where by digesting the snack with pancreatin, the lycopene extraction yield was 250% higher compared to the extraction from the wet snack (Figure 3.6).

Maximum amount of lycopene was extracted after 20 or 60 minutes incubation time, depending on the concentration of pancreatin (Figure 3.6), while the extraction yield obtained after 120 minutes of digestion was lowest at any pancreatin concentration (Figure 3.6). Longer incubation times of more than 6 hours have been employed for the extraction of lipids from extruded products (*Eliasson, 1994; Strange & Schaich, 2000*), while Kean *et al.* (*2008*) extracted the carotenoids present in the extruded maize meal products after a 15 minutes digestion period. Compared to lipids, carotenoids including lycopene, degrade rapidly in the presence of oxygen or light (*Lee & Chen, 2002*).

Although care was taken to reduce the amount of oxygen present in the jars, it can be assumed that some oxygen still remained and the long duration period of 120 minutes provided enough time for the released lycopene to be oxidized by the available oxygen. Prolonged heating has also been shown to deactivate lycopene, however as the incubation temperature used for the purpose of the present experiment was low (37 °C), the effect of heat was considered negligible on lycopene degradation (*Lee & Chen, 2002*).

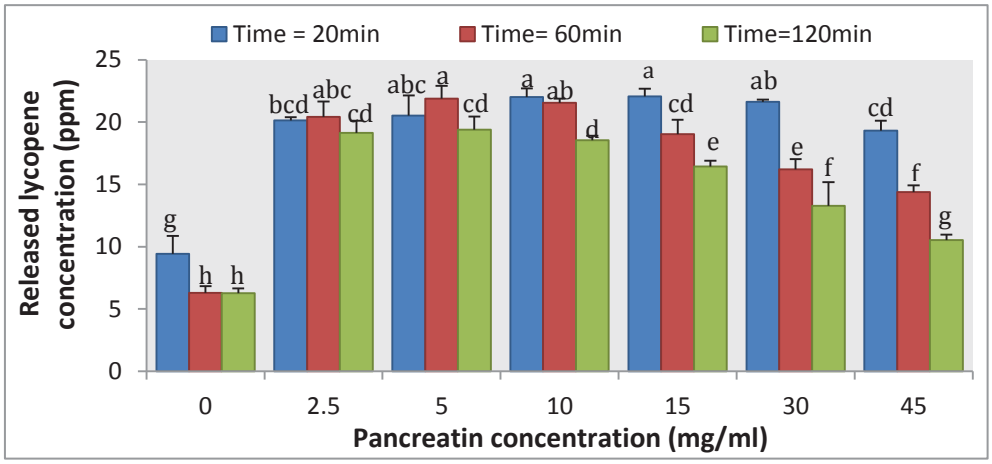

Figure 3-6 Effect of pancreatin concentration and incubation time on lycopene extraction yield from extruded rice product containing 10% tomato skin powder. Error bars represent standard deviations from the means. Different letters above bars (a-g) indicate significant differences (p <0.05) between samples, n =3

Among the experimented conditions, maximum extraction yield for lycopene was obtained after 20 minutes incubation with 10 or 15 mg/ml of pancreatin, or after 60 minutes incubation with 5 mg/ml pancreatin (Figure 3.6). The digestion with 10 mg/ml pancreatin for 20 minutes was chosen as the optimum conditions as it was the most time and cost efficient option compared to the other conditions (Figure 3.6).

3.3.1.4.3. Validation of the extraction method

To validate the optimum conditions obtained from previous experiments and determine the significance of utilizing a pre-digestion step prior to extraction by solvents, another set of samples were produced under eight different extrusion conditions, which were expected to have different lycopene contents. It was found that the extracted lycopene obtained by including the pre-digestion step differed significantly from when the extraction was carried out with solvents only ($p < 0.05$). The incorporation of this additional step enhanced the proportion of the lycopene extracted by solvents by more than 250% (Table 3.3). The positive effect of digestion on extractability is similar to findings of Strange and Schaich (*2000*) who reported that 98.7% of the added lipids to the extruded corn-soy blends were recovered by including the digestion step using 25 mg amylase while in the absence of amylase or at lower concentrations, less than 90% of the lipids were extracted.

As another approach to validate the efficiency of the extraction procedure, the colorimetric a- values of the snacks were determined (Table 3.3) and compared with the extracted lycopene from the digested or undigested products. A significant positive correlation was found between the a-value and extracted lycopene content following the digestion step ($R^2 = 0.86$, $p < 0.05$), while the correlation coefficient between the a-value and lycopene concentration was not significantly different when the extraction was carried out by only using solvents ($R^2 = 0.30$, $p > 0.05$). A similar linear correlation coefficient has been reported by Arias *et al.* (*2000*) between the a*-value and lycopene content of the tomatoes ($R^2 = 0.82$). Although these researchers also reported a high value for polynomial correlation coefficient ($R^2 > 0.90$) between the a*-value and lycopene content, this correlation was not significant for the present experiment.

Table 3-3 Effect of the extraction methods on the extracted lycopene content and the a- value obtained from extruded rice snacks containing 10% tomato skin powder (Feed rate, 6.5 kg/h; Die temperature, 140 °C) †

Sample	Feed moisture (%)	Screw speed (rpm)	Extracted lycopene content using solvent extraction (ppm, dwb)‡	Extracted lycopene content using digestion and solvent extraction (ppm, dwb)*	Redness or the a-value
A	11	250	17.9 ab	35.8 de	6.7 e
B	11	350	17.5 b	33.1 e	6.8 e
C	15	250	15.5 c	38.4 cd	9.4 c
D	15	350	15.6 c	35.3 e	8.5 d
E	19	250	14.8 c	42.2 ab	11.1 b
F	19	350	14.5 c	39.4 bc	11.2 b
G	23	250	15.3 c	44.1 a	12.8 a
H	23	350	19.5 a	39.9 bc	11.6 b

† Data are mean ± SD; n = 3. Different letters indicate significant ($p < 0.05$) differences within each column.

‡ Solvent extraction was carried out three times for 15 minutes each at 50°C and a solvent-to-meal ratio of 40:1.

* Digestion was carried out using 10 mg/ml pancreatin for 20 minutes incubation time.

3.3.1.5. Conclusion

The present study showed that the current technique of extracting lycopene with only solvent is not an efficient method for extruded products. By incorporating a digestion step with 25 mg/ml pancreatin for 20 minutes prior to solvent extraction, the proportion of the lycopene extracted from the extruded material reaches its maximum suggesting that it provides a better estimate for the true lycopene content present in the extruded product.

3.3.2. Optimization of *in-vitro* digestion procedure to simultaneously measure lycopene and starch bioavailability[2]

3.3.2.1. Abstract

To simultaneously measure the *in-vitro* bioavailability of lycopene and starch from the extruded snacks containing tomatoes, a digestion model was developed by using various concentrations of bile (0 to 20 mg/ml) and pancreatin (0, 0.4, 2.4, 4.8 and 6 mg/ml). *In-vitro* bioavailability of starch was determined based on the glucose released, and for lycopene, the amount transferred to the micellar phase of digesta. Furthermore, the digestion process was monitored by measuring the proportion of solids remaining at the end of digestion. The amount of glucose released from the starch after 120 minutes of digestion varied between 683 to 885 mg/g starch at different bile and pancreatin concentrations. The presence of bile reduced the amount of pancreatin needed to maximize starch digestibility. The proportion of micellar lycopene increased from 5 to 43% with increases in both bile and pancreatin concentrations. At constant pancreatin concentration, increases in bile concentration enhanced the *in-vitro* bioavailability of lycopene by up to 500%, while at constant bile concentration, a 150% increase was observed with the increases in pancreatin used. The proportion of solids remaining at the end of digestion varied from 14 to 70%. Increasing the bile and pancreatin concentration enhanced the digestion of the snack. In conclusion, the maximum amount of potentially bioavailable starch and lycopene in this study was

[2] Part of the material presented in this section has been previously published as a peer-reviewed journal article: Dehghan-Shoar, Z., Hardacre, A.K., Reynolds, G.W. (2010). Effect of bile and pancreatin concentration on the *in-vitro* bioavailability of lycopene and starch in extruded snacks containing tomato paste powder. *Food Digestion*, 1(1), 40-46.

obtained by using 4.8 mg/ml pancreatin and 10 mg/ml bile. Furthermore, the results suggest that bile and pancreatin collaborated in the digestion of both starch and lycopene present in the extruded products of tomatoes.

3.3.2.2. Introduction

Starch is gelatinized and cell walls ruptured during extrusion cooking, increasing the susceptibility of the nutrients to the digestive enzymes, and consequently their availability for absorption in the gut (*Camire et al., 1990; Gartner et al., 1997*). Conversely, researchers have shown that the presence of some dietary fibres, such as pectin in the tomatoes, inhibit the absorption of lycopene and starch from food materials (*Altan et al., 2009a; Riedl et al., 1999; Slaughter et al., 2002*). Thus, the bioavailability of lycopene and starch in extruded snacks containing tomatoes or the amount of the ingested nutrient that is absorbed and utilized by the cells in the body, should be determined if nutritional claims are to be made (*Hedran et al., 2002*); simply reporting the levels of these food components does not adequately represent the amount available for absorption.

Although measurements of bioavailability are carried out using clinical trials, these measurements are time consuming and costly. Model systems which simulate the clinical trials offer a cost and time efficient option and are used to predict the potential bioavailability of nutrients (*Woolnough et al.,, 2008; Wright et al., 2008*). Current *in-vitro* methods are generally used to determine one class of a nutrient at a time, while in the gut all nutrients, including starch and lycopene are digested and absorbed at approximately the same time (*Woolnough et al., 2008*). Thus, the simultaneous

measurement of starch and lycopene by a model system may provide a more accurate prediction of the true bioavailability of these nutrients.

In order to simultaneously measure the *in-vitro* bioavailability of starch and lycopene, the *in-vitro* methods conducted for each of these nutrients need to be combined. However, significant differences exist between these *in-vitro* methods because of the variations between the mechanisms involved in the digestion and absorption of each of these nutrients in the gut. For example, during digestion, the starch is broken down to simple sugars which are readily soluble in the aqueous environment of the digesta and are absorbed by the brush border cells (*Englyst & Englyst, 2005*). Whereas lycopene, a lipophilic molecule, needs to enter the mixed micellar phase of the digesta which is formed by the emulsifying action of bile, to reach the brush border cells (*Faulks & Southon, 2005*). Thus the incorporation of bile salts during the digestion of the food material when lycopene is to be analysed is a key difference between the *in-vitro* methods employed to measure starch and lycopene (*Englyst et al., 1999; Garrett et al., 1999; Garrett et al., 2000; Mishra et al., 2008*).

While it has been shown that bile salts improve the transfer of carotenoids such as β-carotene to the micellar phase of the digesta (*Hedran et al., 2002; Wright et al., 2008*), there is limited number of investigations showing the effect of bile on lycopene transfer to the micelles (*Garrett et al., 1999 & 2000*). Furthermore studies on the effect of bile salts on starch digestibility are scarce. It has been proposed that bile emulsifies the lipids trapped within the amylose helix and therefore increase the proportion of the active sites within the starch matrix for the activity of amylase (*Aura et al., 1999; Talanina et al.,, 1984; Woolnough et al., 2008*). There is a lack of knowledge on the effect of bile and pancreatin together on the *in-vitro* bioavailability of starch and lycopene.

Therefore, the aim of the present study was to investigate the effect of simultaneous variation of the bile and pancreatin concentrations on the *in-vitro* bioavailability of starch and lycopene present in an extruded product containing tomato paste powder. The proportion of solids remaining at the end of digestion was also recorded to monitor the digestion progress.

3.3.2.3. Materials and Methods

3.3.2.3.1. Ingredients

In this study, corn grits was substituted with tomato paste powder at a level of 20% (w/w). The preparation of tomato paste powder and corn grits specifications are described in section 3.2.1.

3.3.2.3.2. Methods

3.3.2.3.2.1. Extrusion

The extruder specifications are described in section 3.2.2.1. The temperature in the 7 barrel sections from feed to die were 80/80/80/80/140/140/140 °C. The feed rate of dry ingredients was 11.5 kg, screw speed 350 rpm and water feed rate 0.5 l/hr.

3.3.2.3.2.2. In-vitro *digestion model*

To decrease lycopene degradation, the digestion procedure were carried out in dim light and at a lowered oxygen concentration which was achieved by flushing the

reaction jars with nitrogen. The *in-vitro* bioavailability of lycopene was determined by measuring the amount of lycopene in oil droplets released from the food material into the aqueous phase of the digesta and subsequently incorporated into micelles (*Wright et al., 2008*), after *in-vitro* gastric and duodenal digestion. Starch digestibility was estimated from the rate of glucose release after 20, 60 and 120 minutes of *in-vitro* digestion simulating conditions in the duodenum. Preliminary trials showed that *in-vitro* digestion simulating oral conditions did not have a significant effect on the *in-vitro* bioavailability of lycopene or starch thus this phase was omitted from the experiments reported here. The *in-vitro* digestion model used in this work was based on the methods of Garrett *et al.* (*1999*) for lycopene *in-vitro* bioavailability and Englyst *et al.* (*1999*) and modified from Mishra *et al*. (*2008*) for starch.

Briefly, the snack was ground to pass through a sieve with a mesh size of 2 mm. Then, 4.0 grams of the ground extruded snack was weighed and transferred to 100 ml Duran screw top glass jars to which 15 ml water was added. The gastric phase of digestion was carried out by adjusting the pH to 2.5 using 1 M hydrochloric acid and adding porcine pepsin in 0.1 N hydrochloric acid to reach a final concentration of 3.3 mg/ml. This phase was carried out for 30 min at 37 °C in a shaking water bath (Shak-R-Bath, 3582-1, Barnstead Lab-line, Dubuque, IA, USA) operated at 75 rpm. The pH of samples from the gastric digestion phase was then increased to 5.2 with 0.5 M sodium acetate, and samples were further incubated for 15 minutes at 37 °C at 130 rpm shaking speed to simulate the mechanical disruption of the food matrix during gastric contractions. The duodenal digestion phase was then carried out by increasing the pH to 6.5 using 0.5 M sodium bicarbonate and adding pancreatin and bile extract in 0.1 M sodium bicarbonate buffer at concentrations according to the experimental design and subsequently 0.012

mg/ml of invertase and 0.008 mg/ml of amyloglucosidase was added. The total volume of digesta at the start of each analysis was set to 50 ml and the digestion was carried out for 120 min at 37 °C at a shaking rate of 75 rpm.

To investigate the effect of digestion on the degradation of lycopene, a recovery assay was carried out according to the method of Sadler *et al.* (*1990*).

To measure the starch digestibility, 1.0 ml aliquots were removed from the digestion mix just before the addition of enzymes for the simulated duodenal digestion phase and after 20, 60 and 120 minutes from the start duodenal digestion phase. The samples were added to 4 ml of absolute ethanol in a screw top test tube and immediately vortex mixed to stop enzyme activity. The samples were retained for analysis of sugars accumulating from the digestion of the starch.

3.3.2.3.2.3. Determination of the amount of lycopene transferred to the micelles

Lycopene content of the extruded snack was determined using the method described in Section 3.3.1. The lycopene present in the micelles was considered as the potentially bioavailable lycopene after the *in-vitro* digestion. Micellar lycopene present in the digesta was separated from crystallized forms and undigested material according to the method of Thakkar *et al.* (*2007*). Lycopene was extracted using a mixture of petroleum ether: acetone (3:1 w/w) containing 0.1% (w/v) butylated hydroxytouluene (BHT). Total lycopene content was determined spectrophotometrically at 472 nm using a Helios Epsilon Spectrophotometer (Thermo Electron Corporation, Pittsford, New York, USA) (*Schieber et al., 2001; Sharma & Le Maguer, 1996*).

A set of standards of different concentrations were prepared from pure lycopene in petroleum ether and a calibration curve for lycopene concentration was obtained using the relationship between concentration and absorbance.

3.3.2.3.2.4. Determination of starch digestibility

Glucose release due to the digestion of starch was measured using the dinitrosalicylic acid (DNS) colorimetric method of Mishra *et al*. (*2008*). Glucose content (mg) was calculated based on the proportion of the starch present in the snacks.

3.3.2.3.2.5. Determination of the percentage of the undigested solids

The suspension remaining at the end of digestion was centrifuged at 5000 rpm for 20 minutes to separate the aqueous and solid phases. The solids were dried overnight at 105 °C to constant weight and weighed. The percentage of undigested solids was determined by the weight of solids remaining after digestion divided by the initial weight (dwb) of the sample.

3.3.2.3.2.6. Experimental design and statistical analysis

The concentrations of bile and pancreatin used for the present study were based on previous reports considered as physiologically relevant in the human digestive system *(Baysal et al., 2000; Wright et al., 2008*).

The study consisted of two phases; in the first phase, a full factorial design was used consisting of 6 bile concentrations (0, 3, 5, 10, 15 and 20 mg/ml) in the absence of and

at pancreatin concentration of 2.4 mg/ml on the dependant variables, i.e. starch and lycopene *in-vitro* bioavailability and percentage of undigested solids. In the second phase, a full factorial design consisting of 3 bile concentrations (0, 3 and 10 mg/ml) × 4 pancreatin concentrations (0.4, 2.4, 4.8 and 6 mg/ml) was employed on the same dependant variables.

At least three replicate samples were digested for each treatment. One treatment of three replicates was also digested on all the experimental days to monitor the day-to-day variations. Day-to-day variation for *in-vitro* bioavailability of lycopene and percentage of undigested solids were within 5% of the overall mean and for starch digestibility within 8% of the overall mean. A completely randomized design was used to evaluate the results by one-way analysis of variance (ANOVA). For comparison of the mean values, the Duncan multiple range test was used. Differences were described as differing statistically if the probability of F value was less than 0.5%. All statistics were calculated using SAS software version 9.1 (SAS Inc., Chicago, IL, USA).

3.3.2.4. Results

3.3.2.4.1. In-vitro *bioavailability of starch*

The incorporation of bile significantly enhanced the amount of the glucose released due to the digestion of starch from the snacks when a 2.4 mg/ml pancreatin concentration was used (Figure 3.7). In the absence of bile, the amount of the glucose released was lowest (826 mg/g available starch) but it increased to more than 882 mg/g of the available starch with increases in the bile concentration to 5 mg/ml (Figure 3.7). Further increases in the bile concentration did not change starch digestibility ($p > 0.05$).

In the absence of pancreatin, a similar amount of glucose was obtained at all the bile concentrations used (Figure 3.7).

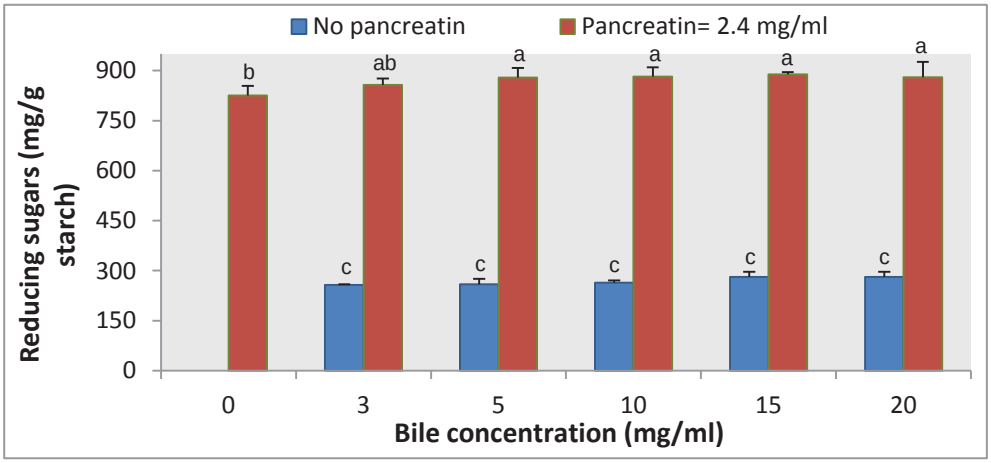

Figure 3-7 The effect of bile concentration in the absence of and at pancreatin concentration of 2.4 mg/ml on glucose released due to the digestion of starch following 120 minutes of simulated digestion. Error bars represent standard deviations from the means. Different letters above bars (a-c) indicate significant differences ($p < 0.05$) between samples, n = 3

At lower pancreatin concentrations, the addition of bile intensified the hydrolysing effect of pancreatic amylase on the starch especially after 120 minutes of digestion (Table 3.4). For example as shown in Table 3.4, at 0.4 mg/ml of pancreatin, increases in the bile concentration from 0 to 10 mg/ml, increased the amount of glucose released from the snack by more than 25% after 120 minutes of digestion, while at a pancreatin concentration of 4.8 mg/ml, maximum starch digestibility after 120 minutes was obtained by adding 3 mg/ml of bile. Increasing the bile concentration to 10 mg/ml did not increase the amount of the glucose released (Table 3.4). Although by using 6 mg/ml pancreatin, the amount of the released glucose after 20 minutes was increased with bile concentration, after 120 minutes of digestion the maximum amount of glucose released were similar in all the treatments with different bile concentrations.

In the absence of pancreatin, the amount of glucose obtained from all treatments with different bile concentrations were statistically similar (Figure 3.7), suggesting that bile alone did not possess amylolytic activity on the starch. However, when combined with pancreatin, bile enhanced the digestibility of the starch significantly. The possibility of a promoting effect of bile on the activity of pancreatic amylase has also been suggested previously by researchers (*Aura et al., 1999; Talanina et al., 1984; Woolnough et al., 2008*).

Table 3-4 The Effect of pancreatin and bile concentration on glucose released due to the digestion of starch following 20, 60 and 120 minutes of simulated digestion

Pancreatin concentration	Bile concentration (mg/ml)	Released glucose (mg/g starch)		
		20 min	60 min	120 min
0.4	0	494 ^{c}	575 ^{b}	683 ^{c}
0.4	3	522 ^{bc}	628 ^{ab}	815 ^{b}
0.4	10	573 ^{abc}	726 ^{ab}	862 ^{a}
2.4	0	561 ^{abc}	608 ^{b}	826 ^{b}
2.4	3	511 ^{bc}	688 ^{ab}	857 ^{ab}
2.4	10	601 ^{ab}	709 ^{ab}	867 ^{a}
4.8	0	579 ^{abc}	613 ^{b}	828 ^{b}
4.8	3	539 ^{abc}	725 ^{ab}	874 ^{a}
4.8	10	611 ^{ab}	771 ^{a}	873 ^{a}
6.0	0	602 ^{ab}	672 ^{ab}	850 ^{a}
6.0	3	569 ^{abc}	639 ^{ab}	885 ^{a}
6.0	10	635 ^{a}	713 ^{ab}	881 ^{a}

Values with different letters (a-c) show significant differences ($p < 0.05$) within each column at individual sampling times, n=3

On the other hand, increases in the pancreatin concentration alone, also enhanced the starch digestibility. The presence of slowly digestible lipid-amylose complexes formed

during the extrusion cooking process may be the reason for this (*Altan et al., 2009a; Tester et al., 2006*). In these conditions, higher concentrations of the amylolytic enzymes and longer incubation times are required to digest the starch (*Strange & Schaich, 2000*), presumably due to the poor accessibility of the pancreatic amylase to the carbohydrate.

3.3.2.4.2. In-vitro *bioavailability of lycopene*

Lycopene recovery after digestion averaged, 91.25 ± 1.46 %, thus the extraction and measurement procedure recovers an acceptable proportion of the lycopene present.

At a pancreatin concentration of 2.4 mg/ml, the addition of bile significantly enhanced the *in-vitro* bioavailability of lycopene (Figure 3.8). In the absence of bile, 5% of lycopene was transferred to the micellar phase of the digesta at this pancreatin concentration, while it was more than 600% higher with increases in the bile concentration to 20 mg/ml (Figure 3.8). In the absence of pancreatin, a slight increase in the transfer of lycopene to the micellar phase of the digesta was observed with increases in bile from 3 to 20 mg/ml.

Simultaneous increases in the bile and pancreatin concentration significantly improved the transfer of lycopene to the micellar phase of the digesta (Figure 3.9). The increases in bile concentration from zero to 10mg/ml, increased the *in-vitro* bioavailability of lycopene by close to 500% at a pancreatin concentration of 4.8 mg/ml, compared to less than 200% increase for all levels of the added pancreatin at constant bile concentration of 10 mg/ml.

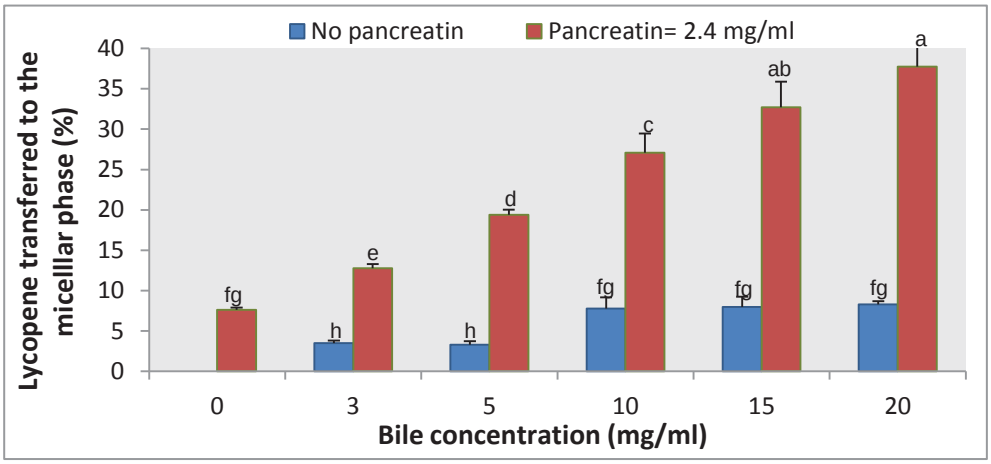

Figure 3-8 The effect of bile concentration in the absence of and at pancreatin concentration of 2.4 mg/ml on* in-vitro *bioavailability of lycopene following 120 minutes of simulated digestion. Error bars represent standard deviations from the means. Different letters above bars (a-g) indicate significant differences ($p < 0.05$) between samples, n = 3

A maximum of 43% of the initial lycopene content from the snack was transferred to the micelles at pancreatin concentration of 4.8 mg/ml and 10 mg/ml of bile (Figure 3.9). At this bile concentration (10 mg/ml), increases in the pancreatin concentration from 4.8 to 6 mg/ml did not further increase the transfer.

The incorporation of bile was necessary to obtain maximum amount of *in-vitro* bioavailable lycopene from the snack. While this corresponds with previous reports (*Garret et al., 1999 & 2000*), the proportion of lycopene that was transferred to the micellar phase of the digesta in this study was generally higher than the values reported earlier. For example, the amount of lycopene present in the micelles was close to 9% at a bile concentration of 3 mg/ml and a pancreatin concentration of 0.4 mg/ml (Figure 3.9). In the report of Garret *et al.* (*1999 & 2000*), 3–5% of lycopene was transferred to the micelles at similar bile and pancreatin concentrations. Also, in the absence of bile, Garret *et al.* (*1999 & 2000*) reported that no lycopene was present in the micelles while

the present study showed that 5% of lycopene was transferred to the micelles under similar conditions (Figure 3.9). Although the type of pancreatin enzyme used by Garret *et al.* (*1999 & 2000*) was not reported, it is possible that the effect of shear and temperature associated with the extrusion processing released the cell components including lycopene therefore improving the transfer of lycopene from the matrix of the extruded product to the digesta. The positive effect of mechanical and thermal processing of food on lycopene bioavailability has been reported previously by researchers (*Gartner et al., 1997; Kean et al., 2008*)

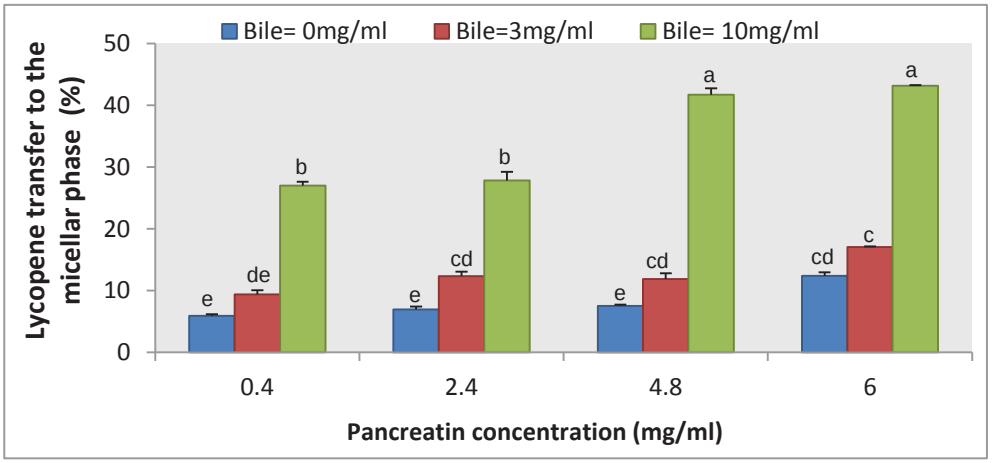

Figure 3-9 The effect of pancreatin and bile concentrations on the* in-vitro *bioavailability of lycopene following 120 minutes of simulated digestion. Error bars represent the standard deviations from the means. Different letters above bars (a-e) indicate significant differences ($p < 0.05$) between samples, n=3

The positive effect of pancreatin on the release of carotenoids, including β-carotene (*Wright et al. 2008*) and lycopene (*Garret et al. 1999*), has been reported previously. It has been suggested by these authors that higher pancreatin concentrations raise the amount of the products of pancreatic lipase action which results in the swelling of the

micelles. Consequently, a higher amount of the lipophilic molecule is transferred to the micelles resulting in its higher availability to the intestinal cells.

3.3.2.4.3. *Percentage of the undigested solids*

The proportion of undigested solids decreased from 26 to 16% as the bile concentration was increased from 0 to 10 mg/ml at a constant pancreatin concentration of 2.4 mg/ml (Figure 3.10). Further increases in bile had no significant effect ($p > 0.05$) on the rate of digestion. In the absence of pancreatin, the lowest proportion of solids was digested.

With simultaneous increases in bile concentration from 0 to 10mg/ml and pancreatin concentration from 0.4 to 6 mg/ml, the proportion of solids remaining at the end of digestion decreased from 32 to 14% (Figure 3.11). The maximum rate of digestion was reached at 4.8 mg/ml of pancreatin and 10 mg/ml of bile (Figure 3.11). The addition of bile increased the efficiency of digestion and less material remained at the end of digestion. For example, at a pancreatin concentration of 4.8 mg/ml, increases in bile concentration from 0 to 10 mg/ml decreased the amount of undigested solids from 25% to 14%.

In comparison with the digestion model system employed by Mishra *et al.* (*2008*), higher pancreatin concentrations were needed to maximize the digestion of the sample in this study. At the end of digestion 14% of the original material remained undigested of which 5% consisted of indigestible dietary fibre. This suggests that the 9% of the sample remaining undigested possibly due to the presence of amylose-lipid complexes that were resistant to digestion.

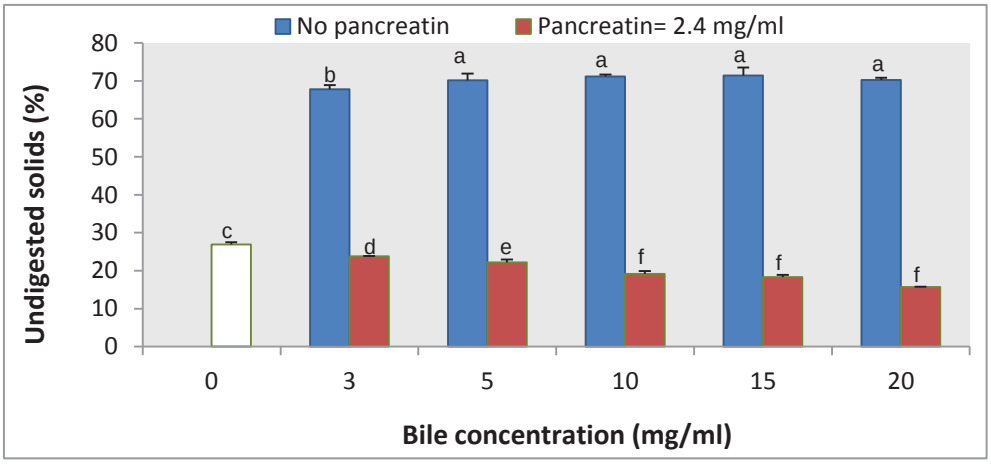

Figure 3-10 The effect of bile concentrations in the absence of and at pancreatin concentration of 2.4 mg/ml on the percentage of undigested solids following 120 minutes of simulated digestion. Error bars represent the standard deviations from the means. Different letters above bars (a-f) indicate significant differences ($p < 0.05$) between samples, n=3

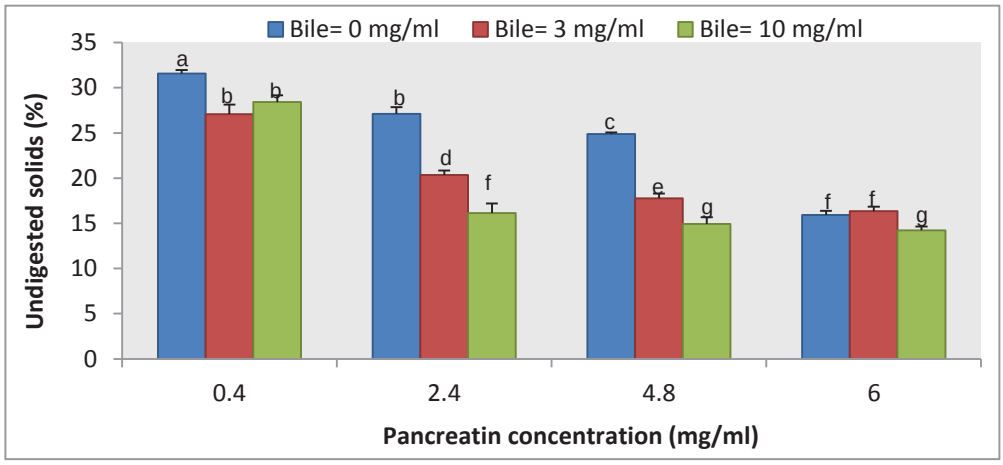

Figure 3-11 The effect of pancreatin and bile extract concentrations on the percentage of undigested solids following 120 minutes of simulated digestion. Error bars represent the standard deviations from the means. Different letters above bars (a-g) indicate significant differences ($p < 0.05$) between samples, n=3

3.3.2.5. Conclusion

This study shows that bile and pancreatin cooperatively affect the *in-vitro* bioavailability of starch and lycopene. The presence of bile is crucial for the transfer of lycopene to the micelles at any pancreatin concentration, while the digestion of the starch was enhanced by bile at pancreatin concentrations of below 4.8 mg/ml but not at higher concentrations. Although the true bioavailability of nutrients can be determined using *in-vivo* trials, it is not possible to manipulate the concentration of bile and pancreatin in those trials. This work provides new information on the synergistic effect of bile and pancreatin on the coincidental digestion of starch and lycopene and may assist in the better understanding of the digestion of lycopene and starch in the gut.

Chapter Four Effect of Raw ingredients on the Physicochemical Characteristics of Extruded Corn-tomato Products

4.1. Effect of starch and tomato source on physicochemical characteristics of extruded products[3]

4.1.1. Abstract

The ingredients used alter the physicochemical properties of extruded snacks but their effect on lycopene content is not known. In this study, crisp low density extruded snacks were manufactured from corn, wheat and rice, with or without 20% dried tomato skin or paste powder extruded at 140, 160 and 180 °C. Lycopene content and the physicochemical properties (expansion, density, hardness, colour parameters and percentage moisture lost during processing) of the extruded products were measured. Extruded products containing tomato skin and tomato paste were generally less expanded and thus denser. More lycopene could be extracted from products containing tomato skin powder and significantly less when wheat flour was used to make the snacks.

4.1.2. Introduction

Consumer acceptability is essential to validate the production of a novel food product. Consumer perception depends on the physical and organoleptic properties of the food. In extruded products, physical characteristics, such as expansion and hardness, mainly depend on the proportion and type of the available starch. Incorporation of tomato

[3] Part of the material presented in this section has been previously published as a peer-reviewed journal article: Dehghan-Shoar, Z., Hardacre, A.K., Brennan, C.S. (2010). The physicochemical characteristics of extruded snacks enriched with tomato lycopene. *Food Chemistry*, 123(4): p. 1117-1122.

derivatives into extruded snacks changes the chemical composition of the melt by reducing the starch content and adding fibre, sugar and other polysaccharides. The presence of components other than starch has a lubricating effect on the melt, which interferes with the air bubble formation as the melt expands on leaving the extruder and may limit the gelatinisation of starch required for expansion of the snacks. As a result, the snacks will be less expanded and harder in texture compared with snacks containing cereal products only (*Ainsworth et al., 2007; Brennan et al., 2008a; Camire, 1998; Yanniotis et al., 2007*). Hard and dense products are not accepted by consumers.

To compensate for the loss in the organoleptic properties, severe extrusion processing at high temperature and shear is needed to decrease the melt viscosity (*Altan et al., 2008a & b; Huang et al., 2006; Yagci & Gogus, 2008*). However, lycopene in the tomato is heat-labile and degrades rapidly at temperatures greater than 100°C (*Lee & Chen, 2002*), and consequently a greater proportion of lycopene may be lost as the severity of the extrusion process is increased.

Raw ingredients such as rice, wheat and corn produce extruded snacks with different physicochemical characteristics. This is related to the variations in the chemical composition of the grain. For example, wheat has a higher protein and lower starch content compared to rice and corn, therefore extruded wheat products are harder and less expanded (*Camire, 1998; Guy, 2001; Riaz, 2006*). It may be possible to improve the physicochemical properties and the lycopene content of the snacks by manipulating the base ingredients.

The aim of this study was to investigate the fate of lycopene and the physicochemical properties of the extruded snacks manufactured from three different base ingredients namely, rice, corn and wheat, as the source of starch and freeze dried tomato paste or

skin powder, as the lycopene source. Nine formulations were extruded at processing temperatures of 140, 160 or 180 °C and the lycopene content, expansion, product density, hardness, percentage of moisture loss during processing and colour parameters of the snacks were determined.

4.1.3. Methods and material

4.1.3.1. Ingredients

The ingredients were prepared as described in Section 3.2.1.

4.1.3.2. Extrusion cooking parameters

The extruder specifications are described in Section 3.2.1. The temperature was controlled at 80 °C in the first 4 barrel sections for all experiments while three different temperatures of 140, 160 and 180 °C were used in the final 3 barrel sections. The feed rate of dry ingredients was kept constant at 11.5 kg/h, screw speed was 350 rpm and water feed rate 0.5 l/h.

4.1.3.3. Physicochemical properties of products

4.1.3.3.1. Physical properties

The extrusion parameters including expansion ratio, density and colour evaluation were determined as the description in Section 3.2.2.

Hardness**:** The hardness of samples was measured using Stable Microsystems TA-HD Texture Analyser (Texture Technologies Corp., Scarsdale, New York, USA) fitted with a 250N load cell. Samples were prepared according to the method of Hardacre *et al.* (*2006*) and gently packed into Kramer shear cell to 80% of the cell height (about 200-300 g). The Kramer probe (5 blades, 3mm thick, 64 mm high, 82mm wide, 11 mm apart) was set to move at a test speed of 0.5 mm/s for a distance of 50 mm representing about 30% of the sample height in the cell. The maximum force needed to break the samples was recorded and analysed by the Texture Exponent software associated with the texture analyser. Measurements are reported as an average of 3 to 4 replicates.

4.1.3.3.2. Chemical composition

Moisture, ash, crude fat and protein, total dietary fibre and starch content were determined as described in Section 3.2.2.

4.1.3.3.3. Lycopene content

Lycopene was extracted from extruded products using the methods of Kaur *et al.* (*2008*) and Sadler *et al.* (*1990*). Briefly, two grams of sample were weighed into brown glass bottles to exclude light. HPLC grade solvents (hexane: acetone: ethanol 2:1:1) containing 0.05% (w/v) butylated hydroxytoluene (BHT) were used to extract lycopene with a solvent-to-meal ratio of 40:1. Diatomaceous earth was added to improve the extraction yield by increasing the amount of mixing. The suspension was stirred for 15 minutes at 50 °C. This procedure was repeated 3 times and the non-polar solvent layers containing lycopene were pooled. The pooled solvent mixture was washed with 40 ml of

cold, distilled water twice. Then the absorbance of the collected solvent mixture was measured at 472 nm using a spectrophotometer (Helios Epsilon Spectrophotometer, Thermo Electron Corporation, Pittsford, New York, USA).

Standards were made using pure lycopene purchased from the Sigma Chemical Company (Sydney, New South Whales, Australia) and used for the preparation of a calibration curve of concentration against absorbance. Lycopene concentration was calculated from this curve from the measured absorbance. All measurements are reported as the mean of duplicate analyses.

4.1.3.4. Experimental design and statistical analysis

The experimental design was a full factorial comprising of three temperature treatments (140, 160 and 180 °C) × 3 starch sources (corn, rice and wheat) × 3 lycopene treatments (tomato paste, tomato skin, no tomato derivative). Extruded samples were prepared from 1.5 kg of raw ingredients and the lycopene content, percentage of moisture loss, colour parameters, expansion ratio, density and hardness of products were measured. Between 2 and 4 replicates of each measurement were made.

A completely randomized design was used to evaluate the results and Analysis of Variance (ANOVA) was carried out to compare the mean values. All significant differences are reported at $p < 0.05$ level. The correlation among dependent variables was determined by the Pearson's correlation coefficient (R^2) using SAS software. The degree of correlation (R^2) was interpreted by the following: ($|R^2| < 0.20$, negligible; $|r| = 0.20–0.40$, low; $|R^2| = 0.40–0.60$, moderate; $|R^2| = 0.60–0.80$, marked; and $|R^2| > 0.80$,

high). All statistics were calculated using SAS software version 9.1 (SAS Inc., Chicago, IL, USA).

4.1.4. Results and Discussion

4.1.4.1. Proximate composition

The chemical composition of the ingredients is shown in Table 4.1. Wheat had approximately 10% less starch compared to rice and corn while the protein content of wheat semolina was about 40% greater than that of rice and corn. The fat content for rice flour was about 6% of that of corn grits or wheat semolina. Ash in rice flour was 50% less than corn or wheat. Dietary fibre content of the ingredient mix increased to up to 17% with tomato skin addition.

A small amount of carotenoids were extracted from corn grits but the amount was considered insignificant compared to that coming from the tomato derivatives (Table 4.1). The lycopene concentration of the raw ingredients containing tomato paste powder was more than 30 times greater than the raw ingredients containing tomato skin.

Table 4-1 Proximate composition of the ingredients (dwb)†

Raw material	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Starch (%)	Fibre (%)	Lycopene (ppm)
Corn grits	11.8	2.3	6.7	0.6	78	1.1	< 1
Corn + 20% tomato paste	11.4	2.1	7.0	0.9	64	4.8	133
Corn + 20% tomato skin	11.4	2.5	8.2	1.8	64	16.6	4
Rice flour	12.1	0.1	6.8	0.3	80	0.8	< 1
Rice + 20% tomato paste	11.7	0.4	7.0	0.6	65	4.6	133
Rice + 20% tomato skin	11.7	0.7	8.2	1.6	65	16.3	4
Wheat semolina	12.5	2.2	10.7	0.6	70	2.5	< 1
Wheat + 20% tomato paste	11.9	2.0	10.2	0.9	57	5.9	133
Wheat + 20% tomato skin	11.9	2.3	11.4	1.8	57	17.7	4

† Calculated from the proximate composition of the raw ingredients and are given as percentages

4.1.4.2. Extrusion parameters

Die temperature, thrust pressure, energy consumption, torque and SME are shown in Table 4.2. The variations in the extruder parameters were mostly due to the differences in the chemical composition i.e. fat, fibre and sugar content, of ingredients (*Camire, 1998; Riaz, 2006*). For example, incorporation of tomato derivatives reduced energy consumption, torque and SME values significantly (Table 4.2), presumably due to the lubricating effect of sugar and fibre from the tomato paste and skin.

Also, rice flour contained the least amount of fat so frictional torque was higher in products containing rice flour (Table 4.2).

4.1.4.3. Physical characteristics

4.1.4.3.1. Expansion ratio

Expansion is an important physical attribute for the extruded snacks that greatly affects consumer acceptability. Expansion of products ranged between 186 – 360%. As expected, incorporation of tomato derivatives reduced the expansion values of up to 25% compared to the controls (Table 4.3).

Expansion was positively correlated with SME ($r = 0.70$) and because the incorporation of tomato derivatives lubricated the melt and therefore SME and torque is reduced, the expansion also decreased (Table 4.2 and 4.3).

Overall, rice flour produced the most expanded products due to the higher starch and lower fibre and fat content compared to corn grits and wheat semolina (Table 4.1 and Figure 4.1).

Table 4-2 Extrusion parameters for products manufactured from rice flour, corn grit or wheat semolina with or without the addition of tomato paste or tomato skin powder (at the 20% level) extruded at a feed rate of 11.5 kg/h, screw speed of 350 rpm and water feed rate of 0.5 l/h

Die Temperature (°C)	Starch Source	Lycopene Source	Thrust Pressure (bar)	Energy Consumption (kW)	Torque (N.m)	SME (kJ/kg)
110	Corn	paste	112	1.15	3.6	106
		Skin	135	1.3	4.8	111
		None	150	1.40	5.6	170
	Rice	Paste	49	1.32	4.8	146
		Skin	49	1.40	4.9	149
		None	78	2.30	7.8	237
	Wheat	Paste	80	1.04	3.7	112
		Skin	149	1.4	5.3	161
		None	205	1.98	6.80	207
125	Corn	Paste	55	1.1	3.7	112
		Skin	145	1.41	5.1	155
		None	170	1.98	6.5	197
	Rice	Paste	49	1.5	5.3	161
		Skin	140	1.5	5.5	167
		None	170	1.81	8.9	270
	Wheat	Paste	180	1.65	5.2	158
		Skin	170	1.95	5.6	170
		None	190	2.5	7.9	270
140	Corn	Paste	26	0.97	3.1	94
		skin	27	1.07	3.7	112
		None	55	1.34	5.0	176
	Rice	Paste	43	0.85	3.5	106
		Skin	45	0.95	5.0	152
		None	78	1.27	5.9	179
	Wheat	Paste	49	1.07	3.9	118
		Skin	120	1.35	4.6	140
		None	140	1.47	5.5	167

Although in the present experiment the effect of temperature on expansion was not significant, Altan *et al.* (*2008a*) reported a reduction of expansion with increase in

temperature from 140 to 166.8 ˚C in extruded products made from barley and tomato pomace. Moraru and Kokini (*2003*) have proposed that the expansion of extruded products is enhanced by increases in temperature to an optimum point and above this temperature expansion is reduced. This optimum temperature depends on the ingredients used. It may be possible that temperature of 160 ˚C for the products made in the present study was this optimum point and the expansion decreased with increase in temperature to 180 ˚C which resulted in the overall insignificant effect of temperature on expansion.

4.1.4.3.2. True density

The true density was used to compare the volume occupied by voids in the extruded products; which could be done as the matrix density was similar for all extrusions. The density of the extruded products varied between 172 and 751 kg/m^3. The true density of the extruded products to which 20% of tomato paste was added, was about twice that of extruded products containing skin or made from base flours only (Table 4.3), due to the presence of sugars and soluble fibre in tomato paste that absorb moisture. The positive correlation between moisture content and density (R^2 = 0.70) further support this.

Table 4-3 Significant main effects of treatments (lycopene source, starch source and temperature) on dependant variables (mean values reported)

Main effect of variables	SME (kJ/kg)	Expansion (%)	Density (kg/m^3)	Hardness (N)	a-value	b-value	L-value	Moisture loss (%)	Lycopene content (ppm)
Effect of lycopene source									
Tomato paste	132.67 ^{b}	242 ^{b}	456 ^{a}	139 ^{ab}	9.42 ^{a}	3.10 ^{a}	89.68 ^{b}	10.7 ^{c}	3.7 ^{a}
Tomato skin	136.45 ^{b}	243 ^{b}	241 ^{b}	224 ^{a}	6.54 ^{b}	0.78 ^{ab}	92.57 ^{b}	32.7 ^{a}	0.5 ^{b}
Control	208.29 ^{a}	330 ^{a}	250 ^{b}	94 ^{b}	5.60 ^{b}	-3.17 ^{b}	96.74 ^{a}	21.2 ^{b}	0.0 ^{c}
Effect of starch source									
Rice flour	175.08 ^{a}	295 ^{a}	*ns*	104 ^{b}	*ns*	*ns*	*ns*	26.3 ^{a}	3.8 ^{a}
Corn grits	134.92 ^{b}	204 ^{b}		148 ^{ab}				17.8 ^{b}	3.6 ^{a}
Wheat semolina	167.01 ^{a}	222 ^{b}		174 ^{a}				20.5 ^{ab}	2.5 ^{b}
Effect of temperature (°C)									
140	154.67 ^{b}	*ns*	419 ^{a}	162 ^{a}	8.88 ^{a}	9.64 ^{a}	82.13 ^{b}	*ns*	*ns*
160	196.98 ^{a}		292 ^{b}	95 ^{b}	4.51 ^{b}	-1.35 ^{b}	95.38 ^{a}		
180	138.24 ^{c}		314 ^{ab}	146 ^{ab}	7.88 ^{a}	1.01 ^{b}	91.55 ^{a}		

^{ns} Not significant ($p > 0.05$)

Increasing the processing temperature from 140 °C to 160 °C decreased true density which is consistent with the available literature (*Altan et al., 2008a; Ilo & Berghofer, 1999*), although the mean density value of products at 160 and 180 °C were similar (Table 4.3).

4.1.4.3.3. Hardness

Hardness was obtained from the maximum force required to fracture the products by the Kramer shear cell. Hardness correlates with the bite hardness that could be expected from eating the product. Hardness varied between 29 and 322 N (Data not shown). Products made with the flours alone were much less hard than those containing tomato skin (Table 4.3). Fibre interferes with air bubble formation and increases the bubble wall thickness (*Ainsworth et al., 2007; Altan et al., 2008b*). During extrusion, it was noticed that the foam structure of products containing tomato skin had more small air bubble and was finer compared to the other products. Substituting starch with tomato skin powder also reduced the proportion of materials capable of forming a melt in the extruder and this would be expected to reduce the foam volume and increase hardness.

Extruded products derived from wheat were generally harder than those made from rice flour and corn grits although, for the latter, the difference was not significant (Table 4.3). This was probably as a result of higher levels of protein in wheat (*Guy, 2001*).

Increasing the temperature from 140 to 160 ˚C almost halved the hardness of the extruded products, although the hardness increased when temperature was raised from 160 to 180 ˚C (Table 4.3). This finding correlated well with product density values (r= 0.59) showing that as expected denser products are harder (*Altan et al., 2008b*). The

tendency of hardness to increase at higher temperature (180 °C) may be due to the higher melt temperature causing changes in the chemistry of the melt.

4.1.4.3.4. Colour parameters

Colour parameters changed with the enrichment with tomato derivatives. The a-value correlates with the lycopene content (*Altan et al., 2008a*) and was greater for products containing tomato paste (Table 4.3). On the other hand products with higher a-values were darker and had lower L-values (R^2 = -0.86) which is similar to previous reports (*Altan et al., 2008a; Ilo & Berghofer, 1999*).

Generally, increasing the temperature from 140 to 160 ˚C decreased the a-value (redness) and b-value (yellowness) suggesting that the degradation of pigments was accelerated at high temperatures (*Altan et al., 2008a; Ilo and Berghofer, 1999*), however this decrease did not continue with a further increase in temperature. Generally with increases in temperature, the products had higher L-values and looked brighter (Table 4.3); this has been attributed to the rise in the number of air bubbles (*Altan et al., 2008a*). However, the L-value at 160 and 180 °C was not significantly different from that at 140 °C.

4.1.4.4. Chemical characteristics

4.1.4.4.1. Moisture loss

Moisture content of the products during extrusion varied between 5.63 to 12.33% across all treatments. The greatest loss of water occurred in the extruded products made with tomato skin (Figure 4.1), which may have been associated with the weaker

water holding capacity of insoluble fibre present in the tomato skin compared to starch and other polysaccharides (*Brennan et al., 2008a; Yanniotis et al., 2007*). Among the starch sources, the greatest moisture loss was achieved in products made with rice flour which were also the most expanded (R^2 = -0.70). The least water loss occurred for the products containing tomato paste powder and therefore containing high levels of soluble polysaccharides with a high water holding capacity.

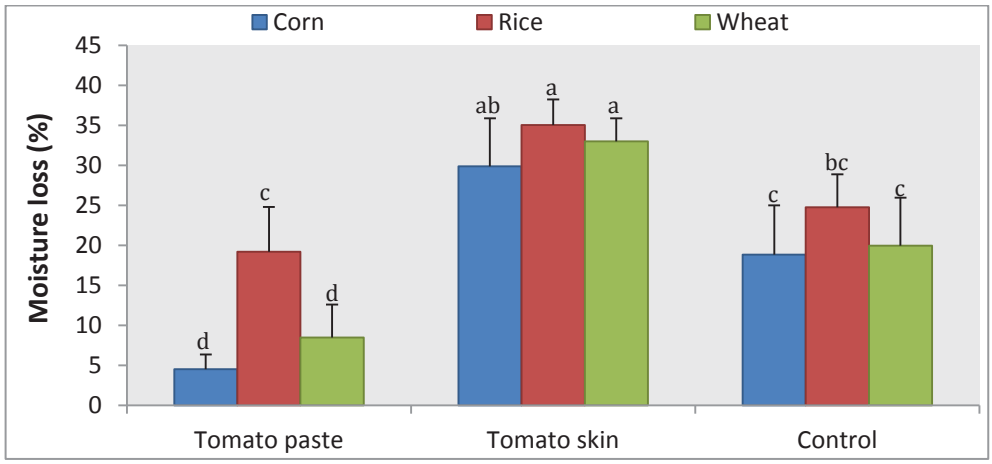

Figure 4-1 Effect of starch and lycopene source on percentage of moisture loss from the extruded products. Error bars represent standard deviations from the means. Different letters above bars (a-d) indicate significant (p < 0.05) differences between samples, n = 3

4.1.4.4.2. *Lycopene content*

The lycopene content of the extruded products ranged between 2.2-4.6 ppm for products containing tomato paste and 0.3-1.5 ppm for products containing tomato skin (data not shown). Although lycopene retention in products containing tomato skin was much higher than for products containing tomato paste, the mean value for lycopene content in products containing tomato skin was about 15% that of products containing

tomato paste (Table 4.3) due to the much higher initial concentration of lycopene in the tomato paste powder (Table 4.1).

The degradation of lycopene was greatest for extruded products containing wheat (Figure 4.2 and Table 4.3). Athar *et al*. (*2006*) have suggested that the chemical composition of the ingredients affects the degradation of heat-labile compounds. As shown in Table 4.1, wheat had a lower starch and higher protein content compared to rice and corn, therefore the starch content may have provided some protection for lycopene.

Although the information on lycopene retention during extrusion cooking is scarce, considerable work has been carried out on the retention of pigments such as β-carotene (*Berset, 1989*) and anthocyanin pigments during extrusion (*Camire et al., 2002 & 2007; Chaovanalikit et al., 2003*). The retention values reported by these authors ranged from 10% to 75%, depending on the pigment type and the food matrix. Compared to those reports, in the present study, the proportion of lycopene lost during extrusion cooking for products containing tomato paste was greater. This may be due to the differences in the stability of lycopene in tomato paste and the extreme processing conditions used.

The temperature range used in this study did not have a significant effect on lycopene content which has also been reported previously for other heat labile components (*Athar et al., 2006*). These authors have suggested a few reasons for the insignificant effect of temperature such as the initial loss of labile forms of the pigment and maintenance of more stable forms of components and also the effect of shear on degradation. The latter would be more logical in the present study, since a negative correlation was observed between the SME and lycopene content (R^2 = -0.63).

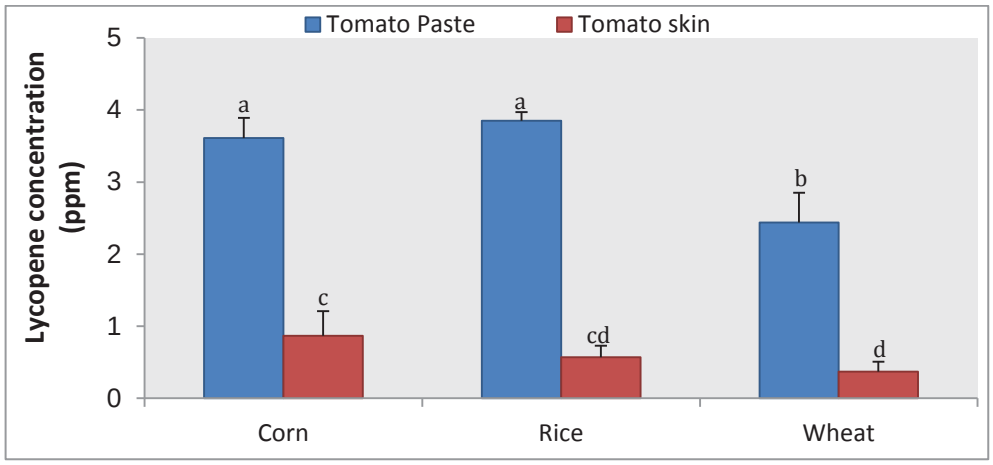

Figure 4-2 Effect of starch and lycopene source on lycopene content of extruded products. Error bars represent standard deviations from the means. Different letters above bars (a-d) indicate significant ($p < 0.05$) differences between samples, $n = 3$

4.1.5. Conclusion

Enrichment of extruded snacks with tomato derivatives enhances their nutritional attributes by adding lycopene and fibre. This work has shown that lycopene can be retained in the extruded snacks. Although the amount of lycopene retained after extrusion was very low, variation in the chemical composition of the base ingredients, especially increases in the starch content, improved the retention values. Furthermore, if a product such as tomato skin that is resistant to shear induced breakdown is used as a source of lycopene, proportionally higher levels can be retained in the final extruded products.

4.2. Effect of various ratios of tomato skin and tomato paste on physicochemical characteristics of corn-based extruded products[4]

4.2.1. Abstract

Blends of tomato skin and/or tomato paste powder at varying ratios (%, w/w) of skin to paste of 0:100, 25:75, 50:50, 75:25 or 100:0 were prepared and used to replace corn grits at 5, 10 or 20% replacement level. The physical properties of the extruded pellets including expansion, density, hardness and colour were measured. In addition, the lycopene content and moisture loss were determined. A sensory trial was also carried out on three snack types with better expected organoleptic characteristics. Adding tomato derivatives to the ingredients reduced the radial expansion ratio but increased product density and hardness ($p < 0.05$). The specific mechanical energy used to produce the snacks was negatively correlated with the lycopene content ($r = -0.89$, $p < 0.01$). Sensory assay showed that the overall acceptability score for all of the extruded corn-tomato products were similar and significantly higher than the control corn snacks.

[4] Part of the material presented in this section has been presented in NZIFST Annual Conference: Dehghan-Shoar, Z., Hardacre, A.K., Reynolds, G.W., Brennan, C.S. (2009). Effect of varying tomato paste and peel concentration on physicochemical and nutritional properties of tomato enriched extruded products. *NZIFST Annual Conference*, 23-24 June, Christchurch.

4.2.2. Introduction

In the previous experiment (Section 4.1), using rice, wheat and corn as the starch sources, it was found that the extruded products made from corn and rice expanded more and contained greater amounts of lycopene compared to those made from wheat. As corn costs less than rice it was chosen as the optimum starch-based ingredient for the production of extruded products containing tomatoes.

In the same study (Section 4.1), when tomato paste or tomato skin were compared as the lycopene sources, it was shown that the products containing tomato paste expanded the most and were softer, while lycopene retention values and dietary fibre content in products containing tomato skin were significantly greater.

Tomato skin contains more than 20 ppm lycopene, protected within the chromoplasts of the cells. In the tomato paste, lycopene is available at a much higher concentration (more than 450 ppm) and is more accessible due to prior processing and generally weaker cell walls enclosing the lycopene compared to the tomato skin. More than 15% (w/w) of tomato paste and 70% (w/w) of the tomato skin consists of dietary fibre. The type of fibre present in the tomato skin is mostly insoluble, while in the tomato paste higher proportions of soluble fibres, mainly pectin, are present. Tomato paste also contains considerable amounts of simple sugars (> 50% w/w), such as glucose and fructose (*Campbell, 2004*).

Therefore, by optimizing the raw ingredients, in particular the concentration of the tomato skin and tomato paste, the organoleptic characteristics and the lycopene content of the expanded corn-based snacks may be improved. In the present study, different formulations were prepared by replacing corn grits with varying concentrations of

tomato skin and tomato paste powder. The physicochemical properties and lycopene content of the snacks were determined. A sensory trial was also carried out on three snack types with better expected organoleptic characteristics indicated by higher expansion values and lower density and hardness values to investigate consumer acceptability.

4.2.3. Methods and material

4.2.3.1. Ingredients

The ingredients were prepared as previously described in Section 3.2.1. Various preparations of tomato skin and paste powders were made by combining them at ratios of 0:100, 25:75, 50:50, 75:25 or 100:0 on w/w basis. The corn grit component was substituted with 5, 10 or 20% of the tomato derivative powder.

4.2.3.2. Extrusion cooking parameters

The extrusion process is described in Section 3.2.2. The temperature profile from feed to die ends of the barrel was 40/60/80/100/140/140/140 °C. The feed rate of the dry ingredients and screw speed were kept constant at 10.5 kg/h and 350 rpm, respectively.

4.2.3.3. Physical properties of products

Product expansion, density, hardness, and colour evaluation is described in Section 3.2.2.

4.2.3.4. Chemical composition

4.2.3.4.1. Proximate composition

The chemical compositions of the formulations were determined as described in Section 3.2.2.2.

4.2.3.4.2. Lycopene content

Lycopene content was determined as previously described in Section 3.3.1 after minor modifications. These modification included transferring the ground (< 200 μ) extruded sample to 50 ml centrifuge tubes and adding 5 ml of porcine pancreatin solution (Sigma product code P7525, 8×USP, 10 mg/ml). The tubes were incubated at 37 °C for 20 minutes. Then 40 ml of the extracting solvents, petroleum ether: acetone (3:1 v/v) containing 0.05% BHT, was added. The mixture was shaken vigorously for 5 minutes and centrifuged at 4500 g at 15 °C for 20 minutes.

4.2.3.5. Sensory analysis:

From the 15 formulations used to produce the snacks, three samples were chosen for the sensory analysis based on their higher expansion ratios and lower hardness and density values, characteristics considered to represent more palatable extruded products. These samples included products containing 5% tomato skin and tomato paste in ratios of 75:25 and 50:50 or samples containing 10% tomato skin and tomato paste at a ratio of 75:25. The control was the extruded corn-based snack without tomato derivative. Cheese powder, salt and oil were added to all samples to replicate the commercial procedure for the products.

A semi-trained panel of 35 students and faculty members from the Institute of Food, Nutrition and Human Health evaluated the extruded snacks for appearance, flavour, texture and overall acceptability using a 9-point hedonic scale (from 1 = extremely dislike to 9 = extremely like). Sensory qualities such as appearance in terms of colour intensity, flavour in terms of tomato flavour and after taste, and texture in terms of crispness were rated based on a 5-point hedonic scale from 1 = very low to 5 = very high. The samples were presented on plates coded with three-digit numbers in individual booths with white light. Panellists rinsed their mouths with water after tasting each sample.

4.2.3.6. Experimental design and statistical analyses

The experimental design was a full factorial completely randomized design consisting of 3 replacement concentrations (5, 10 or 20 %) × 5 tomato skin to tomato paste ratios (100:0, 75:25, 50:50, 25:75 or 0:100). The effect of tomato skin or paste and the percentage incorporated on the physical (colour parameters, expansion ratio, density and hardness) and chemical (lycopene content and moisture content) characteristics of the snacks were evaluated. At least three replicates were evaluated for each treatment. When the F-value was significant, the comparison between the mean values was carried out using the Analysis of Variance (ANOVA). For the sensory evaluation, Duncan's multiple range test was performed to determine the differences between the samples. All significant differences are reported at $p < 0.05$ level. The correlation coefficient between the dependant variables was determined by the Pearson's correlation coefficient (R^2). All statistics were calculated using SPSS software version 13.1 (SPSS Inc., Chicago, IL, USA).

4.2.4. Results and discussion

4.2.4.1. Proximate composition

With the addition of tomato derivatives, the amount of dietary fibre present in the formulations increased from 1% to more than 16%, with the higher levels of fibre associated with a greater proportion of tomato derivatives in the formulation (Table 4.4).

Adding tomato derivatives to the snacks also increased the ash content from 0.6% to 1.8% and decreased the starch content from 78% in the control to 64%.

Increasing the tomato skin to tomato paste ratio increased the amount of dietary fibre by up to 400%, the protein content by up to 150% and ash content by up to 50 (Table 4.4).

4.2.4.2. Extrusion parameters

The extrusion parameters for the production of each snack type are shown in Table 4.5. Increasing the concentration of tomato derivatives in the formulation from 0 (Control) to 20% (Treatments 1 to 15), reduced the energy required to extrude the snacks as was seen from the reduction in torque, thrust pressure, power consumption and SME (Table 4.5). For example, the specific mechanical energy (SME), which measures the amount of energy dissipation in the melt, was decreased by more than 60% compared to the control when 20% tomato paste was incorporated or decreased by 10% compared to the control, when 20% tomato skin was incorporated.

Table 4-4 Proximate composition of the formulations †

Formulation	Replacement concentration	TS‡ ratio	TP* ratio	Moisture	Protein	Fat	Ash	Starch	Fibre
Control	0	0	0	11.8	6.7	2.3	0.6	78	1.1
1	5	100	0	11.2	7.1	2.4	0.9	75	5.0
2	5	75	25	11.2	7.0	2.3	0.8	75	4.2
3	5	50	50	11.2	7.0	2.3	0.8	75	3.5
4	5	25	75	11.2	6.9	2.3	0.7	75	2.8
5	5	0	100	11.2	6.8	2.3	0.6	75	2.0
6	10	100	0	10.6	7.5	2.4	1.2	71	8.8
7	10	75	25	10.6	7.3	2.4	1.1	71	7.4
8	10	50	50	10.6	7.2	2.3	1.0	71	5.9
9	10	25	75	10.6	7.0	2.3	0.8	71	4.4
10	10	0	100	10.6	6.9	2.2	0.7	71	3.0
11	20	100	0	9.4	8.2	2.5	1.8	64	16.6
12	20	75	25	9.4	7.9	2.4	1.6	64	13.6
13	20	50	50	9.4	7.6	2.3	1.3	64	10.7
14	20	25	75	9.4	7.3	2.2	1.1	64	7.8
15	20	0	100	9.4	7.0	2.1	0.9	64	4.8

† Calculated from the proximate composition of the raw ingredients and are given as percentages

‡ Tomato skin

*Tomato paste

The decrease in the energy required to extrude the products can be explained by the reduction of the starch concentration of the ingredients (Table 4.4), which results in a reduction of viscosity of the melt and therefore less shear force applied to the melt (*Ainsworth et al., 2007; Camire, 1998*). Furthermore, apart from soluble fibre such as pectin, tomato paste powder also contains more than 50% of sugars that act as lubricants in the extruder reducing friction and therefore shear resulting from the action of the screws on the melt (*Altan et al., 2008b; Camire, 1998; Lillford, 2008*).

Table 4-5 Extrusion parameters the extruded corn products containing different concentrations of tomato derivatives*

Formulation	Replacement concentration	TS‡ ratio	TP* ratio	Torque (N.m)	Thrust Pressure	Power Consumption	SME (kJ/kg)
Control	0	0	0	7.5 ^{bc}	65 ^{bcd}	1.81^{bcd}	411 ^{a}
1	5	100	0	7.9 ^{a}	105 ^{a}	1.90 ^{a}	382 ^{b}
2	5	75	25	7.7 ^{ab}	95 ^{d}	1.86 ^{ab}	376 ^{bc}
3	5	50	50	7.5 ^{abc}	102 ^{ab}	1.74 ^{de}	356 ^{cde}
4	5	25	75	6.9 ^{de}	101^{abc}	1.60 ^{f}	341 ^{ef}
5	5	0	100	5.7 ^{hi}	69 ^{h}	1.42 ^{h}	332 ^{ef}
6	10	100	0	7.5 ^{bc}	105 ^{a}	1.82 ^{bc}	378 ^{bc}
7	10	75	25	7.3 ^{cd}	101 ^{abc}	1.76 ^{cde}	357 ^{cde}
8	10	50	50	7.2 ^{cde}	103 ^{ab}	1.70 ^{e}	341 ^{ef}
9	10	25	75	6.8 ^{ef}	83 ^{f}	1.62 ^{f}	334 ^{ef}
10	10	0	100	5.4 ^{i}	75 ^{g}	1.29 ^{i}	303 ^{g}
11	20	100	0	6.5 ^{fg}	96^{cd}	1.59 ^{f}	371 ^{bcd}
12	20	75	25	6.3 ^{g}	90^{e}	1.56 ^{fg}	355 ^{cde}
13	20	50	50	6.0 ^{h}	82^{f}	1.50 ^{g}	348 ^{def}
14	20	25	75	5.7 ^{hi}	69 ^{h}	1.41 ^{h}	324 ^{fg}
15	20	0	100	4.6 ^{j}	66 ^{h}	1.17 ^{j}	256 ^{h}

Means ± standard deviations, n = 3. Different letters in the same column are significantly different ($p < 0.05$)

‡ Tomato skin

*Tomato paste

SME was highly correlated with power consumption (R^2 = 0.84, p < 0.01) (Table 4.6), while torque and thrust values were also positively correlated with SME but the correlation coefficients were lower (R^2 = 0.78 and 0.69, respectively).

4.2.4.3. Physical characteristics

4.2.4.3.1. Product Density

Product density is an important physical parameter used for the quality control of the extruded products by food industry. The density of the products in this study was increased by up to 4 times when tomato derivatives were added to the corn grits (Table 4.7). This increase in density may have occurred as a result of the increased concentration of fibre or sugar in the ingredients, which caused a reduction in the melt viscosity and the resulting denser products.

However, the effect of tomato addition on the physical parameters of the extruded products was shown to be concentration-dependant. For example, generally the products containing 5% tomato derivatives had similar density values to the control corn snacks. However, with further increases in the replacement concentration to 10 or 20%, significantly denser snacks were produced (p < 0.05).

The presence of higher proportions of tomato paste produced snacks of greater density compared to products that contained a higher proportion of tomato skin (Table 4.7). This may be due to presence of a greater proportion of simple sugars and pectin in the tomato paste (*Fan et al., 1996*). Pectin in the tomato paste absorbs the moisture present in the melt, resulting in the manufacture of heavier products compared to the products containing tomato skin, which contain a high proportion of insoluble fibre. Insoluble

fibre tends to absorb and loose moisture more easily compared to pectin (*Brennan et al., 2008a & b; Yanniotis et al., 2007*).

4.2.4.3.2. Hardness

Hardness is a physical parameter that can be used to measure the textural properties of extruded products. High values for hardness show that more force is needed to crush the products as it is eaten. The extruded corn-tomato products were up to 4.7 times harder than the control corn snacks (Table 4.7). The addition of components other than starch, such as fibre found in the tomato derivatives, increased the hardness of the extruded products due to the incorporation of fibre into the air bubble walls which increased their thickness (*Stojceska et al., 2008; Yanniotis et al., 2007*).

Table 4-6 Correlation coefficient (R^2) between the variables for extruded corn products containing different concentrations of tomato derivatives

	kW	Torque (N.m)	Thrust (bar)	Density (kg/m^3)	Hardness (N)	Expansion (%)	L-value	a-value	b-value	ML (%)	Lycopene (ppm)
SME	0.84**	0.78**	0.69**	-0.74**	-0.61**	0.34*	0.79**	-0.89**	-0.45**	0.43**	-0.89**
kW	1.00	0.97**	0.85**	-0.71**	-0.55**	0.49**	0.77**	-0.85**	-0.29*	0.57**	-0.82**
Torque		1.00	0.88**	-0.73**	-0.59**	0.57**	0.75**	-0.82**	-0.19 ns	0.62**	-0.81**
Thrust			1.00	-0.63**	-0.45**	0.38**	0.68**	-0.73**	-0.36*	0.61**	-0.73**
Density				1.00	0.93**	-0.70**	-0.62**	0.74**	0.05 ns	-0.69**	0.93**
Hardness					1.00	-0.71**	-0.61**	0.60**	-0.15 ns	-0.64**	0.82**
Expansion						1.00	0.30*	-0.52**	0.59**	0.58**	-0.43*
L							1.00	-0.70**	-0.34*	0.39**	-0.81**
a								1.00	0.34*	-0.42**	0.85**
b									1.00	0.06 ns	0.29 ns
ML										1.00	-0.71**

SME= Specific mechanical energy (kJ/kg), kW= power consumption, ML= Moisture loss (%)

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed).

ns Not significant

Table 4-7 Effect of varying tomato skin and paste concentration on the physicochemical properties of the extruded corn products containing tomato derivatives

No.	Replacement concentration	TS‡ ratio	TP* ratio	Density (kg/m^3)	Hardness (N)	Expansion (%)	L-value	a-value	b-value	Moisture loss (%)	Lycopene content (ppm)
Control	0	0	0	139 ^{g}	208 ^{h}	508 ^{a}	84.27 ^{a}	-1.97 ^{j}	36.89 ^{f}	29 ^{efg}	---
1	5	100	0	129 ^{g}	299 ^{f}	371 ^{hi}	82.66 ^{b}	10.03 ^{i}	35.85 ^{g}	39 ^{bcde}	11 ^{j}
2	5	75	25	134 ^{g}	289 ^{f}	421 ^{ef}	76.45 ^{e}	10.52 ^{i}	39.12 ^{d}	37 ^{cde}	41 ^{i}
3	5	50	50	131 ^{g}	260 ^{g}	448 ^{cd}	75.14 ^{f}	11.79 ^{h}	40.16 ^{c}	43 ^{bc}	60 ^{h}
4	5	25	75	140 ^{g}	245 ^{g}	455 ^{c}	73.53 ^{g}	13.66 ^{f}	40.33 ^{bc}	44 ^{bc}	81 ^{g}
5	5	0	100	154 ^{f}	180 ^{i}	475 ^{b}	73.40 ^{g}	25.13 ^{c}	42.32 ^{a}	41 ^{bcd}	103 ^{f}
6	10	100	0	155 ^{f}	295 ^{f}	301 ^{k}	80.26 ^{c}	13.11 ^{fg}	33.51 ^{i}	42 ^{bcd}	13 ^{j}
7	10	75	25	161 ^{f}	333 ^{e}	363 ^{i}	72.86 ^{g}	12.50 ^{gh}	38.24 ^{e}	49 ^{ab}	82 ^{g}
8	10	50	50	164 ^{f}	300 ^{f}	399 ^{g}	71.13 ^{h}	15.65 ^{e}	39.98 ^{c}	55 ^{a}	132 ^{e}
9	10	25	75	193 ^{d}	294 ^{f}	413 ^{fg}	70.22 ^{i}	16.26 ^{de}	40.92 ^{b}	36 ^{cdef}	151 ^{d}
10	10	0	100	197 ^{d}	254 ^{g}	434 ^{de}	68.83 ^{j}	31.57 ^{b}	40.61 ^{bc}	31 ^{defg}	227 ^{b}
11	20	100	0	178 ^{e}	325 ^{e}	231 ^{l}	78.23 ^{d}	15.63 ^{e}	32.38 ^{j}	39 ^{bcde}	18 ^{j}
12	20	75	25	207 ^{cd}	426 ^{c}	294 ^{k}	68.58 ^{j}	13.26 ^{fg}	34.96 ^{h}	37 ^{cde}	121 ^{e}
13	20	50	50	219 ^{c}	468 ^{b}	300 ^{k}	65.26 ^{l}	16.93 ^{d}	36.02 ^{g}	22 ^{g}	195 ^{c}
14	20	25	75	268 ^{b}	395 ^{d}	343 ^{j}	67.54 ^{k}	16.90 ^{d}	38.14 ^{e}	26 ^{fg}	219 ^{b}
15	20	0	100	594 ^{a}	850 ^{a}	380 ^{h}	63.93 ^{m}	40.68 ^{a}	39.06 ^{d}	10 ^{h}	364 ^{a}

Means ± standard deviations, n = 3. Different letters in the same column are significantly different ($p < 0.05$)

‡ Tomato skin

*Tomato paste

4.2.4.3.3. Expansion

Expansion ratio was negatively correlated to product density (R^2 = - 0.70) and hardness (R^2 = -0.71) and is an important physical parameter that greatly affects consumer acceptability (*Moraru & Kokini, 2003*). Generally consumers prefer products with higher expansion ratios. In the present work, the expansion ratios of products varied from 230 to 508% (Table 4.7). The control snack product without any tomato derivatives expanded the most, while the addition of tomato derivatives decreased the expansion ratio (Table 4.7). Products containing higher proportions of tomato skin expanded less than the products containing higher proportions of tomato paste (Table 4.7), suggesting that the type of fibre is an important parameter affecting the expansion ratio. The presence of a high proportion of insoluble fibre from tomato skin resulted in the least expanded products. This is consistent with the reports of other researchers (*Chang, et al., 1998; Moraru & Kokini, 2003; Yanniotis et al., 2007*).

As the extrusion intensity increased, more expanded products were obtained (Table 4.5, 4.6 & 4.7). This is due to the increase in the proportion of gelatinised starch followed by the reduction of the melt viscosity, therefore higher extensibility and mobility of the melt (*Lillford, 2008*).

4.2.4.3.4. Colour evaluation

The presence of natural pigments in the final extruded product along with the pigments produced during extrusion as a result of caramelisation and Maillard reactions are responsible for the colour of the extruded product (*Ilo & Berghofer, 1999*). The extruded corn-tomato products had colours ranging from yellow to dark red (Figure 4.3) where

the corn based control snacks were of a light yellow colour and had high L-values (Table 4.7). However, increases in the concentration of the tomato derivatives and particularly the proportion of tomato paste decreased the L-value and the extruded products appeared darker in colour (Table 4.7). This is consistent with previous reports (*Altan et al., 2008a*)

Figure 4-3- Variation in colour of the extruded products with/without tomato paste or skin powder

The a-value, which is related to the redness of the products, varied from -1.97 for the control to 40.68 (Table 4.7). The redness of the products increased with the tomato derivative concentration (Table 4.7) due to the increases in lycopene concentration.

Although the lightness or L-value of the products was positively correlated with the extruder parameters especially the SME, the a-value was negatively correlated with the SME ($r = -0.89$, $p < 0.01$) (Table 4.6). Reductions in the redness of the products with the severity of extrusion may have been due to thermal or shear induced losses of lycopene. Higher a-values were recorded in the less expanded, harder and more dense products (Table 4.6), indicating a higher concentration of lycopene in these products. These findings are consistent with previous reports (*Altan et al., 2008a; Ilo & Berghofer, 1999*).

4.2.4.4. Chemical characteristics

4.2.4.4.1. Percentage of moisture loss

Moisture loss from the extruded products ranged from 10 to 55% (Table 4.7). Generally, products containing 5 and 10% of tomato derivatives lost more moisture compared to the control product (Table 4.7). For products containing 20% tomato derivatives, moisture loss decreased at higher concentrations of tomato paste powder (Table 4.7). The presence of sugars and pectin that absorb water in the tomato paste probably reduced the amount of moisture evaporated from the products as they left the die while insoluble fibre present in the tomato skin lost moisture more easily (*Brennan et al., 2008a & b; Yanniotis et al., 2007*). Products that had a greater expansion ratio lost more moisture due to the larger evaporative surface area of these products (Table 4.7).

4.2.4.4.2. Lycopene content

The lycopene content of the extruded products ranged from 26 to 364 ppm (Table 4.7). The increases in the concentration of tomato derivatives and more specifically, the proportion of tomato paste, increased the amount of the lycopene in the products (Table 4.7).

In agreement with previous work (Section 4.1), the lycopene content of the products was negatively correlated with energy input, especially SME (r= -0.89) and energy consumption (r= -0.82) during extrusion processing. The loss of heat labile molecules during extrusion cooking has been suggested to be a result of both thermal and mechanical degradation (*Yajnik et al., 2010*), however further research is required to confirm this effect for lycopene.

Higher lycopene contents were associated with products that were harder and denser, while more highly expanded products contained less lycopene (Table 4.6). More expanded products were also associated with increased SME during processing. The redness of the products (higher a-values) correlated positively with their lycopene content (r= 0.85, $p < 0.01$). Thus, it is possible to use the colorimetric a- value to rapidly compare the lycopene content of these types of products.

4.2.4.4.3. Sensory analysis

Three product types were chosen for their higher expansion and lower hardness and density values. These characteristics are considered necessary for consumer acceptance. The colour intensity and tomato flavour were rated highest in the products containing tomato skin to paste ratio of 75:25, regardless of the concentration (5 or 10%) of tomato derivatives (Table 4.8). Crispness was rated highest for products containing 10% of tomato derivatives with tomato skin to paste ratio of 75:25. This is because fibre increases the number of nucleation sites for the formation of air bubbles therefore the texture of the products will be finer (*Guy, 1994*).

The acceptability scores for the appearance, texture, flavour and overall acceptability are shown in Table 4.9. The score for the texture and flavour acceptability were statistically similar between the products containing tomato derivatives while the appearance of products containing 10% of tomato derivative was rated highest. Although the products containing tomato derivatives were less expanded and harder and denser compared to the control corn-based snack, the overall acceptability score for the products containing tomato derivatives at any level of addition was significantly

higher than the control corn snack ($p < 0.05$). This may have been due to the red colour, tomato flavour and finer texture of the products containing tomatoes.

Table 4-8 Sensory evaluation scores for ratings for colour, flavour, after taste and texture for the extruded products *

	Samples			
Characteristic	Extruded corn grits	5%†, 75:25‡	5%, 50:50	10%, 75:25
Colour intensity	1.4 c	3.8 a	2.8 b	3.5 a
Tomato flavour	1.1 c	3.2 a	2.3 b	3.0 a
Aftertaste	3.3 b	3.8 a	3.9 a	4.0 a
Crispness	4.0 b	4.1 ab	4.1 ab	4.5 a

Mean hedonic scores for attribute ratings (1=low, 5=high) for extruded products evaluated by a consumer panel (n = 35).

* Means within a row with different letters are significantly different ($p < 0.05$), †Replacement ratio, ‡Tomato skin to tomato paste ratio

Table 4-9 Sensory evaluation scores for the acceptability of the extruded products *

	Samples			
	Extruded corn grits	5%†, 75:25‡	5%, 50:50	10%, 75:25
Appearance	5.5 c	6.1 bc	6.7 ab	7.2 a
Flavour	5.6 b	6.8 a	6.4 a	7.0 a
Texture	5.8 b	7.5 a	7.3 a	7.7 a
Overall acceptability	5.5 b	6.9 a	6.9 a	7.1 a

Mean hedonic scores for acceptability trial (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) for extruded products evaluated by a consumer panel (n = 35), * Means within a row with different letters are significantly different ($p < 0.05$), †Replacement ratio, ‡Tomato skin to tomato paste ratio

4.2.5. Conclusion

The utilisation of tomato skin in extruded products is a cost effective option to improve the nutritional value of these products by adding fibre and lycopene, but this incorporation at high levels results in the production of dense products with unpleasant flavour for the consumers. The study showed that although the presence of components other than starch, such as fibre and sugar, can negatively affect organoleptic characteristics, it is possible to optimize the raw ingredients to obtain consumer acceptable extruded products that contain lycopene and fibre. Overall, the extruded products containing 10% tomato derivative powder at a tomato skin to tomato paste ratio of 75:25 were selected as the most acceptable product type as shown by the sensory trial. These products contained more than 81.6 ppm lycopene and more than 7% fibre. This amount is equivalent to 50% of the daily recommended intake for lycopene and 7% of the dietary fibre based on a 30 g serving size. The recommended daily intake of lycopene is 6 mg and of fibre is 20-40 g.

Furthermore, the products gained a high acceptability score, however this score may be increased further by providing more information about the nutritional value of the snacks as compared with the control extruded corn grits to the consumers.

Chapter Five Effect of Raw Ingredients on the Nutritional Characteristics of Extruded Corn-tomato Products[5]

5.1. Abstract

Extruded corn-tomato products were prepared by replacing corn with tomato paste and/or tomato skin powder at ratios of 5%, 10% and 20% and digested using a model digestion system to determine lycopene bioaccessibility and uptake from the extruded products into Caco-2 cells. The digestibility of the starch, the main nutrient component of the extruded products was also investigated. While extrusion cooking reduced the lycopene content of the products, the proportion of bioaccessible lycopene increased. The lycopene uptake by the Caco-2 cells from the extruded products exceeded that of the control in which the lycopene was not extruded by 5% ($p < 0.05$). The starch digestibility in the products varied depending on the type of tomato derivative and its concentration. Optimization of the extrusion cooking process and the ingredients can yield functional extruded snack food products that contain bioavailable lycopene.

[5] Part of the material presented in this section has been previously published as a peer-reviewed journal article: Dehghan-Shoar, Z., Mandimika, T. Hardacre, A.K., Reynolds, G.W., Brennan, C.S. (2011). Lycopene bioaccessibility and starch digestibility for extruded snacks enriched with tomato derivatives. *Journal of Agricultural and Food Chemistry*, 59(22): 12047-12053.

5.2. Introduction

Chemical composition of the raw ingredients can affect the physicochemical characteristics of the extruded products (*Camire, 1998; Lillford, 2008*). Previously (Sections 4.1 and 4.2), the role of raw ingredients on the physical characteristics of extruded tomato-corn products was demonstrated. However, the effect of chemical composition of the ingredients on the nutritional attributes of the extruded tomato-corn products is not known.

Although in Chapter 4 it was shown that lycopene is maintained to some extent in the extruded products, for lycopene to be beneficial to health, it has to be bioavailable. In other words, it needs to become "bioaccessible" or to be released from the extruded food matrix during digestion in a state that allows uptake by intestinal epithelial cells. Further, it needs to be taken-up by these cells in order to be delivered to the target tissue where it can be used or stored (*Faulks & Southon, 2005*).

The bioavailability of nutrients is manipulated by many factors including the processing technique employed to prepare the food (*Failla et al., 2008a; Lemmens et al., 2011*). For instance, extrusion cooking of snack foods is a high shear, high temperature process, typically above 120 °C, that develops a plastic melt from the gelatinized starch undergoing processing in the barrel of the extruder. This gelatinization process increases starch digestibility. The extrusion process also mechanically disrupts cell walls of plant material being processed, releasing the cell contents into the starchy matrix (*Camire et al., 1990; Gartner et al., 1997*).

In tomatoes, lycopene is located within plastids which are inside chromoplasts scattered within the cytoplasm in the cells (*Camire et al., 1990; Hansen & Chiu, 2005*). If

the cells remain intact during processing or passage through the gut, lycopene is unlikely to be bioavailable during digestion. Disruption of the cell walls improves the bioavailability of lycopene, however, it can also increase the susceptibility of the labile lycopene molecule to degradation at the high shear and temperatures that occur during processing, resulting in its partial or complete loss from the food.

Apart from processing itself, the presence of other food components in the ingredients can affect the bioavailability of nutrients. For example, soluble fibres such as pectin and hemicelluloses present in tomato products can reduce shear and processing temperatures, thus reducing disruption of cell walls (*Lillford, 2008*). Furthermore, soluble fibre at high concentrations may interfere with the activity of the digestive enzymes and the absorption of both starch and lycopene at the gut wall (*Brennan, 2005; Riedl et al., 1999; Rock & Swendseid, 1992; Slaughter et al., 2002*). Starch also interacts with other ingredients including lipids during extrusion cooking to form less digestible structures such as amylose-lipid complexes (*Lemmens et al., 2011; Tester et al., 2006*). As lycopene is lipophilic, it may act in a similar way to lipids and interact with the starch component. Thus, the fate of both lycopene and starch during digestion should be investigated when determining the nutritional value of the extruded corn-tomato products.

The bioavailability of nutrients in foods can be predicted using *in-vitro* digestion methods when costly clinical trials are inappropriate. For lycopene to be absorbed it must be released from the food matrix and partitioned into the micellar phase of the digesta in the intestinal lumen with the aid of bile salts (*Failla et al., 2008a*). For this reason, lycopene bioaccessibility is measured as the fraction of lycopene in the food material that is transferred to the micellar phase of the digesta during simulated

digestion. The proportion of micellar lycopene absorbed by cultured Caco-2 cells can be used as a model for uptake by human intestinal epithelial cells (*Garrett et al., 1999; Kean et al., 2008; Lemmens et al., 2011*). Similarly, for starch, *in-vitro* methods that measure the starch digestibility during the simulated digestion of food have been shown to provide a good estimate of the bioavailability of starch (*Englyst & Englyst, 2005*).

In the present study, the effect of varying the proportion of tomato skin and/or tomato paste in corn-based extruded products on the lycopene bioaccessibility and the starch digestibility was investigated. Further, one product type was used to investigate the uptake of lycopene by Caco-2 cell lines.

5.3. Materials and methods

5.3.1. Ingredients

The specifications of the raw ingredients and the chemical reagents are described in Section 3.2.1. Formulations were prepared as described in Section 4.2.3.1.

5.3.2. Proximate composition

The proximate compositions of the formulations were determined according to the method described in Section 3.2.1. These data are shown in Table 4.4.

5.3.3. Extrusion processing

The specifications of the extruder used to make the products are given in Section 3.2.2. The extrusion was carried out as described previously (Section 4.2.3.2).

5.3.4. Simulated digestion

The *in-vitro* digestion method was carried out according to the method described in Section 3.3.2 using 4.8 mg/ml pancreatin and 10 mg/ml bile extract

To separate the micellar lycopene, the digesta was centrifuged at 5000 g for 45 minutes at 4 °C (Multifuge 1S-R, Thermofisher Scientific, Osterode, Germany) and filtered using cellulose acetate filters with a 0.2 μm nominal pore size according to the method of Thakkar *et al.* (*2007*). The filtrate was used to measure the micellar lycopene content of the digesta.

5.3.5. Cell culture

Samples used for the Caco-2 cell model consisted of: 1) digesta obtained from the extruded corn pellets containing 20% of tomato paste powder, 2) digesta from the extruded corn coated with unextruded tomato paste powder at 20% of the total weight. This was the positive control to compare the effect of extrusion on the bioavailability of tomato paste lycopene, and 3) digesta from the extruded corn pellets, as the negative control. The data obtained from the extruded negative control corn pellets were subtracted from treatments 1 and 2, to eliminate the possible errors caused by the interference of other carotenoids present in corn in the test for lycopene.

The cell cultures were prepared according to the method of Garret *et al*. (1999) after minor modifications as follows. Caco-2 cells at passage number 39 were grown in 6 well cell culture plates (Beckton Dickinson Labware, Franklin Lakes, New Jersey, USA) and used for experiments at day 14 after reaching confluency. The monolayers of Caco-2 cells were observed under a light microscope to determine if they showed normal morphological appearance and ensure that the treatments did not have any adverse effect on the cells.

To obtain a similar lycopene concentration of 1 µM for all samples, the test media were diluted with water. Monolayers were washed twice with 1 ml of phosphate-buffered saline (PBS) before the addition of 1 ml of the test media was diluted 1:3 (v/v) with basal Dulbecco's modified Eagle's medium. Cultures were incubated at 37 °C and harvested at 0, 4 and 8 hours after incubation (*Clark et al., 1998; Garrett et al., 1999*). Subsequently, the spent media was separated and stored. Monolayers were washed twice with ice cold PBS containing 2 g/l albumin to remove any residual carotenoids adhering to the surface of the cells before washing twice with cold PBS (*Chitchumroonchokchai et al., 2004*). Cells were collected in 1 mL ice-cold phosphate-buffered saline containing 10% (v/v) ethanol. To assess the stability of micellar lycopene, the media obtained from digestion which contained micellar lycopene was added to plates without cells and incubated under the same conditions as the rest of the samples. Samples including the cells, spent and control media were stored at -70 °C under a blanket of nitrogen before analysis.

5.3.6. Lycopene extraction and analysis of samples

The extraction was performed as quickly as possible in dim light under constant nitrogen gas flushing. To extract lycopene from extruded products, a pre-digestion step was employed using the method described previously (Section 3.3.1).

It was assumed that all reductions in lycopene concentration were due to uptake by the cells, as the lycopene was reasonably stable (> 90%) during the incubation time it was exposed to the Caco-2 cell cultures. The percentage of bioaccessible lycopene was determined by dividing the amount of micellar lycopene in the digesta by the amount of lycopene present in the extruded product.

Spent media and cell samples were extracted twice, once using a mixture of petroleum ether: acetone (3:1 v/v) and once using petroleum ether only. Subsequently, the organic phase was evaporated using nitrogen gas and the residue reconstituted using 200 µl methanol which also served as the mobile phase for the HPLC. The HPLC analysis was performed according to the method of Porrini *et al.* (*1998*) after minor modifications. These included the use of SCL-10AVP liquid chromatograph HPLC apparatus (Shimadzu Scientific Instruments, Columbia, MD, USA). Lycopene separation was achieved by using a reversed phase 5 µm Luna C_{18} column (150 × 4·6 mm, ID) with a guard column. The absorbance was measured at 472 nm using a U.V.-visible detector. The total analysis run was completed within 20 minutes.

Stock solutions of lycopene in petroleum ether were dried under a blanket of nitrogen and stored at -70 °C. Using a range of concentrations for the standards, the concentration range that resulted in a linear response from the HPLC analysis, was used

as the standard range for the experiment. On each experimental day, three standards were analysed along with the test samples.

5.3.7. Glucose analysis

Glucose concentration obtained after the digestion of starch present in the samples was determined using the method described in Section 3.3.2.

5.3.8. Statistical design

The experimental design was a full factorial consisting of 3 replacement concentrations of tomato skin and paste powder at 5, 10 or 20% with corn grits × 5 tomato skin to paste ratios of 100:0, 75:25, 50:50, 25:75 or 0:100. The effect of varying proportions of tomato skin and/or tomato paste on the lycopene bioaccessibility and the starch digestibility in the extruded corn-tomato products was investigated. At least three replicates were carried out for each treatment. A completely randomized experimental design was used and the data statistically evaluated using Analysis of Variance (ANOVA). When the F-value was significant ($p < 0.05$), the comparison between the mean values was carried out using Duncan's multiple range test. All statistics were calculated using SAS software version 9.1 (SAS Inc., Chicago, USA).

The statistical analysis for the Caco-2 cell experiment was carried out on only 2 samples due to time and cost restraints.

The lycopene content of the spent media and Caco-2 cells were collected at 0, 4 and 8 hours from the start of incubation was determined. Five replicates were used for each

treatment. A completely randomized design was used and when $p < 0.05$, the comparison between the means was carried out using Duncan's multiple range test.

5.4. Results and discussion

The chemical compositions of the ingredients have been described previously (Section 4.2.4.1).

5.4.1. Lycopene bioaccessibility

The extrusion cooking improved the lycopene bioaccessibility. The lycopene bioaccessibility from the raw ingredients varied from 16 to 56% (data not shown), while after the extrusion processing it increased to between 19 to 105% (Table 5.2). Furthermore, the proportion of bioaccessible lycopene increased as torque, power consumption and SME increased (Table 5.3). In the tomato derivatives, lycopene is present inside the cells. The cell walls, particularly those of the skin fraction are tough and resistant to rupture during factory processing and mastication. The process of extrusion cooking involves severe mechanical and heat processing treatments which rupture cell walls allowing the lycopene in the chromoplasts to escape from the previously digestion resistant cells. During extrusion some of the cell wall non-starch polysaccharides, also referred to as soluble fibre components, are broken down. The breakdown of these resistant structures frees the cell components and also increasing their release during digestion (*Camire et al., 1990; Failla et al., 2008a & b; Gartner et al., 1997; Lemmens et al., 2009*). Obviously, lycopene is also destroyed by the conditions

present during extrusion and the final available lycopene in the product is a result of the balance between release and destruction.

As the concentration of tomato derivatives increased, the proportion of the lycopene present that was bioaccessible decreased, but the absolute amount of bioaccessible lycopene increased (Table 5.2).

The proportion of lycopene taken up by the Caco-2 cells after 8 hours of incubation from the extruded corn-tomato paste products exceeded that of the extruded corn snacks coated with tomato paste powder (unextruded control) by up to 5%. The majority of uptake by the cells occurred in the first 4 hours of incubation (Figure 5.1). This finding suggests that the exposure of tomato derivatives to temperature during extrusion processing can result in a greater uptake of lycopene by the Caco-2 cells ($p < 0.05$). This may be due to the presence of higher concentrations of *cis*-lycopene in the extruded products compared to the unextruded tomato paste powder. It is suggested that the *cis*-isomers of lycopene are more soluble in oil; thus, they are transferred more easily from the aqueous component of the digestion system into the micellar phase of the digesta (*Boileau et al., 2002; Failla et al., 2008b*). Heat processing has been shown to promote the isomerization of carotenoids (*Boileau et al., 2002*).

The amount of lycopene taken up by the Caco-2 cells reported in this study (Figure 5.1) was greater than previous reports on commercial baby food preparation and stir-fried vegetables containing tomato paste (*Garrett et al., 1999 & 2000*). These products are processed at much greater moisture content and lower shear conditions compared to the conditions used in the present work. In addition, differences between the simulated digestion and lycopene extraction methods, specific attributes of the cell culture used, the nature of the food matrix and the processing itself may separately or in combination

contributed to have caused this variation. Clinical trials are required to validate these hypotheses.

Table 5-1 Lycopene concentration in the extruded products, digesta and proportion of lycopene transferred from the extruded product to the digesta

No.	Replacement concentration	TS‡ ratio	TP* ratio	Lycopene content of extruded products (ppm)	Micellar Lycopene in the digesta (ppm)	Proportion of lycopene bioaccessibility
1	5	100	0	26 ^{j}	27 ^{h}	105 ^{a}
2	5	75	25	56 ^{i}	42 ^{def}	75 ^{b}
3	5	50	50	75 ^{h}	32 ^{gh}	42 ^{d}
4	5	25	75	96 ^{g}	37 ^{fg}	39 ^{d}
5	5	0	100	118 ^{f}	39 ^{efg}	37 ^{d}
6	10	100	0	28 ^{j}	19 ^{i}	68 ^{b}
7	10	75	25	97 ^{g}	50 ^{d}	52 ^{c}
8	10	50	50	147 ^{e}	62 ^{c}	38 ^{d}
9	10	25	75	166 ^{d}	60 ^{c}	36 ^{d}
10	10	0	100	242 ^{b}	104 ^{a}	40 ^{d}
11	20	100	0	34 ^{j}	24 ^{hi}	71 ^{b}
12	20	75	25	137 ^{e}	46 ^{de}	34 ^{d}
13	20	50	50	234 ^{b}	50 ^{d}	21 ^{e}
14	20	25	75	210 ^{c}	39 ^{def}	20 ^{e}
15	20	0	100	364 ^{a}	71 ^{b}	19 ^{e}

Different letters represent the significant differences between samples within each column ($p < 0.05$), n=3.

‡ Tomato skin

* Tomato paste

Table 5-2 Correlation coefficients (R^2) between the extrusion parameters with the amount of glucose released from the starch after 20 and 120 minutes of digestion (G20 and G120)

	Lycopene content			Starch digestibility	
	Extruded product	Micellar phase of digesta	Bioaccessibility (%)	G20	G120
SME (kJ/kg)	-0.56**	-0.69**	0.24^{ns}	0.05^{ns}	0.04^{ns}
Torque (N.m)	-0.83**	-0.60*	0.65*	0.66*	0.54*
Power consumption (kW)	-0.84**	-0.64*	0.68*	0.63*	0.53*

* Significant at $p < 0.05$

** Significant at $p < 0.01$

ns Not significant

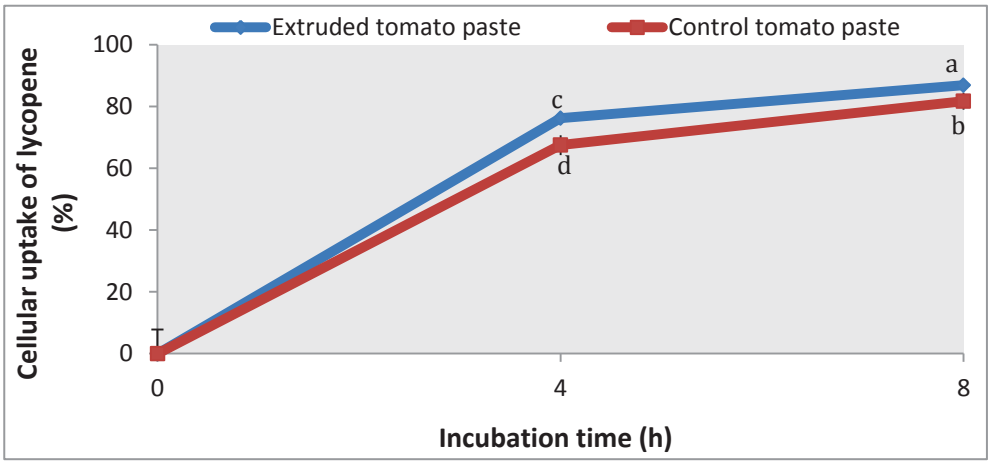

Figure 5-1- Relative uptake of micellar lycopene by Caco-2 cultures during 8 hours of incubation. Different letters above the error bars indicate significant differences between the values at each incubation time (p < 0.05). Data normalised to proportion of lycopene at time zero. The values for extruded corn grits were used to remove the effect of other carotenoids present in the corn grits, n = 5

Table 5-3 Extrusion conditions for the extruded corn products containing different concentrations of tomato derivatives

No.	Replacement concentration	TS‡ ratio	TP* ratio	Torque (N.m)	Power consumption (kW)	Specific mechanical energy (kJ/kg)	Expansion (%)
1	5	100	0	7.9 ^{a}	1.90 ^{a}	382 ^{a}	475 ^{a}
2	5	75	25	7.7 ^{ab}	1.86 ^{ab}	376 ^{ab}	455 ^{b}
3	5	50	50	7.5 ^{abc}	1.74 ^{de}	356 ^{bcd}	448 ^{bc}
4	5	25	75	6.9 ^{de}	1.60 ^{f}	341 ^{de}	421 ^{de}
5	5	0	100	5.7 ^{hi}	1.42 ^{h}	332 ^{de}	371 ^{gh}
6	10	100	0	7.5 ^{bc}	1.82 ^{bc}	378 ^{ab}	434 ^{cd}
7	10	75	25	7.3 ^{cd}	1.76 ^{cde}	357 ^{bcd}	413 ^{ef}
8	10	50	50	7.2 ^{cde}	1.70 ^{e}	341 ^{de}	399 ^{f}
9	10	25	75	6.8 ^{ef}	1.62 ^{f}	334 ^{de}	363 ^{h}
10	10	0	100	5.4 ^{i}	1.29 ^{i}	303 ^{f}	301 ^{j}
11	20	100	0	6.5 ^{fg}	1.59 ^{f}	371 ^{abc}	380 ^{g}
12	20	75	25	6.3 ^{g}	1.56 ^{fg}	355 ^{cd}	343 ^{i}
13	20	50	50	6.0 ^{h}	1.50 ^{g}	348 ^{cde}	300 ^{j}
14	20	25	75	5.7 ^{hi}	1.41 ^{h}	324 ^{ef}	294 ^{j}
15	20	0	100	4.6 ^{j}	1.17 ^{j}	256 ^{g}	231 ^{k}

Different letters represent the significant differences between samples within each column ($p < 0.05$), n=3

‡ Tomato skin concentration

*Tomato paste concentration

5.4.2. Starch digestibility

The amount of glucose released from the unprocessed ingredients after 20 minutes (G20) of digestion varied from 100 to 184 mg/g and after 120 minutes (G120) from 208 to 345 mg/g (data not shown). For the extruded products, glucose released at G20 was much greater than for the unextruded product and varied from 488 to 780 mg/g and at G120 had increased to between 687 and 924 mg/g (Figures 5.2 and 5.3). This agrees with previous reports which state that extrusion cooking results in the gelatinisation of starch, thus improving its susceptibility to the digestive enzymes (*Altan et al., 2009; Brennan et al., 2008a*).

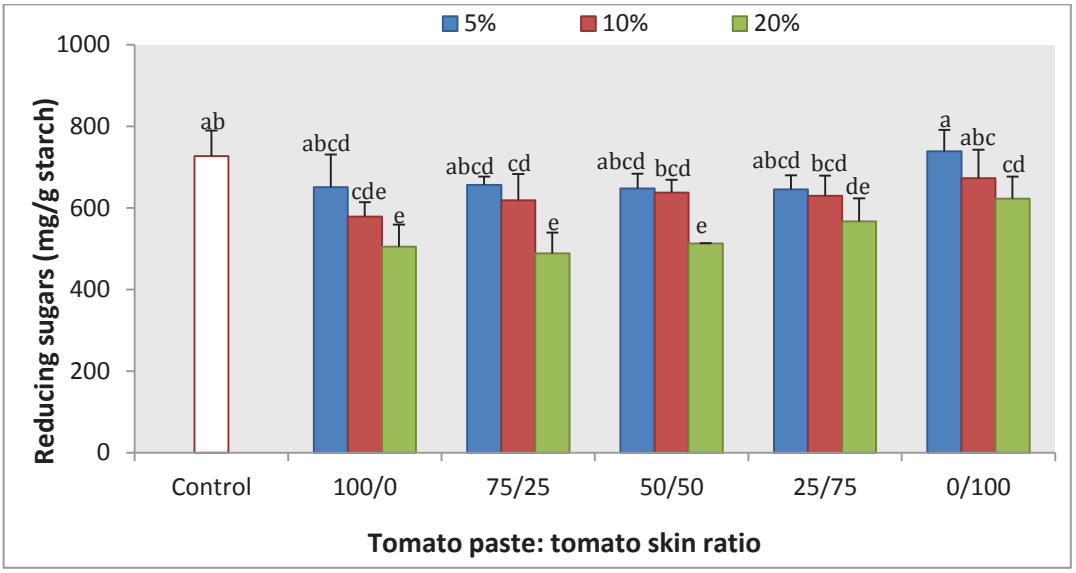

Figure 5-2 The amount of glucose released by the digestion of starch after 20 minutes incubation. The control is extruded corn snack, while the treated extruded products contained various concentrations of tomato paste and tomato skin at three different inclusion levels. Error bars represent standard deviations from the means. Different letters above bars (a-e) indicate the significant ($p < 0.05$) differences between the samples, n=3

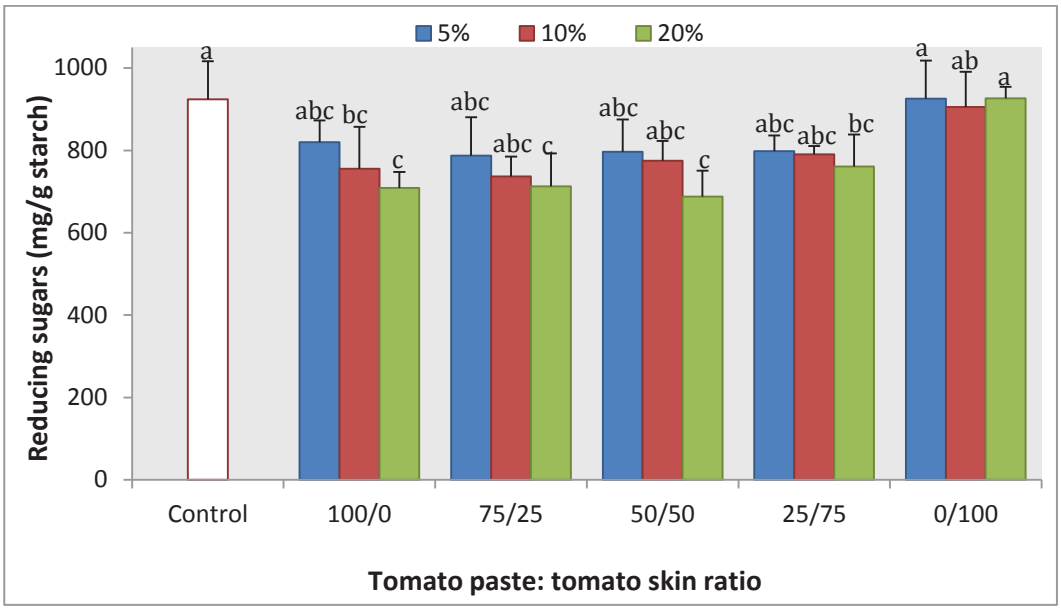

Figure 5-3 The amount of glucose released by the digestion of starch after 120 minutes incubation. The control is extruded corn snacks while the treated extruded products contained various concentrations of tomato paste and tomato skin at three different inclusion levels. Error bars represent standard deviations from the means. Different letters above bars (a-c) indicate the significant ($p < 0.05$) differences

Extruded products containing high proportions of tomato paste powder, were less expanded (Table 5.4) and had the lowest G20 and G120 values (Figures 5.2 and 5.3). It is possible that adding tomato paste reduces the starch concentration and increases sugars and other polysaccharides, thus reducing the shear force developed during processing (Table 5.1), which in turn reduces the proportion of gelatinized starch and slows the rate of digestion (*Altan et al., 2009; Lillford, 2008*). The positive correlation between the power consumption and torque values with the amount of starch digested at G20 and G120 support this hypothesis (Table 5.3).

Another reason for the reduced starch digestibility in these products could be due to the competition for the available water by the fibre components, limiting gelatinisation of

the starch and thus reducing the susceptibility of the starch to digestion (*Tester & Sommerville, 2003*). Dietary fibre can also reduce the rate of amylolytic activity by directly binding to the amylolytic enzymes (*Slaughter et al., 2002*), although this has only been reported at much higher fibre concentrations than those occurring in this work.

Altan *et al.* (*2009*) have suggested that the reduction in starch digestibility in tomato pomace-enriched barley snacks may be due to the presence of amylose-lipid complexes. The formation of these complexes during extrusion cooking from the raw ingredients containing less than 4% of lipids has been previously reported (*Tester et al., 2006*). However, in this work, the rate of starch digestion was independent of the proportion of lipid present in the samples (Table 5.1).

5.5. Conclusion

The present study investigated the effect of ingredients on the lycopene bioaccessibility from the extruded corn-tomato products. The study also measured the starch digestibility in the products, which made it possible to simultaneously record the changes in these nutritional parameters with ingredient composition and processing conditions.

The study showed that despite the exposure of lycopene to severe heat and shear during extrusion cooking, the pigment entrapped inside the cells are released and become more available for uptake in simulated uptake by the gut wall cells (Caco-2 cells). It was also shown that the mechanical treatment during extrusion cooking plays a major role on the release of lycopene from the matrix of tomato cells. Although,

extrusion processing results in a net loss of lycopene from the products, there is a net gain of bioaccessible lycopene in the extruded products due to the breakdown of resistant cell structures. It is suggested that by optimization of the extrusion processing, extruded corn-tomato products can be produced that contain significant amounts of potentially bioavailable lycopene and fibre.

Starch digestibility of the products decreased with the proportion of tomato paste powder they contained, but not with the proportion of tomato skin powder. This indicates that by carefully proportioning these ingredients when designing a snack food, the rate of starch digestion from the snacks can be controlled.

Chapter Six Effect of Extrusion cooking on the Physicochemical Characteristics of Extruded Corn-Tomato Products

6.1. An investigation on the effect of feed moisture content and screw speed on physicochemical characteristics of extruded corn-tomato products

6.1.1. Abstract

Tomato skin obtained from industry has a high moisture content that requires further drying prior to be used in the manufacture of extruded products. It would be a less costly process to add the wet skin to the raw ingredients used to make the extruded products. Therefore, the aim of this study was to determine the maximum amount of moisture that can be present in the ingredients used to produce expanded tomato-corn extruded products. Corn grits were replaced with 10% tomato skin with various moisture contents to reach final moisture contents of 11, 15, 19 or 23% and processed using a laboratory-scale twin screw extruder operating at 250 or 350 rpm. Colour parameters, expansion, and lycopene content of the products were measured to determine the optimum moisture content of the ingredients. Increasing the moisture content to 15% did not change the expansion ratio of the products significantly, but further increases in moisture produced less expanded and denser products. Increasing the moisture content to 19% improved the amount of lycopene recovered from the products, while further increasing moisture content to 23% had no significant effect. Overall, extrusion at 250 rpm of ingredients containing 15% moisture was chosen as the optimum condition. These products contained more than 30ppm lycopene and 7% dietary fibre.

6.1.2. Introduction

The ingredients used to manufacture extruded snacks should generally contain less than 12% moisture (*Guy, 2001*). Higher moisture contents negatively affects the physical characteristics of the extruded products, and as moisture levels in the ingredients increase above about 14%, the products are less expanded and denser (*Lue et al., 1991*). However, in its native state, tomato skin obtained from the processing plant has a moisture content of more than 60%. Thus, an extra processing step is required to dry the tomato skin.

The aim of this study was to determine the maximum amount of moisture that can be present in the raw ingredients without detracting from the expansion ratio of the products containing tomato skin. Thus, the effect of raw ingredients containing moisture contents of 11, 15, 19 and 23% and extruded at two screw speeds of 250 and 350 rpm on the expansion ratio, colour parameters and lycopene concentration of extruded products containing tomato skin was investigated.

6.1.3. Materials and methods

6.1.3.1. Ingredients

The ingredients were prepared as described in Section 3.2.1.

6.1.3.2. Sample preparation

The ingredients were prepared by incorporating 10% of dry (6% moisture) tomato skin powder into corn grits containing about 12% moisture. The samples were conditioned using tap water where necessary to final moisture contents of 11, 15, 19 and 23%,

mixed thoroughly and placed in sealed buckets overnight in room temperature to obtain uniform moisture distribution.

6.1.3.3. Extrusion cooking

The extruder specifications are described in Section 3.2.2. The temperature was kept constant at 80 °C in the first 4 barrel sections and 100, 120 and 140 °C in the final 3 barrel sections, respectively. The screw speed was set to either 250 or 350 rpm. The feed rate of dry ingredients was kept constant at 6.5 kg/h. The extruder parameters were determined as specified in Section 3.2.2.1.

6.1.3.4. Physicochemical properties of products

The product characteristics including expansion ratio, colour evaluation, moisture were determined as described in Section 3.2.2 and lycopene content was determined as in Section 3.3.1.

6.1.3.5. Experimental design and data analysis

The experiment was designed as a full factorial with four moisture contents (11, 15, 19 and 23%) × two screw speeds (250 or 350 rpm). Extruded samples were prepared from 2 kg of raw ingredients and lycopene content, colour parameters, expansion ratio of products were measured. Between 3 and 4 replicates of each measurement were made.

A completely randomized design was used to evaluate the results and Analysis of Variance (ANOVA) was carried out to compare the mean values. All significant

differences are reported at $p < 0.05$ level. All statistics were calculated using SAS software version 9.1 (SAS Inc., Chicago, IL, USA).

6.1.4. Results and Discussion

6.1.4.1. Extrusion parameters

Increases in the moisture content of the ingredients significantly decreased the energy required to process the ingredients (Figure 6.1), showing that moisture lubricates the melt, reducing the shear force and energy dissipation on the melt (*Lillford, 2008; Vainionpaa et al., 1989*).

On the other hand, the increase in screw speed from 250 to 350 rpm increased the SME (Figure 6.1) as a result of the increase in the mechanical energy required to turn the screws and energy dissipated on the melt (*Ilo et al., 1996*).

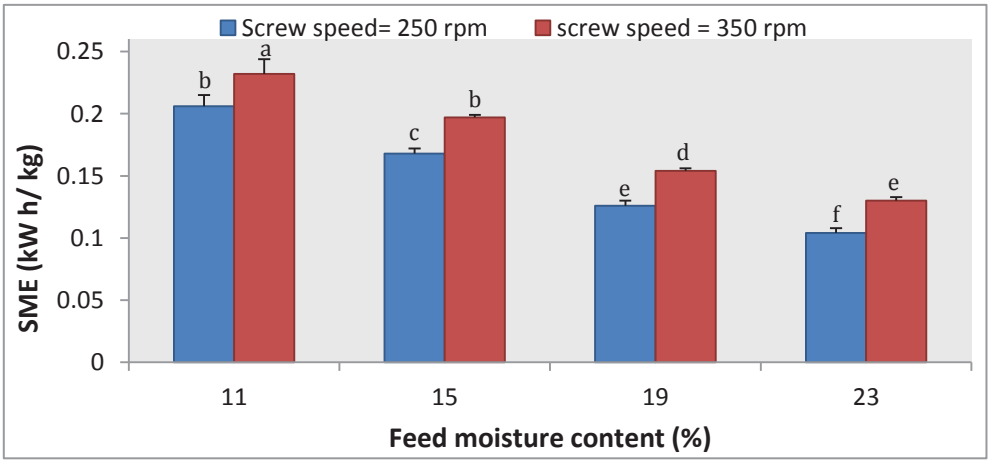

Figure 6-1 Effect of feed moisture and screw speed on the specific mechanical energy used to process the extruded products containing 10% tomato skin powder. Different letters above bars (a-f) indicate the significant ($p < 0.05$) differences between the samples, n=3

6.1.4.2. Expansion ratio

While increases in moisture content to 15% did not significantly affect the expansion ratio of the products, further increasing moisture content above this level reduced expansion (Figure 6.2). To obtain an expanded product, energy is required to gelatinize or "melt" the starch and also superheat the water in the melt. The main source of this energy is from the friction and shear force imparted by the screws to the ingredients. However, with the presence of larger amounts of moisture, the screws are lubricated and the friction is reduced, thus reducing the amount of energy applied to the melt. This limits the gelatinization of starch progress and thus the expansion of the extruded products.

Although increases in the screw speed are expected to improve the expansion of the snacks (*Moraru & Kokini, 2003*), the effect of screw speed alone was not significant in the present study. This may have been due to the screw speeds used (250 and 350 rpm). Meanwhile, the interaction effect of screw speed and moisture content was significant and was more pronounced at feed moisture contents of 23% (Figure 6.2).

6.1.4.3. Colour evaluation

The a-value was significantly influenced by the feed moisture content (Figure 6.3). The lowest a-value was found in the products manufactured from ingredients containing low moisture content and extruded at high screw speeds. Although the screw speed alone did not affect the a-value significantly, the interaction effect of moisture content and screw speed was significant (*Ilo & Berghofer, 1999*).

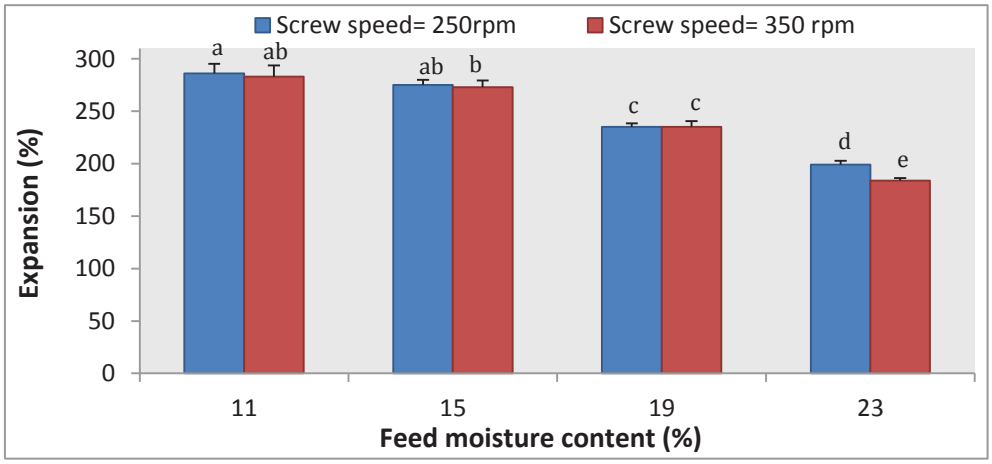

Figure 6-2 Effect of feed moisture and screw speed on expansion of the extruded products containing 10% tomato skin powder. Different letters above bars (a-e) indicate the significant ($p < 0.05$) differences between the samples, n=3

Significantly higher lycopene contents were obtained from the ingredients containing higher moisture contents. The effect of moisture on pigment retention has been previously reported to be related to the reduction in the extrusion intensity and shear stress on the raw ingredients (*Ilo & Berghofer, 1998*). Similar lycopene contents were obtained from ingredients having 11 and 15% moisture extruded at 350 rpm (Figure 6.4).

Screw speed also had a significant effect on the lycopene content of extruded products containing tomato skin ($p < 0.05$). The products extruded at the higher screw speed of 350 rpm had consistently lower lycopene contents.

A high negative correlation was found between the SME and lycopene content (Table 6.1), suggesting that the increase in the intensity of the extrusion process is detrimental to lycopene. It has been suggested that the loss of β-carotene (*Yajnik et al., 2010*) and

thiamine (*Ilo & Berghofer, 1998*) during extrusion cooking is a result of both thermal and mechanical effects, however further work is required for lycopene.

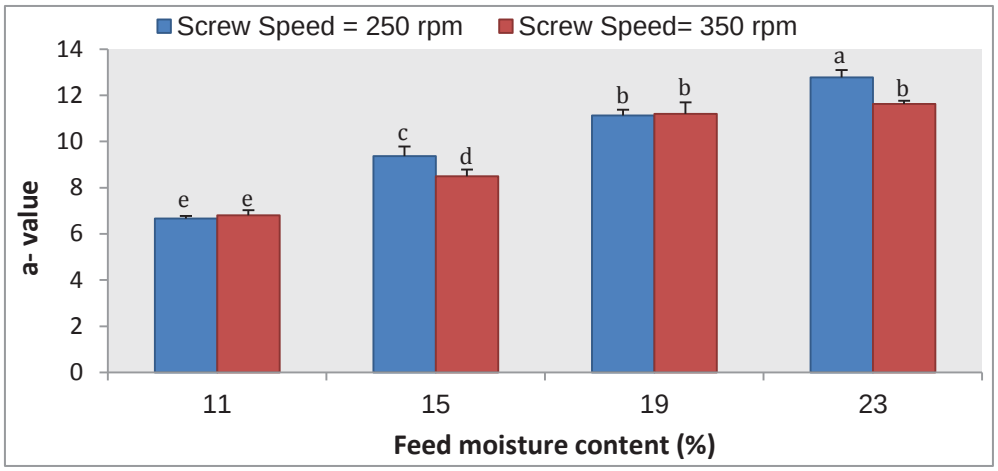

Figure 6-3 Effect of feed moisture and screw speed on the a- values of the extruded products containing 10% tomato skin powder. Different letters above bars (a-e) indicate the significant (p < 0.05) differences between the samples, n=3

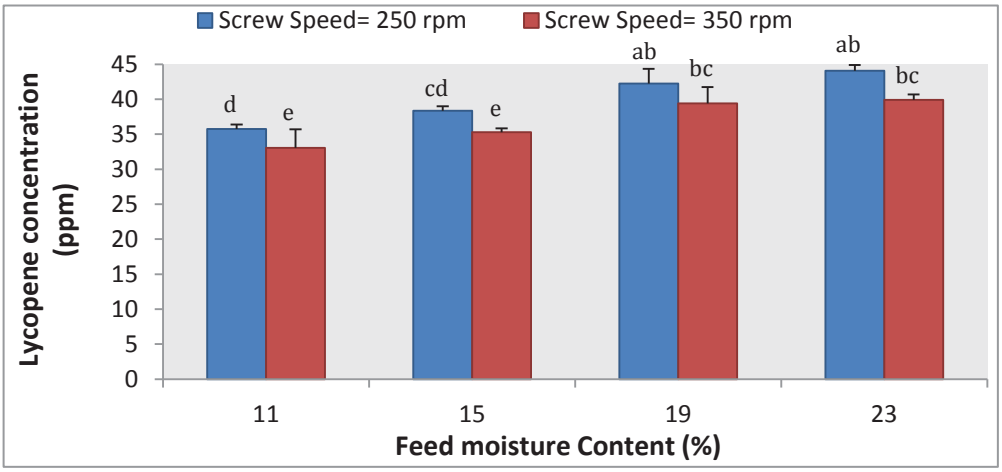

Figure 6-4 Effect of feed moisture and screw speed on lycopene contents of the extruded products containing 10% tomato skin powder. Different letters above bars (a-e) indicate the significant (p < 0.05) differences between the samples, n=3

Table 6-1 The correlation coefficient (R^2) between lycopene content of the extruded corn products containing 10% tomato skin powder and the extrusion parameters, a-value and expansion values

	SME	Torque	kW	Thrust	a-value	Expansion
Lycopene content	-0.92*	-0.63*	-0.89*	-0.63*	0.87*	-0.75*

* Significant at $p < 0.05$

Furthermore, the product redness or a-value was highly correlated with the lycopene content. Earlier studies have speculated that the a-value is related to the presence of lycopene pigment in the products containing tomatoes (*Altan et al., 2008a; Arias et al., 2000*), the present finding confirms this speculation (Table 6.1).

6.1.5. Conclusion

It can be concluded that by employing the suggested formulation, it is possible to utilize raw ingredients that contain up to 15% moisture, for the production of expanded extruded products containing tomato skin. Thus, by using 10% tomato skin that has 49% moisture content and adding to 90% corn grits containing 11.2% moisture, the utilization of expensive drying techniques to completely dry the tomato skin is unnecessary.

6.2. Effect of extrusion cooking on lycopene content and some physicochemical parameters of extruded corn-tomato products

6.2.1. Abstract

Corn grit was replaced with 10% tomato derivatives blend containing 75% tomato skin and 25% tomato paste and extruded using a co-rotating twin-screw extruder to produce snacks with improved nutritional value. Using response surface methodology, the effect of extrusion temperature between 140-160 °C, screw speeds between 250 and 350 rpm and moisture content of the ingredients between 11 and 15% on the processing parameters (SME and power consumption) and product characteristics (expansion, density, hardness, colour and lycopene retention) was evaluated. The moisture content of the ingredients had the greatest influence on the responses. Expansion was correlated positively with SME, while density, hardness and lycopene retention values were correlated negatively with expansion ($p < 0.05$). Extrusion of corn-tomato based ingredients containing 12.7% moisture at 160 °C using a screw speed of 350 rpm was considered as the optimum conditions to produce extruded products containing maximum lycopene content and highest expansion values using this formulation.

6.2.2. Introduction

Previously it was shown that the addition of tomato derivatives such as tomato skin and tomato paste improves the nutritional value of the extruded snacks (Chapter 5),

however this enrichment reduces the organoleptic quality of the products. For instance, the resulting products will be harder and more dense (Sections 4.1 and 4.2) which may result in the reduction in consumer acceptability (*Moraru & Kokini, 2003*). Further, it was shown that by increasing the SME or the severity of the extrusion, the organoleptic properties of the snacks may be improved (Chapter 4), possibly due to the changes in the melt characteristics as suggested by researchers (*Camire, 1998; Lillford, 2008*). However, lycopene was shown to degrade with increases in the severity of extrusion processing (Sections 4.1 and 4.2). Therefore, by optimizing processing parameters, a balance between the optimal maintained lycopene content and acceptable organoleptic properties of the snacks can be achieved.

A typical commercial extruded snack has an expansion ratio of between 350-500% and hardness of between 200-400N. From the sixteen formulations used in Section 4.2, blends of 7.5% tomato skin and 2.5% tomato paste with corn grits, fitted well within this range (Table 4.7) and gained high consumer acceptability scores in the sensory trial (Table 4.9), in addition to containing significant amounts of fibre and lycopene. In the present study, using that formulation, the effect of extrusion parameters, feed moisture content, screw speed and temperature, on lycopene retention, expansion ratio, true density, hardness, colour and moisture content of the products were determined.

6.2.3. Materials and methods

6.2.3.1. Ingredients

The ingredients were prepared as described in Section 3.2.1.

6.2.3.2. Sample preparation

The tomato derivative mixture was prepared by mixing 75% dry tomato skin powder with 25% tomato paste powder. A dry blend was then made comprising 90% 220 spec corn grit and 10% of the tomato derivative mixture. This formulation was chosen based on previous studies (Section 4.2.5). The samples were conditioned to different moisture contents, mixed thoroughly and placed in sealed buckets overnight at room temperature to obtain uniform moisture distribution.

6.2.3.3. Extrusion cooking

The extruder specifications are described in Section 3.2.2. The temperature was kept constant at 80 °C in the first 4 barrel sections for all experiments and either 133, 140, 150, 160 or 167 °C were used in the final 3 barrel sections. The feed rate of ingredients was kept constant at 15.5 kg/h.

The extruder parameters were determined as described in Section 3.2.2.1.

6.2.3.4. Physical properties of products

The product characteristics including expansion ratio, density, hardness and colour evaluation were determined as specified in Section 3.2.2.

6.2.3.5. Chemical composition

6.2.3.5.1. *Proximate composition*

The chemical analysis of the ingredients has been described in Section 3.2.2.2. The proximate composition of the tomato-corn grit mixture is shown in Table 6.2.

Table 6-2 Proximate composition of corn grit, 7.5% tomato skin and 2.5% tomato paste mixture

Moisture (%)	10.6
Protein (%)	7.3
Fat (%)	2.35
Ash (%)	1.07
Starch (%)	71
Fibre (%)	7.4
Lycopene (ppm, dwb)	140

6.2.3.5.2. Lycopene content

Lycopene content was determined as described in Section 4.2.3.

6.2.3.6. Experimental design and data analysis

A central composite rotatable design was used to determine the effect of the three independent variables: temperature of the last three barrels (X_1), screw speed (X_2) and feed moisture content (X_3) at five levels (Table 6.3). The ranges chosen for each parameter were determined based on previous experiments. The experimental design, along with the coded and actual levels is given in Table 6.4. Response surface methodology was used to investigate the effect of independent variables on the

responses, i.e. specific mechanical energy (SME) and power consumption as the processing parameters, and expansion ratio, product density, colour, hardness and lycopene retention as the product characteristics. Response surface graphs were plotted between two independent variables at a time while keeping the other variables constant at zero level to investigate the effect of different levels of the independent variables on the dependent variables.

Table 6-3 Independent variables and their levels used for the central composite rotatable design for the extrusion conditions of extruded corn products containing 7.5% tomato skin powder and 2.5% tomato paste

	Symbol	Coded variable levels				
		-1.682	-1	0	1	1.682
Temperature (°C)	X_1	133	140	150	160	167
Screw speed (rpm)	X_2	215	250	300	350	384
Feed moisture (%)	X_3	9.6	11	13	15	16.4

The related response surface plots and statistical analysis was carried out using Design Expert software version 6.0.2 (Statease Inc, Minneapolis, MN, USA). To be able to optimize the procedure, data was modelled by multiple regression analysis at linear, interaction and quadratic levels. Only the variables significant at $p < 0.05$ levels were selected for discussion. Analysis of variance (ANOVA) was used to determine the statistical significance of the variables for each response. The Pearson's correlation coefficients between the product characteristics and process parameters were determined using SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA). The reported

data are the average of at least three replicates with a coefficient of variation (CV) of less than 10%.

Table 6-4 Experimental design for the extrusion conditions for the production of extruded corn products containing 7.5% tomato skin powder and 2.5% tomato paste powder

Run number	Coded levels			Actual levels		
	X_1	X_2	X_3	Temperature (°C)	Screw speed (rpm)	Moisture (%)
1	1.00	1.00	-1.00	160	350	11.00
2	1.68	0.00	0.00	167	300	13.00
3	-1.00	1.00	1.00	140	350	15.00
4	-1.00	1.00	-1.00	140	350	11.00
5	-1.00	-1.00	-1.00	140	250	11.00
6	1.00	1.00	1.00	160	350	15.00
7	1.00	-1.00	1.00	160	250	15.00
8	0.00	1.68	0.00	150	384	13.00
9	0.00	0.00	1.68	150	300	16.36
10	0.00	0.00	0.00	150	300	13.00
11	-1.68	0.00	0.00	133	300	13.00
12	0.00	0.00	0.00	150	300	13.00
13	0.00	0.00	0.00	150	300	13.00
14	0.00	0.00	0.00	150	300	13.00
15	0.00	0.00	0.00	150	300	13.00
16	0.00	0.00	0.00	150	300	13.00
17	-1.00	-1.00	1.00	140	250	15.00
18	1.00	-1.00	-1.00	160	250	11.00
19	0.00	-1.68	0.00	150	216	13.00
20	0.00	0.00	-1.68	150	300	9.64

6.2.4. Results and discussion

6.2.4.1. Specific mechanical energy (SME)

The effect of the independent variables on the specific mechanical energy (SME) gave the regression coefficients shown in Table 6.5. Temperature and feed moisture content had a negative linear effect on the SME, while screw speed had a significant positive effect ($p < 0.05$). The quadratic effects of all variables were significant, with temperature being significantly and negatively correlated with SME. A quadratic model was considered significant for the experimental data. The analysis of variance showed a high correlation ($R^2 = 0.99$) between the experimental data and the fitted model (Table 6.6).

The values for SME ranged from 0.078 to 0.117 kWh/kg (data not shown). Increases in the feed moisture reduced the SME (Figure 6.5) due to the lubricating effect of water on the melt (*Vainionpaa et al., 1989*). Conversely, increases in screw speed, increased the SME due to the higher shear rate applied to the melt. The increases in temperature also decreased the SME because higher temperatures reduce the melt viscosity and lower amount of energy is dissipated (*Altan et al., 2008b; Baik et al., 2004*).

Table 6-5 The regression equations for the dependent variables using independent variables temperature (X_1), screw speed (X_2) and feed moisture content (X_3) of extruded corn products containing 7.5% tomato skin powder and 2.5% tomato paste powder produced using different extrusion conditions based on coded factors

	SME	kW	EXP	D	H	L-value	a-value	b-value	ΔE	LYC
Intercept	0.097	1.51	317.6	119.5	174.7	96.30	-0.14	0.66	4.79	81.08
X_1	-1.112E-003	0.011	1.44	-6.26	-11.56	-0.35	ns	-1.00	-0.22	1.71
X_2	6.862E-003	0.080	4.10	-7.36	-7.67	0.36	-0.25	-0.52	-0.45	ns
X_3	-0.011	-0.18	-6.67	15.32	14.40	0.23	0.28	4.05	2.15	3.53
X_1^2	-4.142E-004	ns	ns	-1.12	ns	-0.26	ns	-0.70	0.05	ns
X_2^2	9.416E-004	ns	ns	0.11	ns	-0.29	ns	-0.12	0.07	ns
X_3^2	1.071E-003	ns	ns	3.93	ns	-0.65	0.39	0.76	1.41	ns
$X_1 X_2$	3.166E-003	0.046	20.92	0.32	ns	0.13	ns	0.69	0.03	ns
$X_1 X_3$	3.231E-003	0.041	1.53	-2.22	ns	0.15	ns	-0.25	-0.54	ns
$X_2 X_3$	9.685E-004	0.013	-5.58	-3.85	ns	-0.08	ns	-0.05	-0.04	ns

X1= Temperature, X2= Screw speed, X3= Feed moisture, kW= Power consumption, EXP= Expansion, D= Density, H= Hardness, LYC= Lycopene retention

^{ns} Not significant ($p > 0.05$)

Table 6-6 Analysis of variance (ANOVA) results for the fitted models of dependant variables for extruded corn product containing 7.5% tomato skin powder and 2.5% tomato paste powder produced using different extrusion conditions

Response	Source	df	Sum of squares	Mean squares	F-value	*p*-value	R^2
SME	Regression	9	2.518E-003	2.798E-004	112.83	< 0.0001	0.99
	Lack of fit	5	2.045E-005	4.089E-006	4.70	0.0573	
	Pure error	5	4.349E-006	8.697E-007			
	residual	10	2.479E-005	2.479E-006			
	total	19	2.543E-003				
Power consumption	Regression	4	0.56	0.14	65.53	< 0.0001	0.95
	Lack of fit	10	0.03	3.157E-003	27.86	0.0009	
	Pure error	5	5.67E-004	1.13E-004			
	residual	13	0.03	2.23E-003			
	total	19	0.59				
Expansion	Regression	6	4633.09	772.18	22.93	< 0.0001	0.91
	Lack of fit	8	307.17	38.40	1.47	0.3489	
	Pure error	5	130.64	26.13			
	residual	13	437.81	33.68			
	total	19	5070.90				
Density	Regression	9	4896.45	544.05	61.89	< 0.0001	0.98
	Lack of fit	5	62.82	12.56	2.50	0.1684	
	Pure error	5	25.10	5.02			
	residual	10	87.91	8.79			
	total	19	4984.36				
Hardness	Regression	3	5460.89	1820.30	9.62	0.0007	0.64
	Lack of fit	11	2504.86	227.71	2.18	0.2015	
	Pure error	5	522.97	104.59			
	residual	16	3027.83	189.24			
	total	19	8488.72				

Response	Source	df	Sum of squares	Mean squares	F-value	*p*-value	R^2
L-value	Regression	9	11.71	1.30	5.06	0.0092	0.82
	Lack of fit	5	1.28	0.26	0.99	0.5026	
	Pure error	5	1.29	0.26			
	residual	10	2.57	0.26			
	total	19	14.29				
a-value	Regression	3	4.11	1.37	21.81	< 0.0001	0.80
	Lack of fit	11	0.67	0.061	0.91	0.5870	
	Pure error	5	0.34	0.067			
	residual	16	1.01	0.063			
	total	19	5.12				
b-value	Regression	9	262.86	29.21	34.83	< 0.0001	0.97
	Lack of fit	5	4.85	0.97	1.37	0.3693	
	Pure error	5	3.54	0.71			
	residual	10	8.39	0.84			
	total	19	271.25				
ΔE	Regression	9	97.79	10.87	20.95	< 0.0001	0.95
	Lack of fit	5	2.24	0.45	0.76	0.6134	
	Pure error	5	2.94	0.59			
	residual	10	5.19	0.52			
	total	19	102.98				
Lycopene	Regression	2	210.51	105.26	13.87	0.0003	0.62
retention	Lack of fit	12	84.10	7.01	0.78	0.6660	
	Pure error	5	44.89	8.98			
	residual	17	129.00	7.59			
	total	19	339.51				

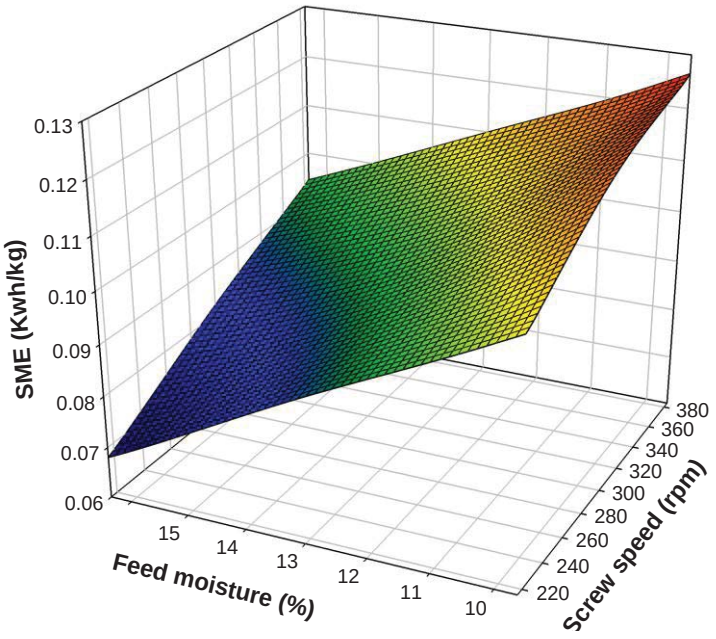

Figure 6-5 Predicted effect of screw speed and feed moisture content on SME of extruded corn products containing 10%w/w tomato derivatives extruded at constant temperature of 150 °C

6.2.4.2. Power Consumption

The regression analysis carried out using the power consumption values and the independent variables showed a significant negative linear effect of feed moisture content and positive linear effect of screw speed and temperature on the power consumption values ($p < 0.05$, Table 6.6). The interaction effect of all of the variables and especially temperature with screw speed and temperature with feed moisture were significant ($p < 0.05$).

A two-factor interaction model (2FI) was fitted to the data (Table 6.6). Despite that the lack of fit test was significant for the model, a high coefficient of determination (0.95),

and a low coefficient of variation (3.13%) was found, which suggests that the model was an acceptable description of the data for these experimental conditions.

The average power consumption during processing ranged from 1.19 to 1.79 Kw (data not shown). Increases in the feed moisture content decreased power consumption (Figure 6.6), while increases in the screw speed required more power input ($p < 0.05$). Power consumption values correlated highly with the results obtained from SME values ($R^2 = 0.96$, $p < 0.01$) (Table 6.7).

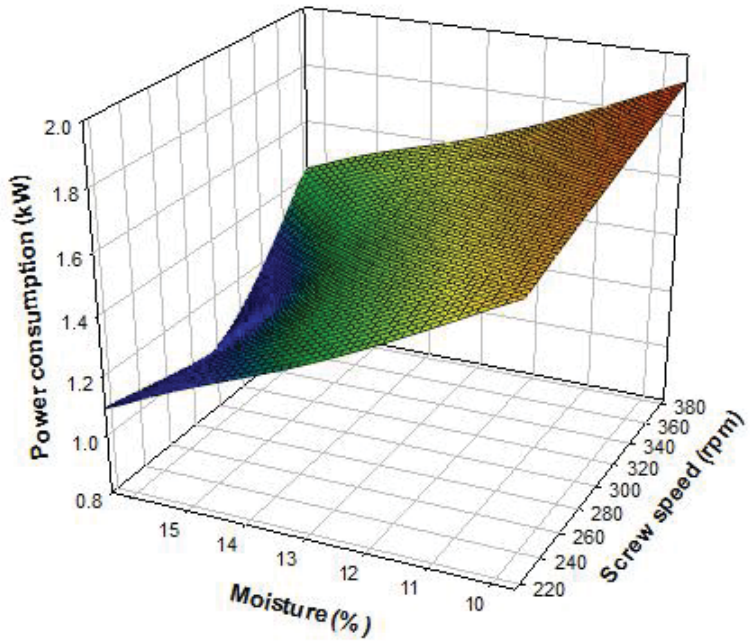

Figure 6-6 Predicted effect of moisture content and screw speed on power consumption of extruded corn products containing 10% (w/w) tomato derivatives extruded at constant temperature of 150 °C

Table 6-7 Correlation coefficient (R^2) between dependant variables for extruded corn product containing 7.5% tomato skin powder and 2.5% tomato paste powder produced using different extrusion conditions

	kW	EXP	D	L	a	b	ΔE	H	LYC
SME	**0.958****	**0.527***	**-0.819****	0.005	**-0.543***	**-0.742****	**-0.698****	**-0.590****	**-0.560***
kW		**0.511***	**-0.868****	0.027	**-0.637****	**-0.819****	**-0.789****	**-0.653****	**-0.602****
EXP			-0.364	0.163	-0.276	-0.278	-0.386	-0.166	-0.068
D				0.031	**0.636****	**0.905****	**0.851****	**0.867****	**0.477***
L					**-0.615****	0.188	-0.249	-0.020	0.029
a						**0.502***	**0.760****	**0.509***	0.372
b							**0.874****	**0.734****	**0.563****
ΔE								**0.710****	**0.553***
H									0.372

kW- Power consumption, EXP= Expansion ratio, D= Density, H= Hardness, LYC= Lycopene retention (%), All significant coefficients are in bold

* Significant at $p < 0.05$

** Significant at $p < 0.0$5

6.2.4.3. Expansion

The expansion of the extruded products ranged between 286 to 350% (data not shown). According to the regression coefficients shown in Table 6.5, for temperature (X_1), screw speed (X_2) and feed moisture (X_3), the increases in the feed moisture decreased expansion, while the increases in temperature and screw speed improved the expansion ratios.

Increases in screw speed and temperature result in an increase in the amount of energy dissipated in the melt thus improving the gelatinisation and break down of the structure of the starch within the melt (*Moraru & Kokini, 2003*). This reduces the viscosity of the

melt, thus favouring foam development and expansion of the bubbles as they form. The modelled effect of temperature and screw speed is shown in Figure 6.7. However, Moraru and Kokini (2003) have suggested that the expansion of extruded products increases with temperature to a point depending on the nature of the raw ingredients, then it decreases due to the extensive break down of the starch, thus weakening of the melt structure.

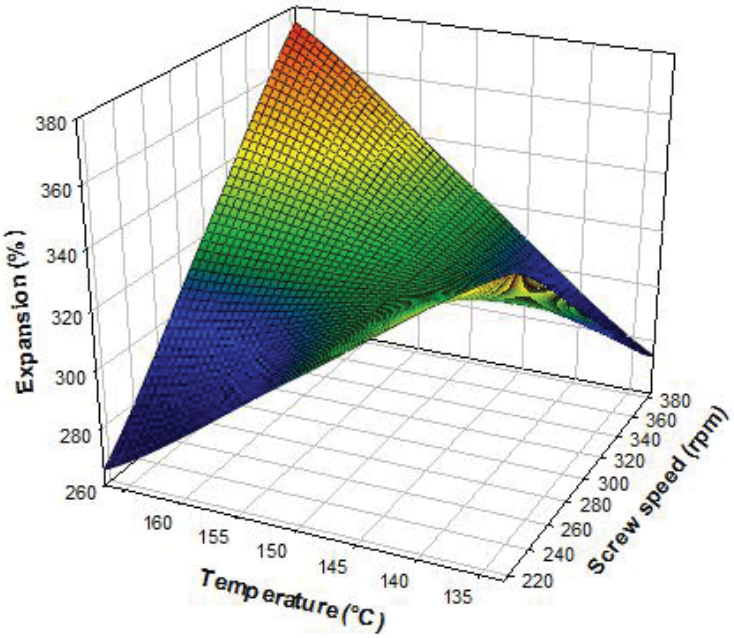

Figure 6-7 Predicted effect of temperature and screw speed on the expansion of extruded corn products containing 10%(w/w) tomato derivatives with an initial feed moisture content of 13%

6.2.4.4. Density

The regression analysis of the data for density values of the products fitted a quadratic model (Table 6.5). As shown in Table 6.5, extrusion temperature (X_1) and screw speed (X_2) had a significant negative linear effect on density of extruded products, while the

feed moisture content (X_3) had a positive effect on product density ($p < 0.05$). The interaction effect of feed moisture, screw speed or temperature negatively affected the density while the interaction effect of temperature and screw speed positively affected the density (Table 6.5).

The density of the extruded products varied between 99 to 158 kg/m^3. Increasing temperature and screw speed slightly decreased the density and increasing feed moisture content also resulted in denser products (Figure 6.8).

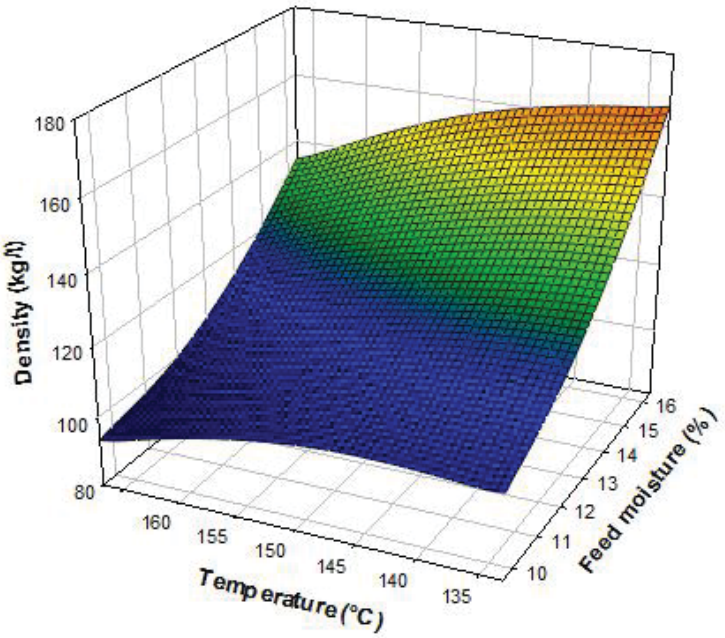

Figure 6-8 Predicted effect of feed moisture content and temperature on the density of extruded corn products containing 10% (w/w) tomato derivatives extruded at 300 rpm

6.2.4.5. Hardness

Hardness is the maximum force needed to bite into a product and can be used to predict the consumer perception of bite hardness of the snacks. Generally, hard products are not accepted by consumers.

A linear model was chosen to best fit the experimental data for hardness. Although the R^2 was low (0.64), the significant F-value and non-significant lack-of-fit showed that the model was appropriate for the experimented conditions (Table 6.6).

Hardness of the products ranged from 143 to 227N. The value decreased with temperature and screw speed and increased with feed moisture content ($p < 0.05$), (Figure 6.9). Previous researchers have also reported that increased screw speed reduced the hardness of extruded snacks. The range of screw speeds used in this work, had less effect on hardness compared to the other variables which is consistent with the reports of other authors (*Altan et al., 2008a; Liu et al., 2000*). Hardness was highly correlated with the density ($R^2 = 0.87$, Table 6.7).

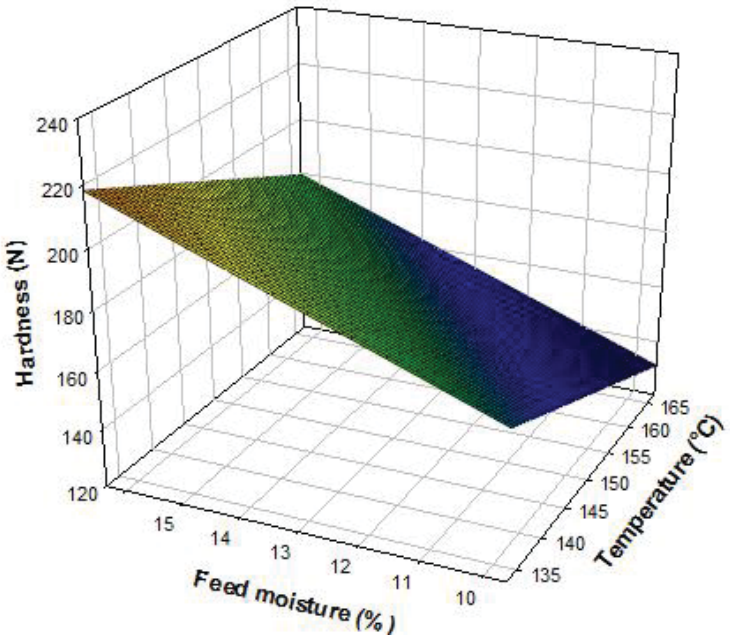

Figure 6-9 Predictive effect of feed moisture content and temperature of extrusion on the hardness of extruded corn products containing 10% (w/w) tomato derivatives extruded at 300 rpm

6.2.4.6. Colour

The colour of a food product has a significant role in consumer acceptability. In extruded corn-tomato products, colour can also indicate the presence of lycopene (*Altan et al., 2008a*). As shown in Table 6.5, feed moisture content was the most intensified variable in the developed models for colour parameters ($p < 0.01$).

The feed moisture content had a quadratic effect on lightness of the products ($p < 0.01$), while temperature and screw speed effected the lightness in linear terms ($p < 0.05$). Screw speed and feed moisture affected the redness of the product in linear terms while feed moisture affected the redness in quadratic terms ($p < 0.01$).

The non-extruded ingredients had an L-value of 98.39, an a-value of 2.26 and a b-value of -2.80, while the colour parameters in the products varied from 93.4 to 97.1 for the L-value, from -0.61 to 1.46 for the a-value and from -4.93 to 9.11 for the b-value. While the increases in screw speed from 250 to 350 rpm increased the lightness of the products, increases in temperature from 140 to 160 °C resulted in the darker products (Figure 6.10). The formation of non-enzymatic brown pigments have been suggested to darken the colour of the extruded products (*Altan et al., 2008a; Ilo & Berghofer, 1999*).

On the other hand, the increases in feed moisture significantly increased the redness of the products possibly due to greater retention of lycopene pigment within the product. Although in Section 6.1, it was shown that the increases in screw speed from 250 to 350 rpm reduced the a-value, in this study, a slight increase in a-value (< 0.25) was found with the increases in screw speed at low moisture levels (Figure 6.11).

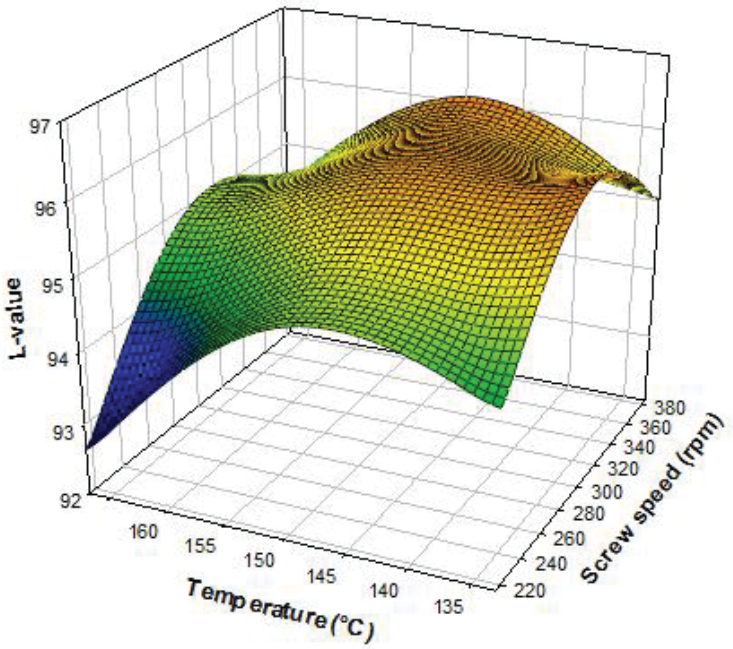

Figure 6-10 Predicted effect of extrusion temperature and screw speed on the L-value of extruded corn products containing 10% (w/w) tomato derivatives extruded with an initial feed moisture content of 13%

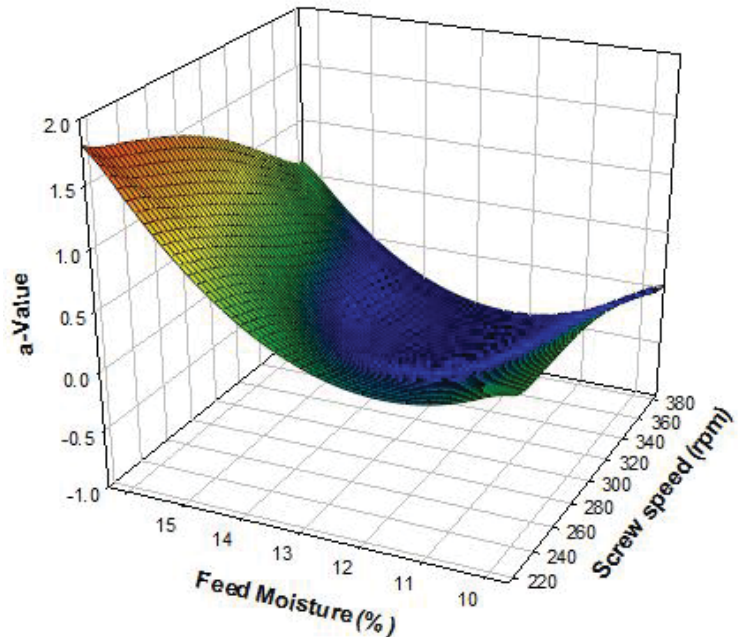

Figure 6-11 Predicted effect of extrusion Moisture content and screw speed on the a-value of extruded corn products containing 10% (w/w) tomato derivatives extruded tomato enriched snacks extruded at constant temperature of 150 °C

The differences between the raw ingredients and tomato skin in the study reported in Section 6.1 compared to a mixture of tomato derivatives in the present study may be the reason for the variation in observations.

The lightness and redness values for the products correlated negatively (Table 6.7), which is similar to previous reports (*Altan et al., 2008a; Ilo & Berghofer, 1999*). Total colour change had a high negative correlation with the SME (Table 6.7).

6.2.4.7. Lycopene retention

A positive linear effect was found between lycopene retention and temperature or feed moisture content. However, the effect of screw speed on lycopene retention was not significant ($p > 0.05$). Analysis of variance for the developed linear model of lycopene retention is shown in Table 6.6.

Lycopene content of the products varied between 91 to 111 ppm (dwb), which corresponded to retention values of between 73 to 91%. As lycopene retention values correlated negatively with SME (Table 6.7), it is possible that the lubricating effect of moisture reducing the SME was the reason for higher lycopene retention in products produced at higher moisture contents (*Killeit, 1994*).

Previous reports have suggested that the loss of β-carotene during extrusion cooking was thermally induced (*Guzman-Tello & Cheftel, 1987 & 1990*), however, in the present study, increases in the extrusion temperature from 133 to 168 °C, slightly (< 3%) improved lycopene retention (Figure 6.12).

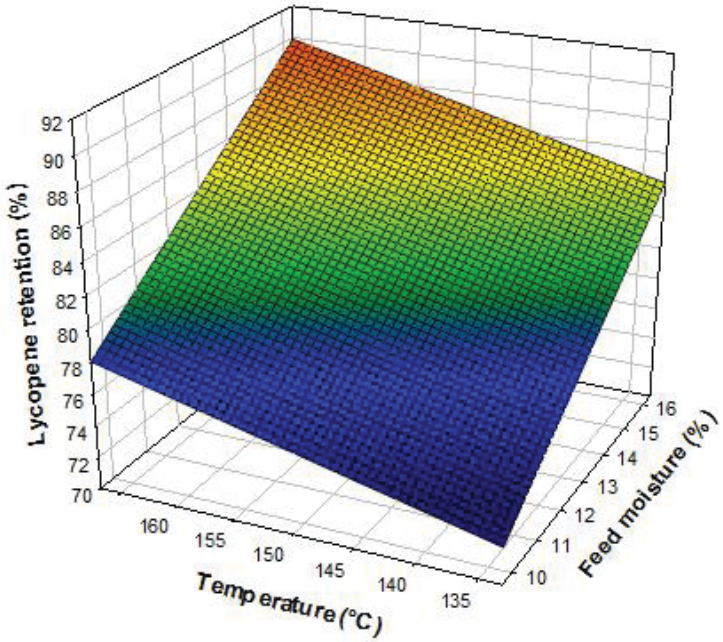

Figure 6-12 Predicted effect of temperature and feed moisture content on percentage of lycopene retention of extruded corn products containing 10% (w/w) tomato derivatives extruded at 300 rpm

Researchers have proposed that thermal degradation of heat labile molecules during extrusion cooking is dependent on the residence time of the melt in the extruder barrel (*Ilo & Berghofer, 1999; Yajnik et al., 2010*).

Research reporting the extrusion of carotenoid pigments has concentrated on adding pure pigments to the extruded products (*Guzman-Tello & Cheftel, 1990; Yajnik et al., 2010*); however, in this study the lycopene was present within the tissue of the tomato skin and paste. In this case, the thermal and mechanical processing during extrusion cooking may have released the lycopene from the cells (*Gartner et al., 1997*), and only upon release was some of this lycopene being thermally degraded. To conclude, it can be proposed that the final amount of lycopene present in the extruded tomato-corn products is a result of the amount of lycopene released from the cells less the amount

degraded during extrusion. The amount of lycopene released and degraded depends on parameters such as the extrusion temperature, mean residence time, the shear history, and the amount of energy dissipated on the melt. In the present study, maximum analysable lycopene occurred at 160 °C and SME of 0.108 kWh/kg.

The screw speed had no significant effect on the concentration of lycopene detected in the extruded products in the present work. Guzman-Tello and Cheftel (*1987 & 1990*) have reported that the degradation of heat labile molecules such as thiamine and β-carotene increased as screw speed increased. Plunkett and Ainsworth (*2007*) showed that the effectiveness of screw speed on the degradation of L-ascorbic acid depends on the range of screw speeds used. For the extruder they used, speeds of more than 200 rpm did not further increase the rate of loss of L-ascorbic acid. Other researchers have also shown that the highest rate of degradation of heat labile molecules occurs at low screw speed and temperature. It may be that at higher screw speeds and temperatures, the residence time of the sample in the extruder barrel is insufficient for the destruction of lycopene (*Cha et al., 2003; Fonseca et al., 2008; Killeit, 1994*).

6.2.5. Conclusion

The study showed that between the processing conditions feed moisture is the most effective parameter on the physical characteristics and lycopene content of the extruded corn-tomato products. By optimizing the extrusion processing conditions, it is possible to maximize the amount of retained lycopene in the corn-tomato products and obtain consumer acceptable products. The optimum conditions in the present study were 12.7% moisture content, extruded at 160 °C at a screw speed of 350 rpm. The obtained

products had a lycopene content of 103 ppm and 7% fibre and an expansion ratio of 346% and hardness of 153 N which is comparable to the characteristics of similar conventional commercial products available in the market.

Chapter Seven Extrusion Cooking Affects Lycopene Bioaccessibility and Starch Digestibility in Extruded Corn-Tomato Products

7.1. Abstract

Response surface methodology was used to investigate the effect of extrusion processing conditions; screw speeds of between 250 and 350 rpm, temperatures of between 140 and 160 °C and ingredient moisture contents of between 11 to 15%, on the amount of lycopene transferred to micellar phase of digesta (bioaccessible lycopene). Furthermore, the rate of digestion of starch in these products was also measured. The work showed that moisture content had a major role on the experimental parameters. Increasing the feed moisture improved the lycopene micellarisation but reduced the starch digestibility. The screw speed had a positive effect on both lycopene bioaccessibility and starch digestibility. Increases in the energy used to extrude the products reduced lycopene concentration in the micellar phase of the digesta, while increasing the starch digestibility within the products. Using the developed models, optimum conditions to obtain tomato-corn products that had maximum values for the starch digestion and lycopene bioaccessibility were predicted to be obtained by using feed ingredients containing 12.55% moisture and extruded at 140 °C with a screw speed of 300 rpm.

7.2. Introduction

The incorporation of tomato derivatives into extruded products provides lycopene which is contained within the cells of the tomato derivatives. Mechanical and thermal processing during extrusion disrupts the cell walls, releasing lycopene and improving its accessibility to enzymes during digestion. Mechanical processing can also destroy the released pigments during cell disruption (*Clinton, 1998; Southon & Faulks, 2003*). By optimizing extrusion processing, a balance between the amount of lycopene released from the cells and potentially available for absorption, and the proportion that is degraded may be obtained. By varying the extrusion process, the extent of starch gelatinisation and other intermolecular interactions such as starch with lipid is altered, and therefore the digestibility of the starch in the products is also changed (*Camire et al., 1990; Singh et al., 2007; Strange & Schaich, 2000).* Thus, to gain a better understanding of the nutritional value of extruded corn-tomato products, the starch digestibility should also be investigated.

The aim of this study was to determine the effect of extrusion processing conditions, namely screw speed, extrusion temperature and feed moisture content, on the lycopene bioaccessibility and starch digestibility in extruded corn-tomato products. Using the data obtained from the study, the optimum extrusion processing conditions required to produce functional extruded products that contain bioavailable lycopene can be determined.

7.3. Materials and methods

7.3.1. Ingredients

The raw ingredients were prepared as described in Section 6.2.3.1.

7.3.2. Methods

The extrusion cooking was carried out according to the description in Section 6.2.3.3. The digestion was carried out according to Section 5.3.4. The digestion process was carried out according to Section 3.3.2. Lycopene content in the extruded products, in the digesta and the micellar phase of the digesta was determined according to Section 3.3.1.

7.3.3. Experimental design and data analysis

The experimental design was carried out according to Section 6.2.3.6. A central composite rotatable design was used to determine the effect of the extrusion cooking parameters, namely, temperature of the last three barrels (X_1), screw speed (X_2) and feed moisture content (X_3) at five levels, as the independent experimental variables (Table 6.3).

The experimental design and the coded and actual levels have been presented in Table 6.4. Response surface methodology was used to investigate the effect of extrusion cooking conditions on the lycopene bioaccessibility and starch digestibility.

7.4. Results and discussion

7.4.1. Lycopene bioaccessibility

The lycopene content of the raw ingredient containing tomato derivatives was 124 ppm (dwb), while that of the extruded products varied between 91 to 111 ppm (dwb). In the unextruded control, 21% of the initial lycopene was transferred to the digesta while 13% of lycopene was incorporated into micelles (data not shown). In the extruded products, 50 to 89% of the lycopene present entered the aqueous phase of the digesta while 11 to 17% of the lycopene entered the micellar phase, depending on the extrusion conditions employed to make the product. Although lycopene that has entered the digesta can be potentially bioavailable, the majority of lycopene present in the micellar phase can be readily taken up by the cells. A linear regression equation was derived from the extrusion processing parameters and the amount of lycopene released into the aqueous phase of the digesta (Equation 7.1):

Equation 7-1 Linear regression of the effect of extrusion parameters on lycopene release from extruded corn products containing 7.5% tomato skin powder and 2.5% tomato paste powder

Lycopene concentration in the digesta (ppm) = 66.02 - 6.19*A + 0.21* B + 4.45*C

Where A, is the temperature of extrusion (°C), B is the screw speed (rpm) and C is the feed moisture content (%).

Increases in extrusion temperature significantly decreased the amount of lycopene in the digesta, while increases in the feed moisture content increased this value (Figure

7.1). It has been proposed that the loss of carotenoids, including lycopene is thermally and mechanically induced (*Guzman-Tello & Cheftel, 1990; Yajnik et al., 2010*). Increasing the feed moisture decreases the amount of work carried out by the extruder on the melt thus reducing the amount of lycopene degradation (*Killeit, 1994*).

On the other hand, increasing the screw speed from 250 to 350 rpm (Eq. 7.1), slightly improved the release of lycopene from the food matrix. This suggests that although shear force applied by the screws can mechanically destroy lycopene, it can also break down the fibrous cell walls enclosing the chromoplasts to release the lycopene. Extrusion of ingredients containing 15% moisture at 140 °C at 250 rpm resulted in the highest lycopene release into the digesta.

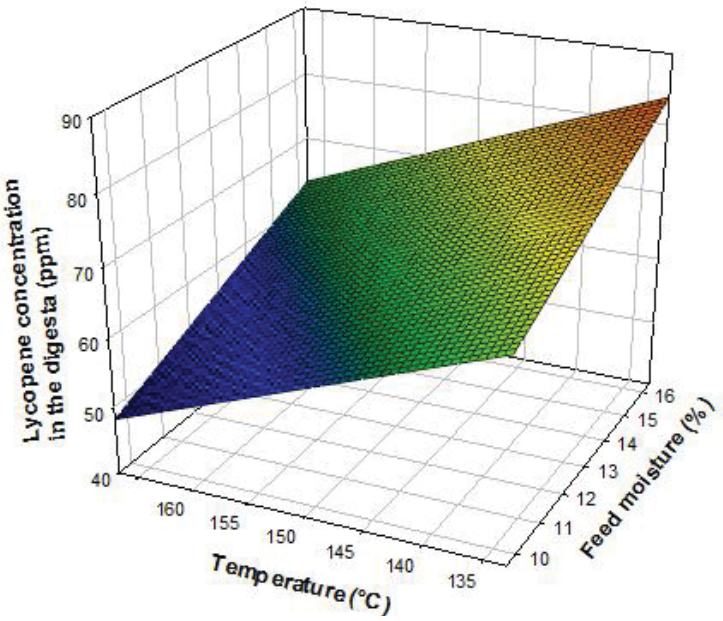

Figure 7-1 Predicted effect of feed moisture content and temperature of extrusion on lycopene concentration in the digesta of extruded corn products containing 10% tomato derivatives extruded at 300 rpm

A linear regression model was found to best predict the effect of extrusion parameters on the proportion of lycopene micellarisation. The equation was as follows (Equation 7.2):

Equation 7-2 Linear regression of the effect of the extrusion parameters on proportion of lycopene micellarisation from extruded corn product containing 7.5% tomato skin powder and 2.5% tomato paste powder

Proportion of lycopene micellarisation= 12.99 – 0.51*A + 0.23*B + 1.34*C

Where A, is the temperature of extrusion (°C), B is the screw speed (rpm) and C is the feed moisture content (%).

Moisture content was most effective extrusion parameter on lycopene micellarisaton. Screw speed slightly improved lycopene micellarisation, while extrusion temperature reduced the amount of lycopene entering the micellar phase of digesta (Figure 7.2).

The extrusion parameters including SME, torque and power consumption values were negatively correlated with lycopene micellarisation (Table 7.3), indicating the detrimental effect of extrusion on lycopene survival during processing. On the other hand, the correlation between the extrusion parameters and lycopene transfer to the digesta was not significant. This may be as a result of the two-sided effect of extrusion on lycopene whereby extrusion releases the entrapped lycopene from the cells, thus improving lycopene transfer to digesta while it may also destroy the pigment that had been released previously during processing.

Optimum conditions that resulted in the maximum lycopene micellarisation were using ingredients containing 15% moisture and extrusion at 140 °C and 350 rpm.

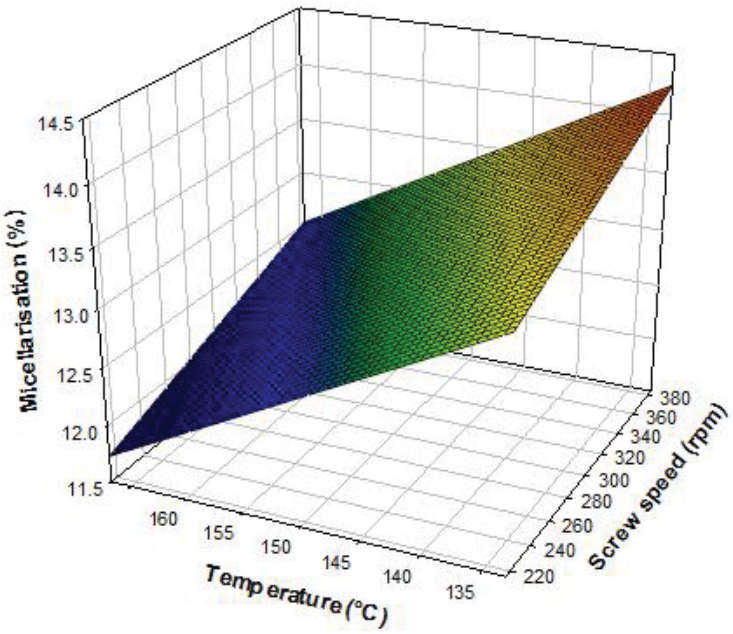

Figure 7-2 Predicted effect of temperature and screw speed on micellar lycopene of extruded corn products containing 10% tomato derivatives extruded with an initial moisture content of 13%

Table 7-1 Pearson's correlation coefficients (R^2) between the extruder parameters and lycopene transfer to the digesta and micellarisation in extruded corn product containing 7.5% tomato skin powder and 2.5% tomato paste powder produced using different extrusion conditions.

	SME	Torque	Power consumption
% Transfer to digesta	-0.34 ^{ns}	-0.37 ^{ns}	-0.38 ^{ns}
% Micellarisation	-0.51*	-0.69*	-0.59*

* Significant at $p< 0.05$,

^{ns} Not significant

7.4.2. Starch digestibility

The amount of glucose released after 20 minutes of digestion (G20) from the unextruded control mixture was 214 mg/g starch, while from the extruded tomato-corn

products it varied between 600 to 837 mg/g starch. The G20 represents the amount of rapidly digestible starch present in food. The raw ingredients are expected to have lower G20 values as they contain ungelatinized starch (*Englyst & Englyst, 2005*). In the extruded products, G20 increases with the amount of gelatinized starch present in the products, while the formation of slowly digestible starch or resistant starch such as amylose-lipid complexes can decrease G20 (*Brennan, 2005*).

Overall, increases in feed moisture decreased the G20 values of the products, while increases in the screw speed improved G20. The effect of temperature on G20 was dependent on the temperature at which the products were extruded. For instance, increasing temperature from 140 to 150 °C decreased G20, while further increases to 160 °C increased G20 (Figure 7.3).

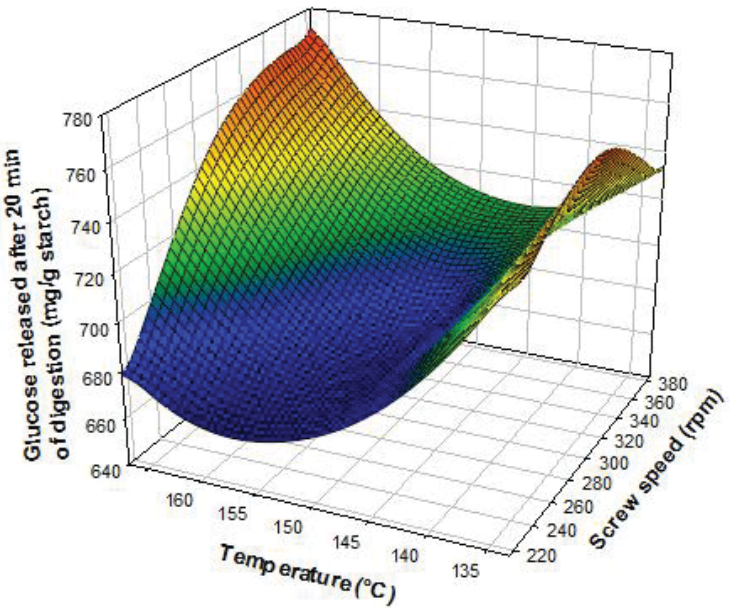

Figure 7-3 Predicted effect of screw speed and temperature on the amount of glucose released after 20 minutes of digestion of extruded corn products containing 10% tomato derivatives extruded at constant temperature of 150 °C

A quadratic model was developed to predict the relationship between extrusion parameters and the proportion of starch digested at G20 (Equation 7.3) and G120 (Equation 7.4):

Equation 7-3 Quadratic regression effect of extrusion parameters on G20 in extruded corn product containing 7.5% tomato skin powder and 2.5% tomato paste powder

$$G20\ value = 681.52 - 4.14*A + 12.22*B - 68.86*C + 26.78*A^2 + 2.93*B^2 - 2.42*C^2 + 11.39*A*B + 20.45*A*C + 22.27*B*C$$

Equation 7-4 Quadratic regression effect of extrusion parameters on G120 in extruded corn product containing 7.5% tomato skin powder and 2.5% tomato paste powder

$$G120 = 909.30 + 10.68*A + 11.78*B - 24.55*C + 6.82*A^2 - 2.46*B^2 - 18.85*C^2 + 24.98*A*B + 17.45*A*C - 3.85*B*C$$

Where A, is the temperature of extrusion (°C), B is the screw speed (rpm) and C is the feed moisture content (%).

At the end of digestion of the raw ingredients (G120), 406 mg glucose/g starch was released, while for the extruded products between 805 to 966 mg of glucose/g starch was released by G120. Compared to the report of Altan *et al.* (*2009*), higher G120 values were obtained in the present study, possibly due to the differences in the raw ingredients used. Increases in temperature and screw speed increased the digestion of starch at G120 showing that increasing these parameters increased the proportion of gelatinised starch. Although increasing the feed moisture content from 11 to 12 % did not significantly affect the proportion of starch digested at the end of digestion (G120), increases above 12% significantly reduced the proportion of starch digested at G120 (Figure 7.4).

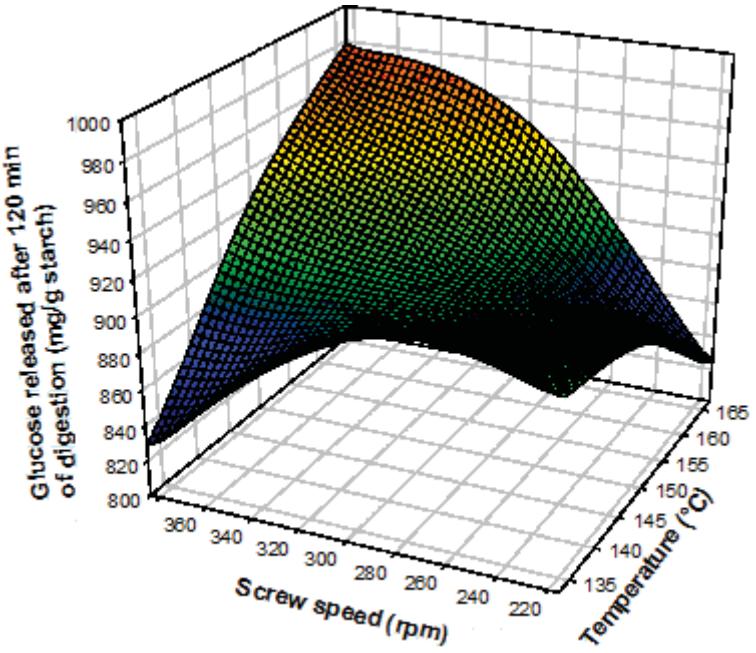

Figure 7-4 Predicted effect of screw speed and feed moisture content on the amount of glucose released after 120 minutes of digestion of extruded corn products containing tomato derivatives extruded at constant temperature of 150 °C

(Table 7.4), confirming that extrusion cooking promoted the gelatinisation of starch and its digestibility.

Table 7-2 Pearson's correlation coefficients (R^2) between the extrusion parameters and G20 and G120 in extruded corn product containing 7.5% tomato skin powder and 2.5% tomato paste powder produced under different extrusion conditions

	SME	Torque	Power consumption
G20	0.70 *	0.52 *	0.80 *
G120	0.57 *	0.59 *	0.63 *

* Significant at $p < 0.05$

7.5. Conclusion

The present study showed that although maximum amount of lycopene bioaccessibility is achieved at low temperature and high feed moisture content, these conditions do not result complete gelatinisation and digestibility of starch. On the other hand, maximum starch digestibility after 20 minutes can be achieved by using the extrusion temperatures of below 150 °C. The formation of less digestible starch complexes at higher temperatures may be the reason for the lower G20 values. According to the developed models, extruding feed ingredients containing 12.55% moisture at 140 °C and at a screw speed of 300 rpm is the optimum conditions to produce extruded corn-tomato products from the formulation. The products obtained in these conditions contained maximum amounts of both potentially bioavailable lycopene and digestible starch after digestion.

Chapter Eight Enzymatic Hydrolysis of Extruded Corn-Tomato Products

8.1. Abstract

To determine the possible interaction between lycopene and starch or protein in the extruded tomato-corn products, the effect of digestion of an extruded corn-tomato product by amylase, amyloglucosidase and protease enzymes and the subsequent extractability of lycopene by solvents was examined. The optimum concentration for each enzyme in respect of release of the highest amount of lycopene was determined. Subsequently, a comparison was made between the amount of lycopene released by using these enzymes at optimum concentrations and the amount released by the addition of pancreatin. The control was water without any enzymes. Amylolytic enzymes and pancreatin released the maximum amount of lycopene, whilst protease released the least amount and was similar to the control ($p > 0.05$). The amount of lycopene released by protease was 6% of the total extractable lycopene. It was concluded that more than 90% of the lycopene in the extruded products was associated with the starch. Using an amylolytic enzyme for digestion, the bonds between starch and lycopene were broken and the lycopene was released thus becoming accessible to solvents for extraction.

8.2. Introduction

In the work described in the previous Chapters, the effect of raw ingredients and extrusion process conditions on the physicochemical and nutritional characteristics of extruded corn-tomato products was investigated. It was shown that lycopene retention and *in-vitro* bioavailability varied with the changes in the formulation. For example, it was found that lycopene is retained more in rice and corn-based products compared to wheat (Chapter 4). Furthermore, the results suggested that the majority of lycopene within the extruded product was not available in free form and became inaccessible to solvents after extrusion. Digesting the extruded product with pancreatin released the inaccessible lycopene and allowed extraction by solvents (Section 3.2). However, in this inaccessible form, the chemical properties of lycopene did not seem to be affected as it was still taken-up by the Caco-2 cells at rates similar to the unextruded lycopene after *in-vitro* digestion (Chapter 5).

It is hypothesized that during extrusion cooking, lycopene is incorporated into the protein or starch component of the melt. Although there are some reports of the formation of protein-lipid complexes in food (*Alzagtat & Alli, 2002; de Planque & Killian, 2003*), the formation of amylose-lipid complexes during extrusion cooking is well described (*Eliasson, 1994; Strange & Schaich, 2000; Wicklund & Magnus, 1997*). In this case, the aliphatic part of a fatty acid, particularly mono-glyceride, is incorporated into the hydrophobic cavity of the amylose single helix. Also it is possible that parts of the amylopectin molecule can also be involved. The inclusion into this structure makes the lipid less accessible to solvents (*Morrison & Coventry, 1989*). The majority of the incorporated lipids are released as the starch is digested with amylolytic enzymes such

as α-amylase. It may be possible that the lipophilic nature of lycopene and its similarities with fatty acids enables it to form structures similar to those formed by lipids with amylose.

The aim of this of the present study was to determine if protein or starch may have interacted with lycopene in an extruded corn-tomato product. This knowledge may provide invaluable information on a method to stabilize the pigment during extrusion cooking. Therefore, extruded tomato-corn product was digested using various concentrations of α-amylase, amyloglucosidase and protease. The amount of lycopene released using these enzymes at optimum concentrations was compared with the amount of lycopene released without digestion and the amount released by using pancreatin.

8.3. Materials and methods

The raw ingredients for the extruded products made with corn and tomato were prepared as described in Section 6.2.3.2. An extruded product was digested using protease (Megazyme cat.no. E-BSPRT), α-amylase (Megazyme cat no. E-BLAAM), amyloglucosidase (Megazyme cat no. E-AMGDF), or pancreatin from porcine pancreas (Sigma product code P7525, 8×USP).

8.3.1. Methods

Corn grits was replaced with 20% tomato paste and extruded at 140 °C in the last 3 barrels, at a feed rate of 6.5 kg/h and a screw speed of 300rpm, according to section

3.3.1.3.2. This formulation and extrusion conditions were chosen to result in a high lycopene concentration to be able to distinguish between digestion treatments. Lycopene recovery assay was carried out according to Section 3.3.1.3.6. The digestion was carried out as described in Section 3.3.1 after some modifications. These modifications were: the enzymes included protease, amyloglucosidase and amylase was used at concentrations of 0, 0.01, 0.05, 0.1, 0.15, 0.2 mg/ml. The pancreatin concentration used in this study was 10 mg/ml as determined from the results of Section 3.3.1. This concentration resulted in the highest amount of lycopene released in the least amount of time in an earlier study (Section 3.2). The extraction and quantification of lycopene was carried out as described in Section 4.2.3.

8.3.2. Experimental design

For the first part of the experiment, a full factorial design was employed that included 3 enzyme types (protease, amylase, amyloglucosidase) at 6 concentrations of 0, 0.01, 0.05, 0.1, 0.15, 0.2 mg/ml. Based on the results from the first experiment, the concentration that gave the maximum amount of released lycopene was used for the second experiment. For the second part of the experiment, a full factorial design using 4 enzymes (pancreatin, protease, amylase and amyloglucosidase) and a water control was used and the amount of lycopene released from the extruded product determined.

A completely randomized design was used to evaluate the results and Analysis of Variance (ANOVA) was carried out to compare the mean values. All significant differences are reported at $p < 0.05$ level. All statistics were calculated using SAS software version 9.1 (SAS Inc., Chicago, IL, USA).

8.4. Results and discussion

8.4.1. Determining the optimum concentration of enzymes to release lycopene

The amount of lycopene released from the extruded product varied between 29.5 to 693 ppm depending on the type of enzyme used for the digestion. The amount of lycopene released by amyloglucosidase and amylase were similar ($p > 0.05$). The maximum amount of lycopene released was obtained at a concentration of 0.05 mg/ml for both amylase and amyloglucosidase (Figure 8.1). At concentrations of above 0.05 mg/ml, no significant increase in lycopene release was found ($p > 0.05$).

Protease released the lowest amount of lycopene regardless of concentration (Figure 8.1).

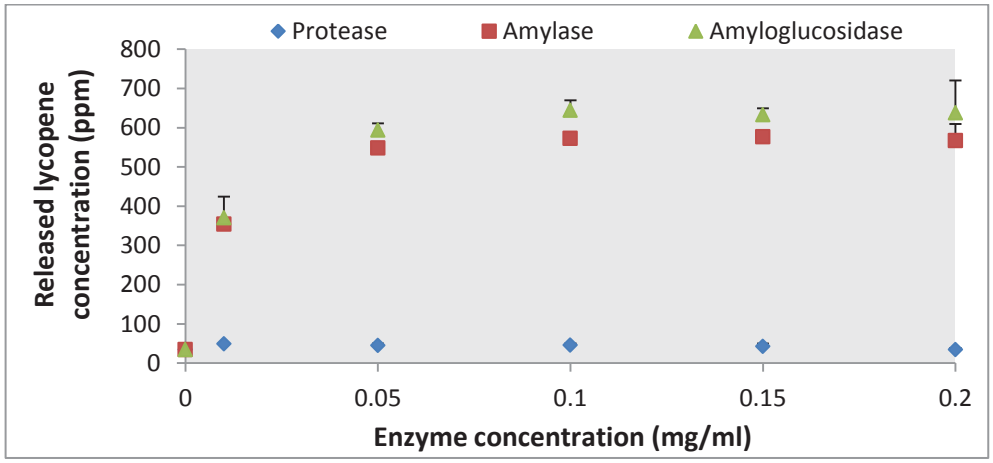

Figure 8-1 Effect of different concentrations of protease, amylase and amyloglucosidase on the amount of lycopene released from the extruded corn-tomato products. Different letters above bars (a-e) indicate the significant ($p < 0.05$) differences between the samples, n= 3

8.4.2. Determining the most efficient enzyme to release lycopene

The amount of lycopene released by pancreatin at 10mg/ml was similar to that released by 0.1 mg/ml amylase or amyloglucosidase ($p > 0.05$). In the absence of amylolytic enzymes, only 6% of the extractable lycopene in the extruded product was detected (Figure 8.2). Digestion by the protease at any concentration resulted in similar concentrations of extracted lycopene to when no enzyme was used, suggesting that protein does not interact with lycopene in extruded snacks.

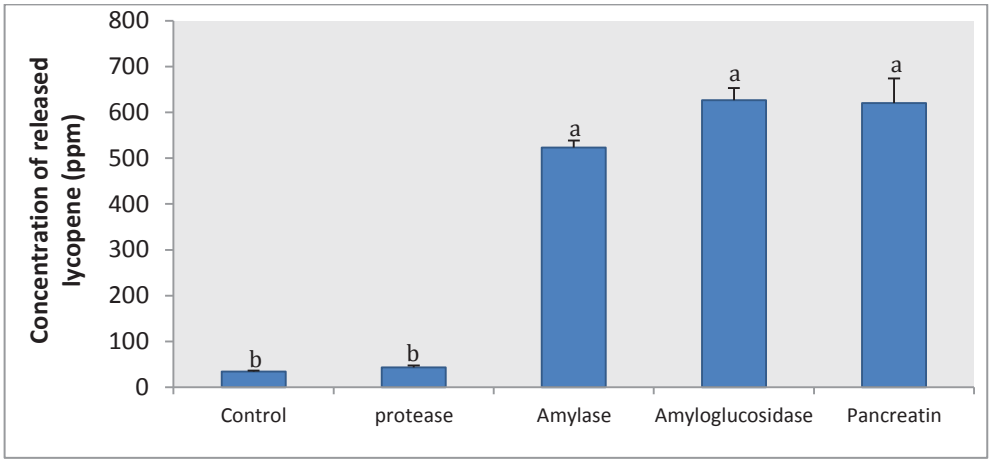

Figure 8-2 Effect of various enzymes on the maximal amount of lycopene released from an extruded corn products containing 10% tomato derivatives. Error bars represent the significant ($p < 0.05$) differences of the means. Different letters above bars (a-b) indicate significant differences between samples, n= 3

It has been shown by researchers that complexes formed between mono-glycerides and starch are less accessible to solvents (*Morrison & Coventry, 1989*). With digestion, the bonds between the lipid and amylose components are broken and accessibility to lipase is increased (*Strange & Schaich, 2000; Wicklund & Magnus, 1997*). This work provides some evidence for the hypothesis that lycopene may bind to the starch in a similar way

to mono-glycerides. Further experiments are required to confirm the mechanism of interaction between lycopene and starch during extrusion cooking.

8.5. Conclusion

The present study suggests that during extrusion cooking, the lycopene becomes associated with the starchy portion of the product. This starch-lycopene complex reduces the extraction of lycopene by solvents. So to release the lycopene, a digestion step with an amylolytic enzyme is necessary to digest the starch and release the lycopene. Formation of starch-lycopene complex could have applications in the food industry by forming a lycopene source that is more stable during storage or processing.

Chapter Nine General Discussion and Conclusions

9.1. Introduction

Expanded snacks are a popular class of convenience food products that have had a rapid market growth over the last decade. They are made using extrusion cooking process. Extrusion processing is a short time, high temperature, high shear process that is known to destroy DNA (*Murray et al., 2007*), vitamins (*Athar et al., 2006*), pigments (*Camire et al., 2002; Marty & Berset, 1986*) and other relatively labile plant materials; and under extreme conditions of extrusion cooking starch may also be dextrinised (*Camire et al., 1990*). During extrusion, starch, the principle ingredient of the extruded product is fully gelatinised. This gelatinisation process occurs as a result of mechanically rupturing the starch granules in low moisture conditions, as opposed to gelatinisation that occurs after heating the starch in a high moisture environment. Gelatinisation converts the starch from a relatively slowly digested carbohydrate to a rapidly digested product. Extruded products contain a high proportion of readily digested carbohydrate and therefore are a good source of energy but are limited in other nutritional qualities.

In this study, dried tomato powders derived from either a paste made from pulp or the ground skins were added to extruded products. Neither product contained the seed fraction. These tomato derivatives are rich in both soluble and insoluble fibre and contain relatively large amounts of the red pigment, lycopene, regarded as a valuable dietary component (*Giovannucci, 1999; Kun et al., 2006*). Tomato paste is available in the market while the skin, although rich in fibre and lycopene, is usually discarded in

the waste stream. Thus, the main focus of this study was to utilize the tomato skin waste stream to improve the nutritional properties of extruded foods. In the work, the raw ingredients and extrusion cooking process were varied to optimize the organoleptic properties and lycopene content of the extruded products containing tomato. Also, the proportion of lycopene available for digestion from extruded products containing paste and/or skin powder was investigated using varying extrusion processing conditions. Furthermore, the changes in the starch digestibility in these products were also investigated.

9.2. Effect of raw ingredients and extrusion processing on the organoleptic characteristics

This study showed that by using only tomato skin powder as the source for lycopene, the resulting extruded products were hard, dense and tasteless. Combining tomato skin with tomato paste resulted in products that were acceptable to consumers (Section 4.2.4.4.3). The optimum combination of tomato skin and paste powders resulted in the well expanded low density products described in Chapter 4. The optimization of the extrusion process for the formulation further improved the physical organoleptic characteristics of the final products. For example, the density was reduced by 36% and hardness by 46% (Chapter 6).

Overall, extruded corn-tomato products were less expanded and more dense compared to the control products made from corn grits only, however, as shown in Chapter 4, products containing tomato powders were more acceptable to consumers compared to

the control products made with corn possibly as result of the red colour, tomato flavour and finer texture of these products compared to extruded corn grits.

9.3. Effect of raw ingredients and extrusion processing on lycopene content and bioavailability

This study showed that a significant proportion of tomato lycopene survived during the extrusion processing of products containing tomato paste and/or skin powder. Although it was evident that a proportion of the lycopene was destroyed by heat and shear during extrusion, a balance was found between the amount of lycopene lost and the amount of biologically available lycopene released from intact tomato cells in the paste or skin powder. Evidence from measurements made during this work suggests that this balance between loss and release determines the concentration of bioavailable lycopene in the extruded products.

Compared to previous reports on carotenoid stability during extrusion cooking (Table 2.1, Chapter 2), generally higher retention values were obtained in the present study. This confirms that supplying the pigment in its natural environment provides some protection which may reduce the losses during extrusion cooking.

It was also shown that it is possible to improve the amount of retained lycopene by optimizing the ingredients used. For example, the utilization of raw ingredients that contain higher concentrations of starch such as rice and corn, as compared with wheat, resulted in products with higher concentrations of lycopene (Chapter 4). It was further confirmed by the results from Chapter 8, that during extrusion cooking, the lycopene

appears to interact with the starch. While the lycopene-starch interaction makes the pigment inaccessible to solvents during extraction, it may also provide some protection to the pigment during the extrusion process. Furthermore, the release of pigment during digestion (Chapter 3 and 8), and its high level of uptake by the Caco-2 cells (Chapter 5), suggest that this interaction may be manipulated to control release rates.

The extruded corn-tomato products that contained the highest lycopene content and had the best organoleptic properties were made using corn grits replaced with 7.5% tomato skin and 2.5% tomato paste powder with initial moisture content of 12.7% and were extruded at a feed rate of 15.5 kg/h at 160°C using a screw speed of 350 rpm, to achieve a specific mechanical energy of 0.108 kWh/kg (Chapter 6).

During the study, colour parameters especially the a-value was shown to be a useful tool to predict the lycopene content of the products. Although, the colour parameters do not provide an accurate estimate of the concentration of the pigment, they will measure the redness and therefore rank products based on their lycopene content.

9.4. Effect of raw ingredients and extrusion cooking on starch digestibility

In the present study, the starch digestibility in the extruded products was investigated as it has been shown that adding fibre-rich ingredients may affect the extent of starch gelatinisation and therefore its digestibility (*Altan et al., 2009; Lue et al., 1991*). Variation in raw ingredients and the extrusion cooking process, resulted in products with a range of starch digestibility values. For example, it was shown that by increasing

the proportion of tomato paste (> 5%), the digestion of starch decreased significantly, while tomato skin had less effect on reducing the starch digestibility (Chapter 5). Utilizing processing temperatures below 150°C resulted in products with a lower proportion of rapidly digestible starch (RDS), while applying higher temperatures increased this proportion (Chapter 7).

The starch digestibility in the corn-tomato products was also considered to be partly dependent on the presence of starch complexes possibly with lipids (Section 3.3.2). Evidence for this hypothesis is provided by the finding that higher concentrations of amylolytic enzyme and bile were required to completely hydrolyse the gelatinised starch in these products. Furthermore, the presence of lycopene-starch complexes as suggested in Chapter 8 may have played a role on reducing the proportion of RDS. However, the significance of these complexes on reducing the starch digestibility requires further investigation.

9.5. Optimum conditions to produce well expanded functional extruded corn-tomato products

One of the aims of the thesis was to investigate the optimum conditions required to produce extruded corn-tomato products that are acceptable to consumers and contain maximum amount of bioavailable lycopene and digestible starch. Common commercial products in the market have an expansion value of between 350-500% and hardness of between 200-400N. Using the models developed in Chapters 6 and 7, extrusion of ingredients with moisture content of 13.33% at 350 rpm and 160°C were the optimum conditions. In these conditions, the extruded products had an expansion ratio of 340%,

density of 106 kg/m^3 and hardness of 158 N. The lycopene content of the products was 102 ppm, of which 13% was accessible for absorption *in-vitro*, and RDS and SDS were 726 and 230 mg/g starch, respectively.

9.6. Further notes

In the present study, due to the limitations in time and finance, a model digestion system was employed to investigate the potential bioavailability of starch and lycopene in the extruded products. These models have been shown to correlate well with the actual bioavailability values of starch and carotenoids (*Englyst & Englyst, 2005; Failla et al., 2008a*). However, performing *in-vivo* studies on the extruded corn-tomato products is necessary to reflect their true nutritional value.

Another reason for the incorporation of tomato derivatives into the extruded products was to increase the proportion of dietary fibre. Although the fibre content of the raw ingredients was determined during the course of the study, determining the ratio of insoluble to soluble fibre, in the extruded products was beyond the scope of the study.

Based on the large body of literature available, it was concluded that lycopene may be beneficial on health (*Aggarwal, 2003; Giovannucci, 1999; Omoni & Aluko, 2005; Rao & Agarwal, 1999; Shi & Maguer, 2000; Singh & Goyal, 2008; Stahl & Sies, 1996; Weisburger, 2002; Willcox et al., 2003*). It can be argued that most of these reports are based on epidemiological studies and not direct observations, nevertheless, experiments on lycopene are on-going and there is considerable evidence available on its potential to promote wellbeing mainly due to its high antioxidant activity. Furthermore, lycopene in the present study served as a model for carotenoid pigments and the results from this

work can potentially assist should the other pigments in this family, such as β-carotene, be incorporated into extruded products.

Finally, throughout the thesis, freeze-dried tomato derivatives (with a moisture content of less than 5%) were used to produce the extruded food products. However, drying of the tomato derivative adds additional cost to the final product and thus may not be commercially viable. According to results from chapter 6, the addition of tomato derivatives containing greater moisture contents (up to 42%) did not significantly affect the expansion ratio or the moisture content

of the extruded products. It should also be noted that the tomato waste coming from the processing plant has high moisture content (> 60%) and is susceptible to microbiological deterioration, thus, processing should be done in the least amount of time.

9.7. Recommended future research

The following recommendations can be suggested for future research:

- In the present study, a model digestion system was developed to simultaneously determine lycopene bioaccessibility and starch digestibility. Using this model, it was shown that extrusion cooking can be used to produce extruded products that contain bioavailable lycopene. However, *in-vivo* studies are required to validate the method developed and also confirm the range of 'true' bioavailabilities of lycopene in these products.

- Lycopene from tomatoes has been reported to be one of the most effective antioxidant carotenoid pigments and was utilised in the present study. However many other vegetables contain carotenoid pigments including pumpkins and carrots. These vegetables also have the potential to be used to make functional extruded products.
- The present study showed that some of the lycopene present in the products became inaccessible to solvents during extrusion cooking. The pigment became much more susceptible when the extruded product was digested *in-vitro* using an amylolytic digestion system but not with a proteolytic system. It was proposed that the lycopene and starch were interacting during extrusion cooking. However, the mechanism of this interaction is not clear but may be similar to that of mono-glycerides and starch. Further exploration in this area may provide a tool to protect lycopene in foods.
- Lycopene retention was investigated in extruded products manufactured using various raw ingredients and extrusion conditions. To better understand the mechanism of lycopene degradation during extrusion cooking, kinetic studies are needed. This information could be used to measure the rates of thermal or mechanical degradation of lycopene during extrusion processing to aid further optimisation of the process.
- Apart from lycopene which was the main focus of this study, tomatoes are also a good source for fibre. Although the concentrations of total dietary fibre in the utilized formulations were reported, the insoluble: soluble fibre ratio was not determined. The changes in this ratio may be beneficial to better reflect the nutritional value of these extruded products.

- The stability of lycopene in the final product and shelf life studies are required to be able to determine the optimum preservation conditions to maintain the lycopene concentration during storage.
- Lycopene degradation can occur due to its oxidation. Previous reports on anthocyanin have shown that by using naturally derived antioxidant compounds such as rosemary extract, it is possible to improve pigment retention during extrusion cooking. Thus, it is recommended to investigate the effect of similar compounds on the lycopene retention in the extruded products.
- Although the present study investigated the consumer acceptability of extruded products containing tomato derivatives, information regarding the nutritional value of the products was not provided to the panellists. Thus, the effect of providing this information on consumer choices warrants further research.

Appendix I Statement of Contribution to Doctoral Thesis Containing publications

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(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Zeinab Dehghan-Shoar

Name/Title of Principal Supervisor: Dr. Gordon Reynolds

Name of Published Research Output and full reference:
Dehghan-Shoar, Z., Hardacre, A.K., Meerdink, G., Brennan, C.S. (2011). Lycopene extraction from extruded snacks containing tomato skin. International Journal of Food Science and Technology, 46(2): 365-371.

In which Chapter is the Published Work: Three

Please indicate either:

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G W Reynolds
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Name of Candidate: Zeinab Dehghan-Shoar

Name/Title of Principal Supervisor: Dr. Gordon Reynolds

Name of Published Research Output and full reference:
Dehghan-Shoar, Z., Hardacre, A.K., Reynolds, G.W. (2010). Effect of bile and pancreatin concentration on the in-vitro bioavailibility of lycopene and starch in extruded snacks containing tomato paste powder. Food Digestion, 1(1-2): 40-46

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Name of Candidate: Zeinab Dehghan-Shoar

Name/Title of Principal Supervisor: Dr. Gordon Reynolds

Name of Published Research Output and full reference:
Dehghan-Shoar, Z., Hardacre, A.K., Brennan, C.S. (2010). Ingredients affect lycopene content and physico-chemical properties of tomato enriched extruded products. Food Chemistry, 123: 1117-1122.

In which Chapter is the Published Work: Four

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We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Zeinab Dehghan-Shoar

Name/Title of Principal Supervisor: Dr. Gordon Reynolds

Name of Published Research Output and full reference:
Dehghan-Shoar, Z., Mandimika, T., Hardacre, A.K., Reynolds, G.W., Brennan, C.S. (2011). Lycopene bioaccessibility and starch digestibility for extruded snacks enriched with tomato derivatives. journal of Agricultural and Food Chemistry, 59(22): 12047-12053.

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