Boysenberry pomace as a source of antioxidant and dietary fibre in an extruded product

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Abstract

Boysenberry pomace as a source of antioxidant and dietary fibre in an extruded products

Boysenberry, a *Rubus* hybrid *(R.ursinus × R.idaeus),* was processed to acquire its pomace. Pomace was used to prepare extruded breakfast cereal products with maize and rice bases. In total 8 types of products were made: maize and rice extrudates with 4 levels of pomace substitution (control, 5 %, 10 % and 15 % (w/w) pomace mix). Each of the products was analysed for its physio-chemical, textural, availability of carbohydrates for digestion and antioxidants activity.

The expansion of the products decreased significantly ($p < 0.05$) with an increase of pomace substitution. There was no distinct linear trend for the other properties such as moisture, crispiness and hardness for maize products. Although, in the case of rice products, crispiness appeared to decrease and moisture increased with increasing levels of pomace in the products. No significant difference was observed with the hardness of the products, the mean hardness of the products ranged from (2981 g – 3241 g).

L*a*b* colour analysis was used to calculate chroma and hue angles for each of the products and raw mixes. Colour of the products tended to darken as the replacement with pomace increased. Mean L* value of pomace was 19.55 which is darker in appearance, whereas mean L* values for control maize and rice were 96.38 and 96.35 and was whiter in apperance. *In vitro* starch hydrolysis of the extruded products showed that the inclusion of the pomace to the extruded products significantly reduced the rate of sugar hydrolysis. The area under the curve (AUC) significantly decreased with the increase of pomace contents in extruded products, which indicate a potential glycaemic reducing action.

The inclusion of pomace in extruded products significantly increased the product's total phenolic content and antioxidant activity. The extrusion process significantly decreased the antioxidant activity of extruded products in comparison to its raw mixes, although extruded product was able to retain an apprecable level of antioxidant activity. Extruded products

containing 15 % pomace (w/w) showed 300 % surge in the antioxidant activity when compared to the control for both maize and rice.

This research illustrates that the introduction of boysenberry pomace to extruded products was able to decrease potential glycaemic action and increased antioxidant activity of the extruded products.

Keywords: *in vitro* analysis; glycaemic response; antioxidant activity; extrusion; phenolics; pomace

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Chapter 1

Introduction

The consumption of fruits and vegetables has always been perceived as a major part of a healthy diet (Wada & Ou, 2002). The world population is becoming more and more health conscious and selective about the food they consume (Benvenuti, Pellati, Melegari, & Bertelli, 2004). The food industry is trying to include phytonutrients as an important part of consumers diet due to the knowledge that antioxidants lower the incidence of cancer, heart disease, delay the ageing process as well as promoting good health (Cooney, Jensen, & McGhie, 2004; Parry et al., 2005). This class of nutritive components is also known as "natural antioxidants" or "nutraceuticals" and consists of compounds such as ascorbic acid, polyphenols, tocopherols, anthocyanins and flavonoid. These compounds show properties similar to enzymes superoxidase dismutase and glutathione peroxidase which protect living systems from free radical damage. Free radicals attack DNA, proteins, lipids, and damage enzymes, cell membrane and genetic materials (Stoner, Wang, & Casto, 2008). Besides antioxidants, the dietary fibre (DF) content of food has received a lot of attention from both researchers and consumers. Dietary fibres are edible plant cell polysaccharides, which are carbohydrates polymers with 10 or more monomeric units such as lignin and pectin. These fibres escape hydrolytic enzymatic digestion in the upper gastrointestinal tract and reach large intestine where they ferment (Brennan, 2005). DF has been linked to regulation of glucose absorption and insulin secretion, maintaining blood lipids level, reducing the chances of heart disease and cancer, preventing constipation and diverticular disease (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005).

Berry fruits such as blueberries, raspberries, boysenberries, blackcurrants, gooseberry and cranberries have been found to be a rich source of antioxidants and dietary fibres (Bener, Shen, Apak, Finley, & Xu, 2013; Manganaris, Goulas, Vicente, & Terry, 2014; Rosales-Soto, Powers, & Alldredge, 2012). This research work focuses on boysenberries and its pomace extract. Boysenberries are hybrid plants derived from blackberry and raspberry; also known as *Rubus* hybrid *(R.ursinus × R.idaeus).* Boysenberries are a very good source of antioxidants such as anthocyanins and ellagic acids. They contain 912, 33.5, 76, 25.5 and 38.2 mg of total ellagitannin, anthocyanin, proanthocyanins, flavonols and ellagic acids per 100 g of

boysenberries respectively. There have been many studies that have incorporated these nutritive and beneficial compounds into various food products such as extruded and bakery products in order to determine effects of increasing the nutritive value of the food (Camire, Chaovanalikit, Dougherty, & Briggs, 2002; Grigelmo-Miguel, Carreras-Boladeras, & Martín-Belloso, 1999; Wang & Thomas, 1989). New Zealand is the top grower of boysenberries in the world with a total export value of 6 million per year (Horticutural, 2014).

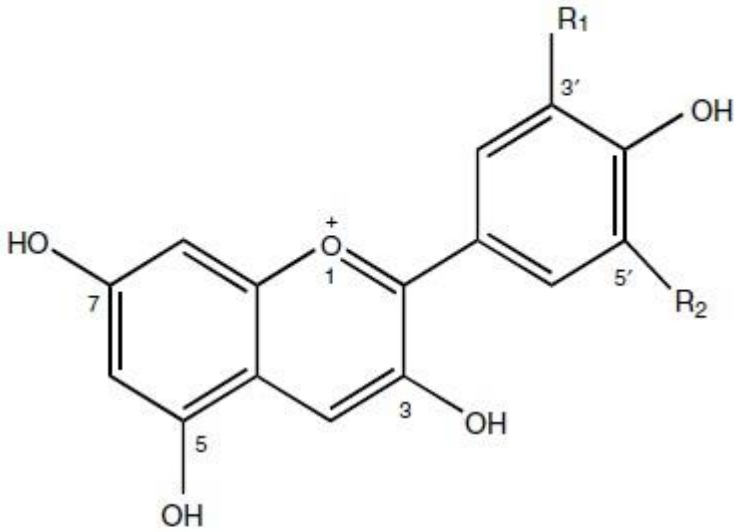
Boysenberry consists range of amino acid, Fatty acids and tocopherol (Table 1.1). Boysenberries have been shown to be as high sources of phytonutrients, antioxidants and dietary fibres (Bushman et al., 2004; Ghosh, McGhie, Zhang, Adaim, & Skinner, 2006; Zyren, Elkins, Dudek, & Hagen, 1983). Four main anthocyanins are present: cyanidin-3-O-sophorose, cyanidin-3-O-glucoside, cyanidin-3-O-2G glucosyl-rutinose and cyanidin-3-O-rutinose; and four ellagitannins: galloyl-sauguniin H-6, sanguiin H-10, sanguiin H-6 and sanguiin H-2 (Kool, Comeskey, Cooney, & McGhie, 2010). Anthocyanins are reported to possess high antioxidant activity inhibiting low-density lipoprotein oxidation and have exhibited vasoprotective and anti-inflammatory activities (Wada & Ou, 2002).

Table 1.1: Nutritional Composition of boysenberry (adapted from Bushman et al. (2004))

Amino acids	mg/100 g fruits	Fatty acids	%fatty acids
glutamic acid	1.56	16:0 (palmitic acid)	3.5
aspartic acid	0.69	16:01	
arginine	0.58	16:02	
leucine	0.46	18:0 (stearic acid)	1.5
glycine	0.44	18:1 Δ6	
alanine	0.3	18:1 Δ9 (oleic acid)	11.6
valine	0.32	18:1 Δ11	0.7
isoleucine	0.32	18:2 (linoleic acid)	59.1
lysine	0.29	18:3 Δ6,9,12	
phenylalanine	0.27	18:3 Δ9,12,15	22.1
proline	0.27	(γ -linolenic acid)	
serine	0.25	20:00	0.7
threonine	0.22	20:01	0.5
histidine	0.2	20:02	0.1
cysteine	0.18	22:00	0.2
tyrosine	0.14		
methionine	0.14	**Tocopherol**	**(mg/100 g seed)**
hydroxyproline	0.03		
tryptophan	0.04	α-tocopherol	2.0 ± 0.003
taurine	0.06	γ-tocopherol	15.4 ± 0.080
ornithine	0.01	total	17.5 ± 0.084

1.1 Antioxidants and Dietary fibres:

Anthocyanins are a group of flavonoid compounds and are o-glycosides and acyloglycosides of anthocyanidins. They consist of core aglycone moiety, sugar or acyl sugar substituents. The most common anthocyanins present in fruits are shown in figure 1.1**.** Various sugars bind to the 3, 5, 7, 3' and 5' carbon of the skeletons leading to a large number of differents anthocyanidin. Among them, the most common are C3 mono, di and triglycoside, and most common sugars being D-glucose, D-galactose, D-xylose, Arabinose and L-rhamnose.



Anthocyanidin	R1	R2	Aglycone MW
Cyanidin (Cy)	H	OH	287
Delphinidin (Dp)	OH	OH	303
Malvidin (Mv)	OMe	OMe	331
Pelargonidin (Pg)	H	H	271
Petunidin (Pt)	OMe	OH	317
Peonidin (Pn)	OMe	H	301

Figure 1.1 Chemical structure of anthocyanidin skeleton (adapted from Cooney et al. (2004))

The main source of dietary fibres (DF) in the human diets are plant materials and cell wall materials (Figuerola et al., 2005). They generally escape digestion in the small intestine and reach the large intestine to undergo fermentation, they also affect the process of metabolism through physiochemical interaction with one another and with other food components (Kristensen & Jensen, 2011). DF are characterised by properties such as hydration and solubility. Generally, dietary fibres are classified into soluble dietary fibres and insoluble dietary fibres. Gums and hydrocolloids are common examples of soluble dietary fibres. They are widely used in food processing industry to form gels and viscous products (McKee & Latner, 2000). DF can increase the food nutritive value and change the physical properties and features of the food. Insoluble dietary fibres are not soluble in water. DF are also known to reduce the transit time in the small intestine. This may be due to the fact that they increase the digests (chime) viscosity and create a diffusion barrier, which alters the digestion rate and nutrient absorption. DF helps in the moderation of postprandial glucose and insulin response, also reduce low-density lipoprotein (LDL) cholesterol (Brennan, 2005).

Antioxidants and dietary fibres are important components in a healthy diet food. Antioxidants neutralise the free radicals – which are by-products of a various oxidative process in the human body. These free radicals can damage membranes, cellular proteins, lipids, DNA and have been linked with cardiovascular disease and cancer (Agbenorhevi, & Marshall, 2011). DF, on the other hand, is beneficial to human health (Kendall, Esfahani, & Jenkins, 2010) in terms of prevention of constipation, maintaining colonic microflora, modulating blood sugar and reducing the risk of colorectal cancer (Brennan, 2005; Brownlee, 2011).

There have been numerous studies about the use of natural oxidants in recent years. Examples of natural antioxidants are polyphenols, ascorbic acids, tocopherol and carotenoids. These substances protect the living system from free radicals- which damage lipids, proteins, DNA, cell membrane, enzyme and genetics materials (Ghosh et al., 2006). Natural defence mechanisms carried by superoxide dismutase and glutathione peroxidase may not be sufficient when there are unusually high loads of oxidants and free radicals (Benvenuti et al., 2004). Hence the natural antioxidants present in the consumed food helps by quenching free radicals. 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) is widely used in the evaluation of natural antioxidants for its free radical scavenging activity (Wendy, Cuvelier, & Berset, 1995).

Plant extracts such as anthocyanosides and polyphenols act as radical scavengers and as an antilipoperoxidants. They also protect degradation of collagens by superoxide anion radicals and inhibit the enzymes xanthine oxidase - an enzyme which generates a superoxide radical. Furthermore, they decrease capillary fragility and permeability and possess high antiphlogistic effects (Benvenuti et al., 2004). Anthocyanins are absorbed and excreted intact and are partially metabolised in human (Cooney et al., 2004). They appear to have limited bioabsorption but reduce the oxidative stress and risk of degenerative disease when they are absorbed and circulated around the body (Ghosh et al., 2006).

1.2 Pomace incorporated to food system

Berry fruits can be a good source of antioxidants and dietary fibre which can be incorporated into various food products to enhance nutritive and functional values of food. There have been numerous studies exploring the potential to develop baked and extruded products enriched with dietary fibres and antioxidants. Fruits, vegetables, and their pomace, have been added to the food products baseline formulation in variable concentrations to produce the desired product. These products have been reported to have higher antioxidant activity and dietary fibre content. For instance, Ramírez-Maganda et al. (2015) used mango processing by-products to develop a muffin, which had higher phenolics compared to a control muffin and reported that the product could modulate the rate of starch digestion. Other research has illustrated that the substitution of 15 % flour by peach pomace in muffins received high consumer acceptance in terms of good flavour and mouthfeel as well as lowering calories intake and increasing DF contents (Grigelmo-Miguel et al., 1999). The nutritional quality of such foods appears to be improved. For instance, there was a significant decrease in postprandial glucose response with 30 % cherry pomace fortified muffin when compared to plain control muffin (Bajerska, Mildner-Szkudlarz, Górnaś, & Seglina, 2015). The inclusion of the flesh fibre concentrate of apple, pears and dates resulted in an improved dough performance as well as potential uses to develop value added products such as cereal bars, biscuits and bread (Bchir, Rabetafika, Paquot, & Blecker, 2014). Muffins enriched with grape pomace have been shown to lower toxic CML (N^6-carboxymethyl lysine) levels without any significant changes in the sensory profile when compared with the control muffins (Mildner-Szkudlarz, Siger, Szwengiel, & Bajerska, 2015). In contrast, there were significant changes in the sensory profile in the grape pomace incorporated muffin and 30-40 percent decrease in antioxidant activity after baking (Bhise, Kaur, & Aggarwal, 2013). Similarly, wine grape pomace fortified muffin were observed to have significant total phenolic content and antioxidant activity and increased dietary fibre content (Walker, Tseng, Cavender, Ross, & Zhao, 2014). Muffins supplemented with 10 % red raspberry juice were reported to retain total phenolic though certain amounts were degraded during baking (Rosales-Soto et al., 2012). Similar outcomes were observed in muffins enriched by strawberry, raspberry and blackcurrant pomace (Górnaś et al., 2016), where there was 36-97 % loss in anthocyanin whereas 100 % recovery of ellagic acid was observed. Cooking at high temperature combined with a short baking period had the best effect in the preservation of anthocyanin. Other researcher have reported that a substitution of 16 % wheat flour by apple skin powder in muffin gave a positive

sensory feedback when evaluated by 66 untrained panellist (Rupasinghe, Wang, Pitts, & Astatkie, 2009). In addition, apple pomace has been used to formulate extruded snack with higher dietary fibre and antioxidants contents (Reis, Rai, & Abu-Ghannam, 2014). Similar outcomes were observed with blackcurrant pomace (Mäkilä et al., 2014), pineapple pomace (Selani et al., 2014), chestnut mushroom (Brennan, Derbyshire, Tiwari, & Brennan, 2012) and blueberry pomace (Khanal, Howard, Brownmiller, & Prior, 2009).

Many studies have been conducted with berry fruits such as blueberries, strawberries, cranberries and their integration in various food products (Sun, Paraman, & Rizvi, 2015). However, very few papers have addressed the boysenberries composition (Allen, Rowan, Shaw, & Franich, 1996; Manganaris et al., 2014) and there is much to be done about its integration with food products. This research report focuses on developing extruded products by incorporating boysenberry pomace and the evaluation of the nutritional value and sensory aspect of the final products. The developed food products were evaluated to determine if they provide health benefits to the consumers.

The research work is focused on determining the contents of boysenberries and its pomace after drying and the use of boysenberries pomace as a source of antioxidants and dietary fibres in model food. Dried and milled boysenberries pomace will be used to prepare extruded products. Chemical, physical and sensory analysis will be carried out with these products and to evaluate their antioxidant properties and dietary fibre contents.

1.3 Objectives

The general aim of this research work was to utilise the quality attributes present in boysenberries pomace to produce an extruded products. To achieve these overall objectives the following objectives were identified to:

- To extract pomace material from boysenberry.
- To produce an extruded product using boysenberries pomace with the range of different concentration (mixture).
- To examine the characteristics of extruded products (such as texture analysis, expansion, density, porosity, colour and sensory analysis).

- To conduct chemical analysis on final products (such as protein, fats and fibre contents, glycaemic impact and antioxidants activity).

1.4 Hypothesis

1. This research work is focused on the development and evaluation of extruded products using the boysenberry pomace. The process is expected to alter the physical characteristic properties such as expansion, colour and crispiness of the product.

2. The total content of bioactive compounds such as antioxidants and phenolic of the boysenberries pomace will be reduced by food processing.

3. The inclusion of berry pomace will reduce the degree of carbohydrate digestion.

Chapter 2

Literature Review

2.1 Boysenberries and their history in New Zealand

Boysenberries are hybrid plants derived from blackberry and raspberry. Boysenberries are a member of family Rosaceae and belong to the genus *Rubus* (*R.ursinus × R.idaeus).* Fruit morphology consists of many loose drupelets clustered firmly to the central column of the receptacle (Vicente et al., 2004). Boysenberries are widely grown in the California & Oregon states of USA, New Zealand and Chile. Total production for the year 2014 was 1156 tonnes and 3100 tonnes in the USA and New Zealand respectively (NASS, 2015). They are self-fertile and a large fruit can be up to 20 g. The colour of the fruits is deep wine red and fruits contain high sugar (around 12° Brix). The sugar- acid balance is good and pH increases as fruits ripen (Hall & Langford, 2008). Boysenberry fruits consists of 8.0 - 13.0 % of soluble solids, 4.26 – 6.43 % of sugar and acid 13.0-40.0 mill equivalent (meq)/ 100 g fruits (Given, 1985). The surface of the fruits have small hairs which make its appearance dull and dusty.

Boysenberries are very perishable fruits, so they are generally frozen as soon as they are harvested (Galletti et al., 1993). On average there are 15-20 canes per plant and cane length reaches 3-4 m. Yield decreases sharply due to cold temperature during flowering time. It is also prone to wilt disease caused by *Verticillium albotrum*. In the past few years, there has been steady growth in New Zealand boysenberries production. Total exports were valued at around 6 million for the year 2014 (Horticutural, 2014).

2.2 Berries and their nutritive constituents:

Rubus berries (raspberries, blackberries, and hybrids such as loganberry, boysenberry and tayberry) are low energy fruits. Fructose is the main sugar component of this variety. They have low natural sodium, cholesterol and long chain omega -3- fatty acids. Instead, they provide dietary intake volume of micronutrients such as vitamin C, potassium and folate (Table 2.1). The overall classification of berry antioxidants is shown in the Fig 2.1. The word phenolic in plants represents group of secondary metabolites. These metabolites consist of one to a number of aromatics rings and are linked to fruit colour, astringency, taste and bitterness. These compounds have a variable degree of hydroxylation, methoxylation and glycosylation.

As shown in Fig 2.1 the main phenolic in berry fruits can be divided into 4 groups: phenolic acid, flavonoids, tannins and stilbenes.

Anthocyanin are flavonoids and high amount of these compounds have been found in berry fruits. Their concentration varies within species and is between 2.43 mg/100 g to 1113.1 mg/100 g besides factors such as harvest time and growth temperature plays an important role in variation of anthocyanin within same variety (Probust, 2015).

Table 2.1: Nutrient composition of fresh Rubus (per 100 g) sourced from Australian (1999, 2010) and New Zealand (2008) food composition databases. (adapted from Probust, 2015)

Nutrient	Raspberry[a]	Blackberry[b]	Boysenberry[3]	Loganberry[c]
Energy, including dietary fibre, kJ	225	211	184	292.8
, kcal	54	50	44	70
Moisture, g	84.6	84.2	85	84.5
Protein, g	1.2	1.4	1.1	1.5
Fat, g	0.3	0.3	0.7	0.3
Dietary fibre, g	6.1	6.1	3	8.1
Fructose, g	3.8	3.9		
Glucose, g	3.1	3.6		
Sucrose, g	0.1	ns		
Maltose, g	ns	ns		
Lactose, g	ns	ns		
Total sugars, g	7	7.5	7.1	4.9
Starch, g	0.3	ns	0.1	
Mannitol, g	0.1			
Sorbitol, g	ns			
Available carbohydrate, without sugar alcohols, g	7.3	7.5		
Available carbohydrate, with sugar alcohols, g	7.4	7.5	7.2	4.9
Lactic acid, g	ns	0.1		
Malic acid, g	0.1	0.2		
Acetic acid, g	ns	ns		
Citric acid, g	2.4	0.4		
Quinic acid, g	ns	ns		
Calcium, mg	28	30	24	27
Copper, mg	0.104	0.16		
Fluoride, µg	ns	ns		
Iron, mg	0.6	0.42	0.8	0.6
Magnesium, mg	22	30		21
Manganese, mg	0.565	0.55		
Phosphorus, mg	37	29	19	26
Potassium, mg	169	114	150	161
Selenium, µg	1.2	2	0.1	
Sodium, mg	1	ns	3	1

Sulphur, mg	14	16		
Zinc, mg	0.36	0.24	0.5	0.3
Iodine, mg			0.2	
Thiamin, mg)	0.037	0.02	0.01	ns
Riboflavin, mg	0.027	0.03	0.02	ns
Niacin, mg	0.36	0.3		0.8
Niacin equivalents, mg	0.56	0.53	1.1	1.1
Pantothenic acid, mg	0.39	0.35		
Pyridoxine, mg	0.05		0.01	
Biotin, µg	5.7	1.4		
Folate, natural, µg	34	36		
Total folates, µg	34	36	63	34
Dietary folate equivalents, µg	34	36		
Alpha carotene, µg	ns	ns		
Beta carotene, µg	28	150		
Cryptoxanthin, µg	ns	340		
Beta carotene equivalents, µg	28	320	301	25
Retinol, µg	ns	ns		
Retinol equivalents, µg	5	53	50	4
Vitamin C, mg	32	38	9	22
Alpha tocopherol, mg	0.8	1.4		
Vitamin E, mg	0.77	1.4		
Total polyunsaturated fatty acids, %	46.2			
, g	0.1	ns	0.5	
Total long chain omega-3 fatty acids, mg	ns		ns	
Cholesterol, mg	ns	ns	ns	

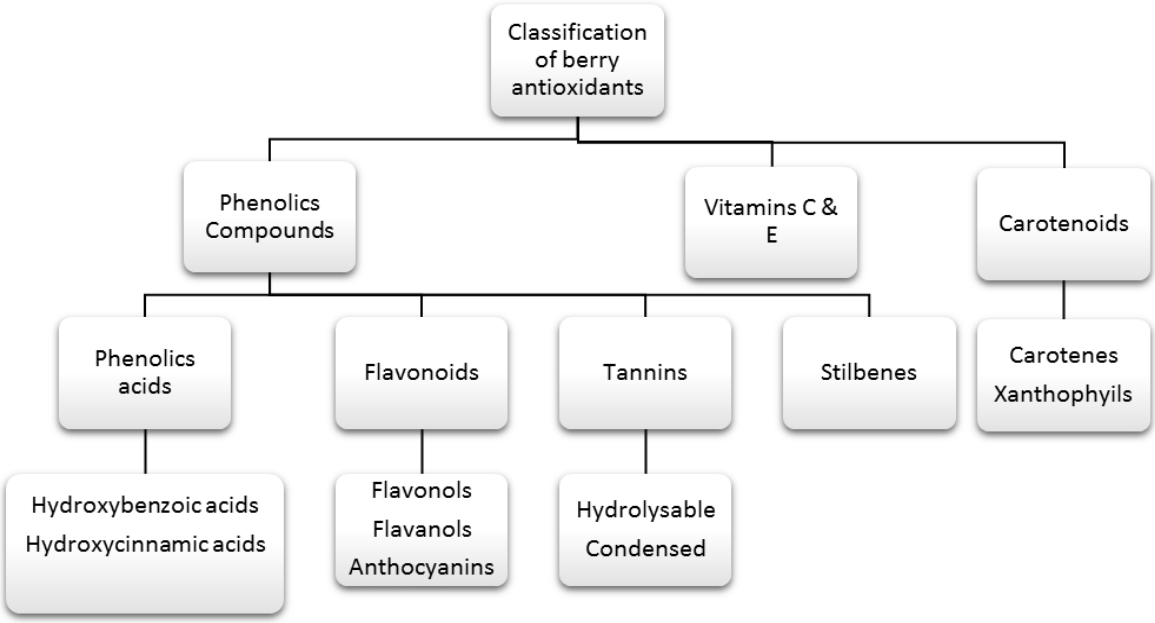


Figure 2.1: Classification of berry antioxidants. (Manganaris et al., 2014)

In the case of blackberries the total anthocyanins present vary from 11.08 mg/100 g to 1660 mg /100 g. Cyanidine -3- glucoside is one of the the major anthocyanin in blackberries. In contrast the mean anthocyanin content for boysenberries has been reported to be around 1514 µg/g and total proanthocyanin from its juice and seeds to be 1.14 mg/ 100 g and 76.3 mg /100 g respectively (Probust, 2015). Similar concentrations have been reported in commercial boysenberry juice where 116 mg total ellagitannins per 100 mL juice and 3.26mg / 100 mL juice total flavonol was reported (Furuuchi, Yokoyama, Watanabe, & Hirayama, 2011). Similarly, Benvenuti et al. (2004) studied berry extracts of blackberry, raspberry, black currant, red currant and black chokeberry for their polyphenol, anthocyanin, ascorbic acids and the radical scavenging activity using the DPPH method. The average total phenol was reported to be 639.8 mg/100 g fresh weight (FW), and the average total anthocyanin was 165.44 mg/ 100 g FW. The highest content of anthocyanin was reported for black chokeberry about 460.5 mg/ 100 g FW. The seeds of berry fruits also have high amounts of antioxidants. Bushman et al. (2004) studied the value added use of seeds from caneberries (red raspberry, black raspberry, boysenberry, Marionberry and evergreen blackberry). Ellagitannins and free ellagic acids were the main phenolics detected in all varieties, the highest amount was detected in boysenberry and blackberry. A considerable amount of tocopherol (γ-tocopherol) was observed in raspberry.

Williams, Edwards, Pun, Chaliha, and Sultanbawa (2014) have reported a different form of ellagic acid (EA) present in boysenberries and strawberries and their antioxidant properties, reactivity, solubility and bioavailability. In nature EA occurs in the different form as a free compound; EA glycosides- which is glycosylated via its hydroxyl groups; and in polymeric form Ellagitannins- polymers esterified with a sugar. EA possess double bonds and it can react with free radicals. The EA glycosides extracted from red raspberries have been reported to have highest antioxidative properties and assumed to be connected with their degree of hydroxylation. Vitamin C and ascorbic acid have been reported to protect EA and EA tannins. Williams et al. (2014) reported 37 mg/100g DW Vitamin C in Boysenberries and 503 mg/100g DW in strawberries and have linked its function in protection of EA, EA glycosides and EA tannins; EA, EA glycosides and EA tannins were reported to be 5.5, 15, 147 mg/100 g DW and 4.8, 8.8, 50.7 mg/100g in boysenberries and strawberries respectively. Recent research published by McGhie, Martin, and Lunken (2012) points to the importance of EA from *Rubus* berries mainly boysenberries in inhibiting mitogen-activated protein kinase. Protein tyrosine

kinase JAK2 is one of the important components in cell signalling process. JAK2 is a mediator used by cytokine to signal prolactin, interleukins 2-6, erythropoietin and IFN_{γ}.

2.3 Extrusion process and extruded food products:

Extrusion is a process where a material is forced or pushed through a die of the desired cross-section to produce a similarly shaped object. It is widely used in the production of metal, plastics and food. In the case of the food industry, ready to eat snacks, breakfast cereals, pasta and sausange are the few examples of produced using extrusion process.. The food material is pushed through an orifice / die of a desired shape using a piston or a screw.

Extrusion cooking is multi-step involving thermal and mechanical processess for the preparation of a large number of food varieties. During the cooking process, the food material is plasticized and cooked in a tube at high temperature for a very short time. Factors such as temperature, moisture, and mechanical shear and pressure results in the chemical reaction and transformation of the molecular structure (Singh, Gamlath, & Wakeling, 2007).

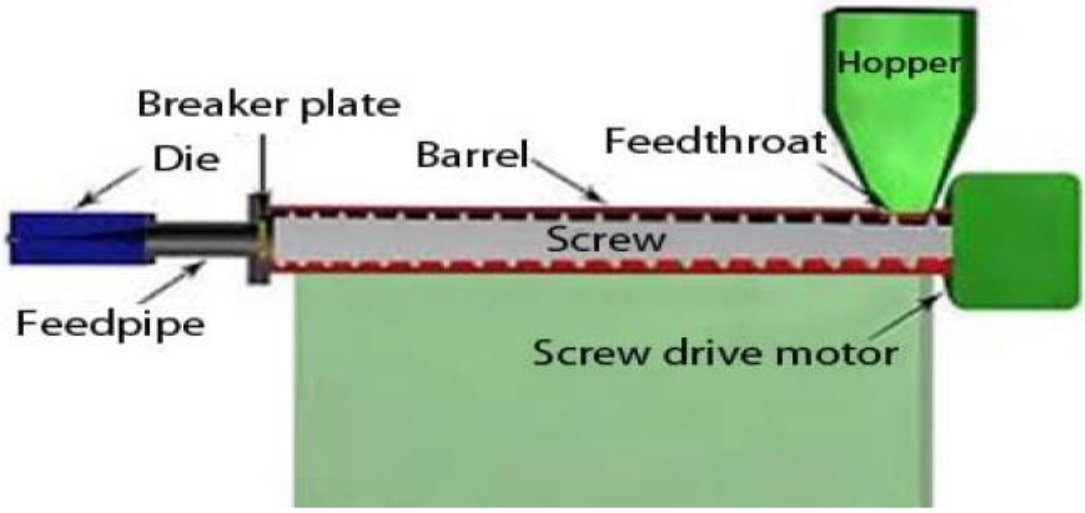


Figure 2.2: Basic extruder adopted from Rao and Thejaswini (2015)

Generally, two type of extruders are being used in food industries on the basis needs and final products. They are:- single-screw; or twin-screw extruder. These two types of equipment perform slightly differently in terms of final product quality, but share the same basic principle. A typical single-screw extruder consists of a feeding screw, extruder barrel, feed hopper,

preconditioning cylinder, die and knife (Figure 2.3). It is very economical in compared with the twin-screw extruder.

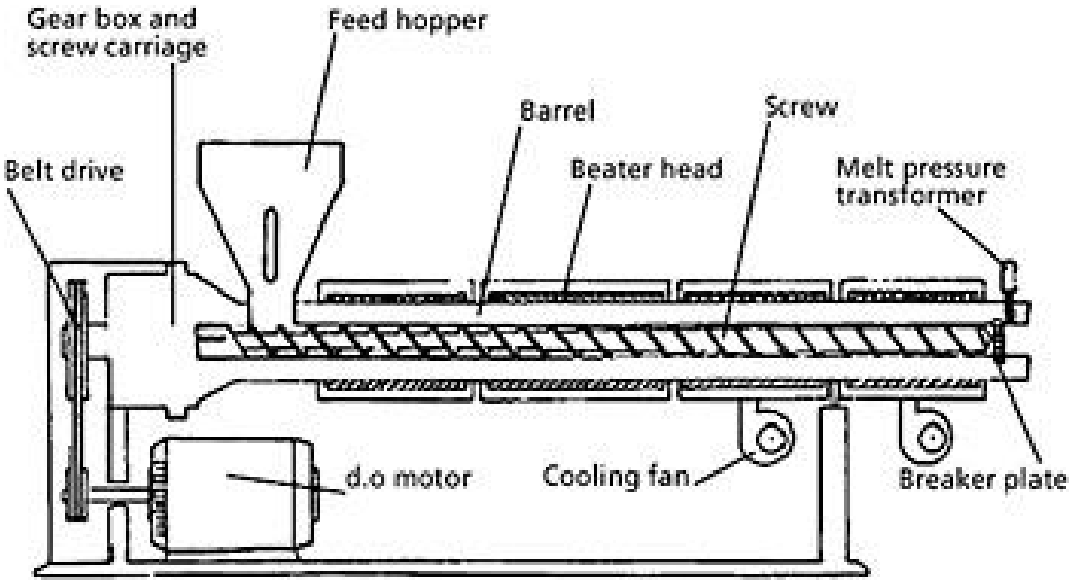


Figure 2.3: Single screw extruder adapted from (www.globalspec.com)

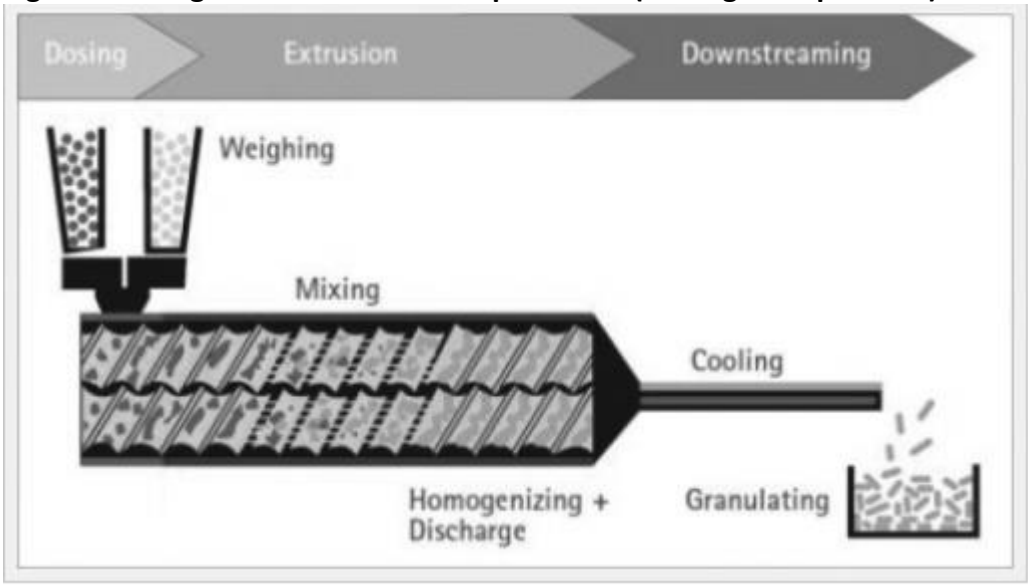


Figure 2.4: Twin-screw extruder adapted from Reddy, Chaitanya, and Rao (2011)

Single-screw extruders often experience slippage and surging of the food product which may affect output. For instance, the high pressure in the barrel leads to the slippage of the products between the screw and the barrel wall. This sometimes results in improper cooking and processing.

The screw moves the product along the barrel and surging occurs when the product is held back from proper flow resulting in a pressure buildup. This results in the product bursting out uncontrollably through the die. Twin-screw extruders use two screws, they intermesh as they turn and mix the product ingredients properly. It can accept a wide range of ingredient size.

2.4 Effects of extrusion on food products:

The effect of extrusion cooking on the nutritional quality of the food can be both beneficial and deleterious. The positive effects can be the reduction of anti-nutritional factors (tannins, phytates, haemagglutinins and trypsin inhibitors) and lipid oxidation, increased soluble dietary fibres and gelatinisation of starch, whereas the negative effects can be the reduction of heat labile vitamins and reduction of valuable proteins (Singh et al., 2007).

The available starch, protein digestibility, dietary fibres and phenolics for an extruded product depends on the food source. Factors such as protein and starch digestibility are influenced by the processing parameters such as process temperature, feed ratio, screw speed and length to diameter ratio. Protein is a biopolymer, consisting long chain of amino acid residue. Protein can undergo many changes during the extrusion process, the disulphide bond between molecules breaks and rearrange (Rao & Thejaswini, 2015). Extrusion cooking has been shown to be able to reduce the trypsin inhibitor activity, phytic acid and tannin content of the extruded products drastically by about 91 %, 44 % and 92 % respectively (Nwabueze, 2007). Reduction of trypsin inhibitory activity has also been correlated strongly with the extrusion parameter such as feed composition and screw speed. The digestibility of protein is also affected by the extrusion process. Stojceska, Ainsworth, Plunkett, İbanoğlu, and İbanoğlu (2008) reported a decrease in the in vitro protein digestibility for the extruded products made from wheat flour with cauliflower.

Starch is a polysaccharide consisting of glucose units which are linked together to form a long chain. Starch also undergoes various changes during the extrusion process. Amylose and amylopectin are the two type of starch molecules. The amount of amylose and amylopectin varies within the food products. For example, waxy corn starch only contains amylose. The varying proportion of the amylose and amylopectin gives the characteristic properties of gel formation and viscosity to the cooked products. During the extrusion process, the shear force and temperature breaks the molecules and releases glucose molecules.

Starch is the main constituent in the extruded products and is linked to the products textural properties such as crispiness, hardness and expansion (Dehghan-Shoar, Hardacre, & Brennan, 2010; Ding, Ainsworth, Tucker, & Marson, 2005; Hagenimana, Ding, & Fang, 2006; Shirani & Ganesharanee, 2009). The starch present in the food melts and gelatinises due to the extrusion process. When the extruded products come out of the die, the difference in the pressure and temperature leads to the products expansion and textural properties (Rao & Thejaswini, 2015). Higher starch content in the food results in higher expansion of the products. Dehghan-Shoar et al. (2010) reported the higher expansion ratio for the rice flour product which consists of more starch component in comparison with corn grits and wheat semolina products. However, the starch source had no significant effect on the density of the extruded products.

It is evident that high pressure, temperature and shear force would reorganise the food constituents at the molecular level, the longer chains and heavier molecules break and an increase in the amount of lighter and short chain molecules in the food sample is observed. White, Howard, and Prior (2010) reported the breakage of longer chain procyanidins oligomer into short chain procyanidins when incorporating the cranberry pomace. There was a significant surge in the amount of procyanidins' monomer, dimer and trimer, whereas the longer chain such as hexamer, heptamer decreased significantly. The authors also reported an increase in the flavonol contents, assumed to be due to disruption of the pomace matrix. The flavonols are bound to the cell wall matrixes, so when cell wall rupture occurred due to extrusion process the flavonol became more available to the extraction process.

The physical and textural characteristic of the extruded products are also very much dependant on the extrusion parameters and the feed constituents (Ding et al., 2005; Stojceska, Ainsworth, Plunkett, & İbanoğlu, 2009). The physical and textural properties such as moisture content, hardness, crispiness, expansion, density, porosity and colour are generally studied and used to describe the quality of extruded products. The gelatinisation of starch is an important step in the extrusion process, hence the starch content in the raw mix plays an important role for the extrudate's physical and textural attribute. Besides other consituents such as protein and dietary fibre (DF), lipids also play an important role in the product textural characteristics. The high lipid content in the feed mix results in the formation

of amylose-lipid complexes. The amylose-lipid complex interferes with the starch gelatinisation and decreases the expansion of the extrudates (Mäkilä et al., 2014).

2.5 Fruit and vegetable pomace in an extruded products:

Extruded ready-to-eat snacks are mainly made from starch or high starch containing food materials. The other nutritionally important constituents such as protein, vitamins, DF, are relatively low which make the extruded product low in nutritional value (Reis et al., 2014). The nutritional value of the extruded product can be altered by manipulating feed mix and by incorporating nutritionally valued ingredient. The development of extrudates with higher nutritional value by using food by-products have been the feature of several previous studies (Altan, McCarthy, & Maskan, 2008; Mäkilä et al., 2014; Mendonça, Grossmann, & Verhé, 2000; Stojceska et al., 2008). During the production of nutritional improved extruded product, special consideration should be given for the amount of nutritional valued ingredient being added, because it could affect the texture and sensory attribute of the new product (Altan et al., 2008; Cardona, Lee, & Talcott, 2009; Reis et al., 2014).

The introduction of fruit pomace has been shown to increase the dietary fibre content of the extruded products with non-significant changes to the texture qualities of the end products (Sun et al., 2015). Similarly, corn flour extruded product fibre content was increased by incorporating 10.5 % pineapple pomace (Selani et al., 2014). For instance, the author reported that when compared with the control extruded product the product with 10.5 % pomace did not show significant changes for qualities such as hardness, water absorption, yellowness and bulk density.

Such results illustrate the importance of conducting research on the possisble changes of product quality characteristics of extruded samples with increasing pomace inclusion.

Chapter 3

Materials and Methods

3.1 Objectives

Commercially available frozen boysenberries (Sujon berries Ltd, New Zealand) were used to prepare an extruded product using rice or maize grains as a base. The prepared extruded products were evaluated for starch content, glycaemic glucose equivalent, texture analysis and antioxidant property. In total 8 different extruded products were prepared and evaluated.

3.2 Preparation of pomace:

Frozen boysenberries (8 kg) were kept at room temperature overnight for thawing. Thawed berries were then crushed by hand and filtered using clean cheese cloth. Berry juice was discarded and the pomace was collected. The collected pomace was evenly spread on the tray and placed in an oven at 55 ^{o}C for drying and 30 % humidity for 48 h. Dried pomace was weighed and sealed in plastic bags and kept at room temperature until required. In total 360 g (dry weight) of pomace was extracted from 8 kg of Boysenberries which amounts to 45g per kg of fruits.

3.3 Preparation of Extrudes:

Maize popping corn (Sunvalley Foods, East Tamaki, Auckland) and long grain brown rice (Budget, Safeway Trader Ltd, Mt Roskill, Auckland) were bought from the local New world supermarket. These rice and maize samples were weighed and mixed with pomace to form control, 5%, 10% and 15% (w/w) pomace mix shown in (Table 3.1).

Table 3.1: Recipe mix used in Extruded Product.

Substitution level	Pomace (g)/ 100g of rice or maize
Control	0
5%	5
10%	10
15%	15

In total 8 types of products were made according to above recipe mix. Maize extrudates with 4 levels of pomace substitution and similarly rice extrudates with 4 levels of pomace substitution.

The recipe was extruded with single screw extruder (Northern Finance, Auckland, New Zealand). The screw diameter was 3 mm and L/D (length to diameter) ratio of the extruder was 10. Prior to extrusion, the feed rate was calibrated for each of the samples dry mix using the feed hopper. The data obtained from actual mass passing through the hopper for a specific time with different speeds was used for the feed rate calibration. Throughout the process, water feed rate was maintained at 0.05 kg/h and shaft speed to 200 rpm. Torque for each of the samples was recorded (table 3.2). An automated product cutter was mounted to the die face. The cutter was set at the same speed as the shaft screw speed to obtain a pelleted product. The final warm expanded products were collected and allowed to dry and cool down to ambient temperature for half an hour, they were then sealed in polyethylene bags for storage.

Table 3.2: Extruder running parameters during production of extrudates

Sample	Shaft speed (rpm)	Feed Rate (kg/h)	Water rate (kg/h)	Current (amps)	Torque (N-m)
Rice sample					
Control	200	10	0.05	5.7	48
5%	200	10	0.05	4.86	62
10%	200	10	0.05	5.49	55
15%	200	10	0.05	5.07	34
Maize Sample					
Control	200	10	0.05	5.61	62
5%	200	10	0.05	4.93	40
10%	200	10	0.05	5.21	56
15%	200	10	0.05	4.76	40

3.4 Quality parameters:

3.4.1 Moisture:

Moisture contents were analysed in triplicate. Milled extrudates (1 g) were weighed into an aluminium dish. The weight of dish and product was measured and kept in an oven at 105 ^{o}C

for 24 h. After 24 h the sample was reweighed and the difference in the weight was used for the analysis of moisture content.

Moisture content (%) $= \frac{(initial\ weight - final\ weight)}{initial\ weight} \times 100$

3.4.2 Texture:

Texture analysis was conducted using a TA.XT.plus Texture Analyzer (Stable Microsystems, Godalming, UK) and data was analysed with texture expert software provided. Texture characteristics were measured using 25 kg load cell and 25 mm cylindrical probe. The test speed for the process was set at 2 mm/sec and was applied with 50 % strain. Crispiness and hardness were calculated using texture expert software respectively as peak count and force exerted. The test was carried out with 10 extrudates for each sample.

3.4.3 Colour:

CIE colour space reading of the raw mix and extruded products were measured by Minolta Reflectance Chroma Meter CR 210 (Minolta Japan). The raw feed mix and extruded products were milled to make a fine powder. The colour value L*, a* and b* was measured and used to calculate Chroma and hue angle.

Chroma = $\sqrt{(a^{*2} + b^{*2})}$

Theta = (ATAN(b/a)/6.2832)*360), ^{o}hue = theta (if a>0 and b>=0),

^{o}hue = 180 + theta (if a<0 and b>=0),

^{o}hue = 180+ theta (if a<0 and b<0),

^{o}hue = 360+ theta (if a>0 and b<0),

3.5 Starch Analysis:

The total starch content was determined using Total Starch Assay Kit – Megazyme, AOAC method 996.11. The assay procedure uses thermostable α-amylase and amyloglucosidase. Starch present in the sample is hydrolysed by α-amylase into soluble branched and unbranched maltodextrins. Further the addition of amyloglucosidase quantitatively hydrolyses maltodextrin to D-glucose. Glucose oxidase then oxidises D-glucose to give hydrogen peroxidase and D-gluconate. Hydrogen peroxidase is quantitatively measured through colorimetric reaction at 510 nm by using peroxide which produces quinoneimine dye.

3.6 In vitro starch hydrolysis:

In vitro starch hydrolysis was analysed for the extruded products similar to method opted by (Gao, Brennan, Mason, & Brennan, 2016). Samples size for both milled raw base and milled extruded products were weighed according to the result obtained from the starch analysis. Weighed samples were then mixed with 30 mL of water and 0.8 mL 1 M HCL. Samples were constantly mixed with a magnetic stirrer and held around 35-40^{o}C throughout the process. A freshly prepared 1 mL pepsin solution (10 % pepsin in 0.05 M HCL) was added and kept for 30 min. Later 2 mL of 1 M $NaHCO_3$ was added followed by 5 mL of 0.1 M Na maleate buffer pH 6. Further, 0.1 mL amyloglucosidase was added to prevent end product inhibition of pancreatic amylase. After that 5 mL of freshly prepared pancreatin (2.5 % in 0.1 M Na maleate buffer pH 6) was added and the volume was adjusted to 53 mL with water. Samples were then incubated, during which 1 mL aliquots were withdrawn at 0, 20, 60, 120 min and were added to 4 mL absolute ethanol and mixed well to stop the reaction. These were stored until analysis of available carbohydrates.

All the collected in vitro digest aliquot (0, 20, 60 120 min) were analysed for available carbohydrate by the DNS (dinitrosalycilate) methods. The aliquots from the *In vitro* digest were centrifuged (Hettich Zentrifugen Rotina 380 centrifuge, Tuttlingen, Germany) at 1000 rpm for 5 min. A 0.05 mL of each aliquot, blank (water), standard (5 mg/ mL glucose) and standard (10 mg/ mL glucose) were added to 0.25 mL enzyme mix (1 % invertase and 1 % amyloglucosidase in acetate buffer pH 5.2) and kept to digest at room temperature for 20 min. A 0.75 mL DNS mixture (0.5 mg/ mL glucose: 4 M NaOH : DNS reagent mixed in a ratio of 1:1:5) was added to the digest and heated at 95-100 ^{o} C for 15 min in covered tubes. Samples were cooled and 4 mL of water was added and observed at 530 nm with spectrophotometer (V-1200 spectrophotometer, Global Science, Auckland, New Zealand). Data were analysed and reducing sugar presented per gram of food was calculated.

Reducing sugar (mg/g food) = $\frac{(A-CF)-P}{SA-P} * D * G * E * \frac{1}{M}$

Where, A = Sample reading, B = mean reagent blank, D = digestion volume, E= ethanol dilution factor, G = standard glucose concentration, M = sample weight, P = blank, Q = 5 mg/ mL glucose, R = 10 mg/ mL glucose, SA = $\frac{((Q*2)-P)+R}{2}$ and CF (correction factor) = B-P.

The area under the curve was calculated and was expressed as mg/ g sample.

3.7 Extraction procedures and measurements for Antioxidants Assay:

3.7.1 Extraction procedures for total phenolics:

The extraction method was carried out similar to methods opted by Vinson, Hao, Su, and Zubik (1998) with a few modification. Dried powered samples (2 g) were weighed into 50 mL plastic screw-cap vials; 20 mL of 1.2 M HCL in 50 % of methanol (v/v) was added to the vials and kept on a magnetic stirrer (500 rpm) for 2 h at room temperature (18 ^{o}C – 25 ^{o}C). Sample extracts were then centrifuged at 1152 rcf for 10 min using centrifuge (Hettich Zentrifugen Rotina 380, Tuttlingen, Germany). Supernatants were collected and stored in -20 ^{o}C until the measurement of total phenolics.

3.7.2 Extraction procedure for DPPH and ORAC assay:

Sample extract for DPPH and ORAC was carried out similar to the methods described by Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Hawkins Byrne (2006) with minor modification. Methanol (70 %) was used as the extraction solvent for DPPH whereas methanol (50 %) was used as the extraction solvent for ORAC assay. Milled sample powder (2 g) was weighed into 50 mL plastic screw- cap vials and 20 mL of solvent was added and kept in the magnetic stirrer (500 rpm) for 24 h at room temperature (18 ^{o}C -25 ^{o}C). The sample extract was then centrifuged at 1152 rcf for 10 min using centrifuge (Hettich Zentrifugen Rotina 380 Tuttlingen, Germany). Supernatant were collected into 10 mL plastics vials and kept in -20^{o}C for further used.

3.7.3 Measurement of total phenolic concentration:

Total phenolic concentrations (TPC) of the samples were measured using 0.2 N Folin-Ciocalteu reagent (Sigma, St Louis, USA) according to the method adapted by Singleton and Rossi (1965). Folin-Ciocalteu reagent is reduced by total phenolic compounds present in the samples to form a blue complex. Diluted extract (0.5 mL) was mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent and 2.0 mL of 7.5 % sodium carbonate. The mixture was mixed throughly and incubated at room temperature for 2h in a dark place. The absorbance reading of the sample was recorded at 760 nm with the spectrophotometer (V-1200 spectrophotometer, Global Science). A standard calibration curve of gallic acid (25-200 µg)(Sigma –Aldrich, Steinheim, Germany) was prepared and the result was expressed as gallic acid equivalent per gram dry weight.

3.7.4 Measurement of antioxidant capacity by DPPH assay:

The ability of the sample to scavenge the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was carried out similar to the methods described by Floegel, Kim, Chung, Koo, and Chun (2011) with minor modification. A 0.1 mM of DPPH stock solution was prepared with 50 % methanol. one mL of DPPH and 1.5 mL of methanol was added to 0.5 mL of the sample extract and incubated for 30 min in the dark. The absorbance reading of the sample was observed at 517 nm with the spectrophotometer (V-1200 spectrophotometer, Global Science). The standard calibration curve was prepared using trolox (0-100 µmol) (Sigma-Aldrich, Steinheim, Germany). The values were expressed as micromoles of Trolox (µmol TE) per gram sample weight.

3.7.5 Measurement of antioxidant capacity by ORAC assay

The ORAC – fluorescence assay was performed similarly to the method described by Thaipong et al. (2006) with minor adjustments. The measurement was carried out using a FLUOstar OPTIMA plate reader from BMG Labtech GmbH (Offenburg, Germany). The plate reader was equipped with the fluorescent filter with excitation of 485 nm and emission of 520 nm. The optimum temperature for the reaction was maintained at 37 ^{o}C and phosphate buffer was used for the peroxyl radical to oxidise fluorescein and to obtain a decay curve. The sample extraction was carried out using methanol as a solvent whereas for all the dilution and blank phosphate buffer was used. A 96-well black microplate (Costar 3915, Corning Inc., Corning, NY, USA) was used. Trolox standard (0-200 µmol) (Sigma-Aldrich, Steinheim, Germany) was made freshly. Initially, samples were diluted to various concentration with phosphate buffer to find a working dilution which gives a fluorescein decay curve similar to trolox standard. Finally, 25 µL of diluted sample extracts, trolox standard (0-200 µmol) were pipetted in triplicate into a 96-well black microplate. Further 150 µL of fluorescence (fluorescein, Sigma-Aldrich, Steinheim, Germany) was added to each well-containing samples and standards except the outer well and blank well where 150 µL of phosphate buffer was added. Addition of phosphate buffer to the outer well was to evenly distribute the heat and maintain the temperature throughout the well. The plate was pre-incubated at 37 ^{o}C for 30 min, plates were covered with parafilm to prevent evaporation. Meantime AAPH (2,2'-azobis-2-methyl-

propanimidamide dihydrichloride, Sigma-Aldrich, Steinheim, Germany) was prepared freshly and maintained to 37 ^{o}C and added to the plates as soon as possible after its 30 min incubation period. The fluorescence was measured every minute for 90 min. Data was analysed using MARS software (V1.20 R2, BMG Labtech GmbH, Offenburg, Germany) and area under the curve (AUC) was obtained. The net AUC is the difference between the AUC of blank and the AUC of the samples. The final values were calculated using the obtained regression equation between trolox standard and net AUC. The ORAC assay values were expressed as micromoles of Trolox (µmol TE) per gram dry weight.

3.8 Statistical analysis:

The statistical analysis was carried using Minitab® version 17.2.1 and Microsoft® Office Excel 2013. The data were reported as mean ± Standard deviation. One-way analysis of variance (ANOVA) (Tukey's test) and correaltion Pearson coefficent were carried out using Minitab statistical software. All analyses were carried out in triplicate unless stated otherwise. Replicates refers to the new repeat samples. The determined significant difference were valid at the $p<0.05$ level of significance.

Chapter 4

Physical Parameter

The physical appearance and textural properties of food products often play an important role in its acceptance among the food consumers. Texture analysis was conducted on the extruded breakfast cereal. In total 8 types of extrudate were made and were studied for their moisture content, expansion, crispiness, hardness and colour appearance. Maize and rice were used as the preliminary base ingredients and was substituted with (5 %, 10 % and 15 %) (w/w) pomace.

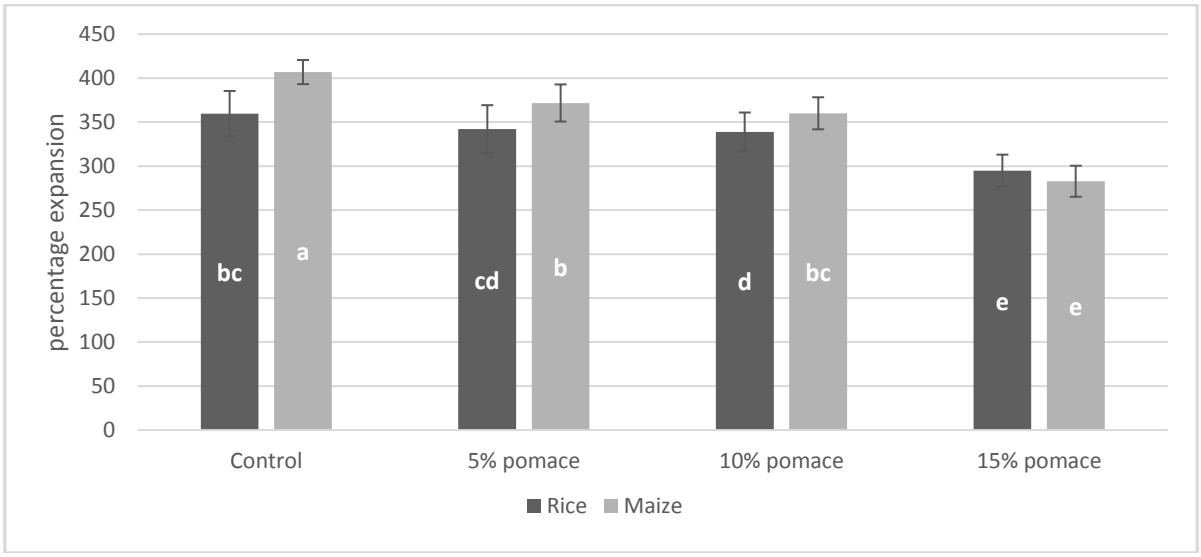
The moisture content of the extrudates tended to increase with an increase in the pomace substitution level. There was a significant increase in the moisture percentage between the control and the 15 % pomace substitution in both the maize and rice products. Previous research has shown that berries fruits contain a high amount of dietary fibres (Camire et al., 2002; Plaami, Kumpulainen, & Tahvonen, 1992). Hence, the introduction of pomace must have increased the dietary fibre contents in the extruded samples. Dietary fibres have been shown to have a high water retention and fat adsorption capacity (Figuerola et al., 2005). Table (4.1) illustrates the moisture percentage of each sample. Maize products generally had higher moisture contents compared to the rice products. This may be due to differences in the basic molecular structure and composition between maize and rice.

Previous research has indicated that high feed moisture decreases the radial expansion ratio of the extrudates (Thymi, Krokida, Pappa, & Maroulis, 2005). Factors such as moisture and pomace content played a significant role in the expansion ratio of the extruded sample. Previous research has shown that the incorporation of the pomace increased the moisture content of the samples and also altered some of the starch characteristics such as the structure of the amylopectin molecules in products as well as the melt elasticity during the extrusion process and radial expansion ratio (Thymi et al., 2005). In the current research it was observed that an increase in the pomace content of the products resulted in a decrease in the expansion percentage of the product figure (4.1).

Table 4.1: Moisture content of the extrudates

Sample	moisture percentage
Extruded maize control	6.59 ± 0.34 [b]
Extruded maize+5% pomace	7.976 ± 0.363 [a]
Extruded maize+10% pomace	6.997 ± 0.349 [b]
Extruded maize+15% pomace	7.93 ± 0.234 [a]
Extruded rice control	5.661 ± 0.09 [c]
Extruded rice+5% pomace	5.49 ± 0.11 [c]
Extruded rice+10% pomace	6.591 ± 0.168 [b]
Extruded rice+15% pomace	6.7 ± 0.279 [b]

Means that do not share a letter in a column are significantly different. P-value < 0.05



Values that do not share a letter are significantly different. P-value <0.05

Figure 4.1: Expansion percentage of extrudates

The pattern for expansion ratio of the extrudates was consistent and followed a similar pattern for both maize and rice extrusion. The expansion percentage for the maize based sample was higher than that of the rice-based samples. For instance, the maximum expansion percentage for the maize and rice were 406.86 ± 25.67 and 359.68 ± 13.71 respectively. However, the maize sample with 15 % pomace showed a lower expansion value than that of the rice sample with 15 % pomace. The increase in the pomace content had a significant effect on the expansion properties of the extruded products. A similar result was reported by Potter, Stojceska, and Plunkett (2013) for extruded snacks where fruit powder was incorporated, the expansion percentage decreased significantly for the banana and apple samples compared to the control made from wheat flour. Pomace is rich in dietary fibre (DF), which when added to extruded mix will increase the DF content in the extruded product. The expansion attribute of the extruded product has been shown to be negatively correlated to the higher content of protein, DF, crude fat (Mäkilä et al., 2014). The fibres interact with the cell wall and can rupture the cell wall, besides it also prevent air bubble formation interfering the expansion of the product (Anton, Fulcher, & Arntfield, 2009).

The incorporation of pomace to the extruded products showed some changes in its crispiness and hardness properties (figure 4.2 and 4.3). The number of peaks recorded by texture analyser indicates the crispiness of the extruded products as they represent individual fracture events occurring during the compression test of the crunchy and crispy products (Obatolu, Omueti, & Adebowale, 2006).

The increase in the hardness of the extrudates showed a decrease in the crispiness. With increasing pomace substitution, the crispiness decreased significantly for both rice and maize based products (figure 4.2). The decrease in the crispiness of the products with the inclusion of pomace was similar to earlier data presented by Sun et al. (2015) where grape and apple pomace were used to prepare starch based extruded products, and a decrease in the crispiness of the products was observed.

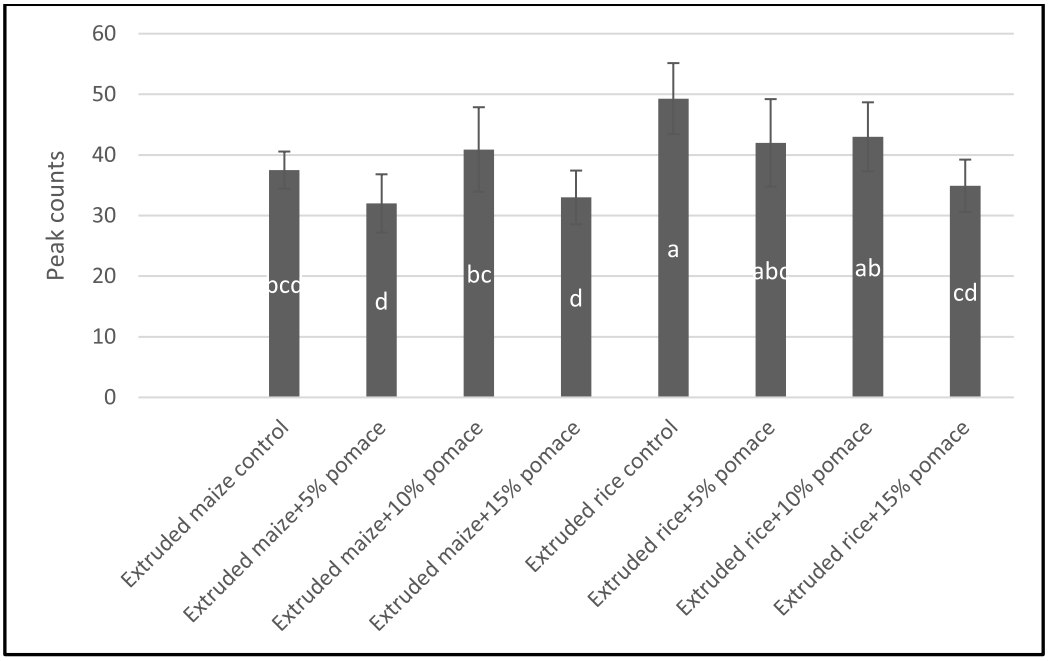
The hardness of products increased with increasing pomace substitution, though this was non-significant for the maize products. Maize and rice base products with 10 % pomace level were hardest when compared with its control, 5 % pomace and 15 % pomace level (figure 4.3). The maximum hardness was 31.78 ± 3.9 and 38.29 ± 2.68 force (N) respectively for maize and rice

samples. The results observed were similar to the results reported by (Dehghan-Shoar et al., 2010) for extruded products enriched with tomato lycopene, where the hardness of the products increased when tomato skin and paste was introduced.

However, in contrast with the rice extruded products, where hardness increased with increasing pomace content, the extruded products with the maize base showed non-significant changes in the hardness attribute. This result was similar to Selani et al. (2014) evaluating extruded products made with corn flour and pineapple pomace. In their study, the authors reported non-significant changes in the hardness of extruded products with 10.5 % pineapple pomace when compared with the control extruded product. A similar report was also made by Stojceska et al. (2008) for the cauliflower fortified extruded product.

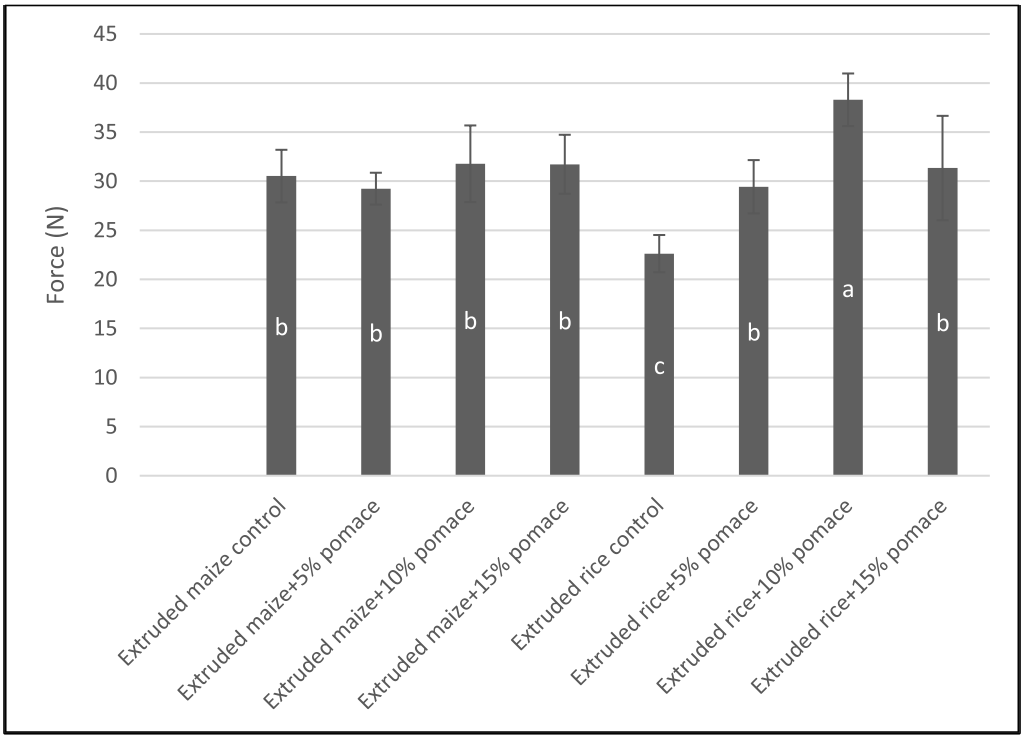
The relatively higher hardness characteristic shown by the rice based extruded product in comparison to maize-based products can be explained by their expansion attributes. When the expansion is less the hardness of the product increases. The expansion of the rice based extrudates was less in comparison with the maize based product. The hardness attribute of the extrudates increases with decreased expansion of the products and increasing feed moisture (Stojceska et al., 2009).

The physical and texture appearance of the products is an important aspect considered by the consumer at the time of purchase. In previous research sensory analysis was carried out for the extruded breakfast cereal incorporated with fruit powder among targeted school children (Potter et al., 2013), where it was observed that the appearance, taste and colour were the main criteria for the acceptance of the product. In this research sensory analysis using a consumer panel was not conducted however the effects on colour due to extrusion cooking and pomace content was studied by the using a colorimeter.



Values that do not share a letter are significantly different. P-value <0.05

Figure 4.2: Crispiness of the extruded products



Values that do not share a letter are significantly different. P-value <0.05

Figure 4.3: Hardness of the extruded products (force g)

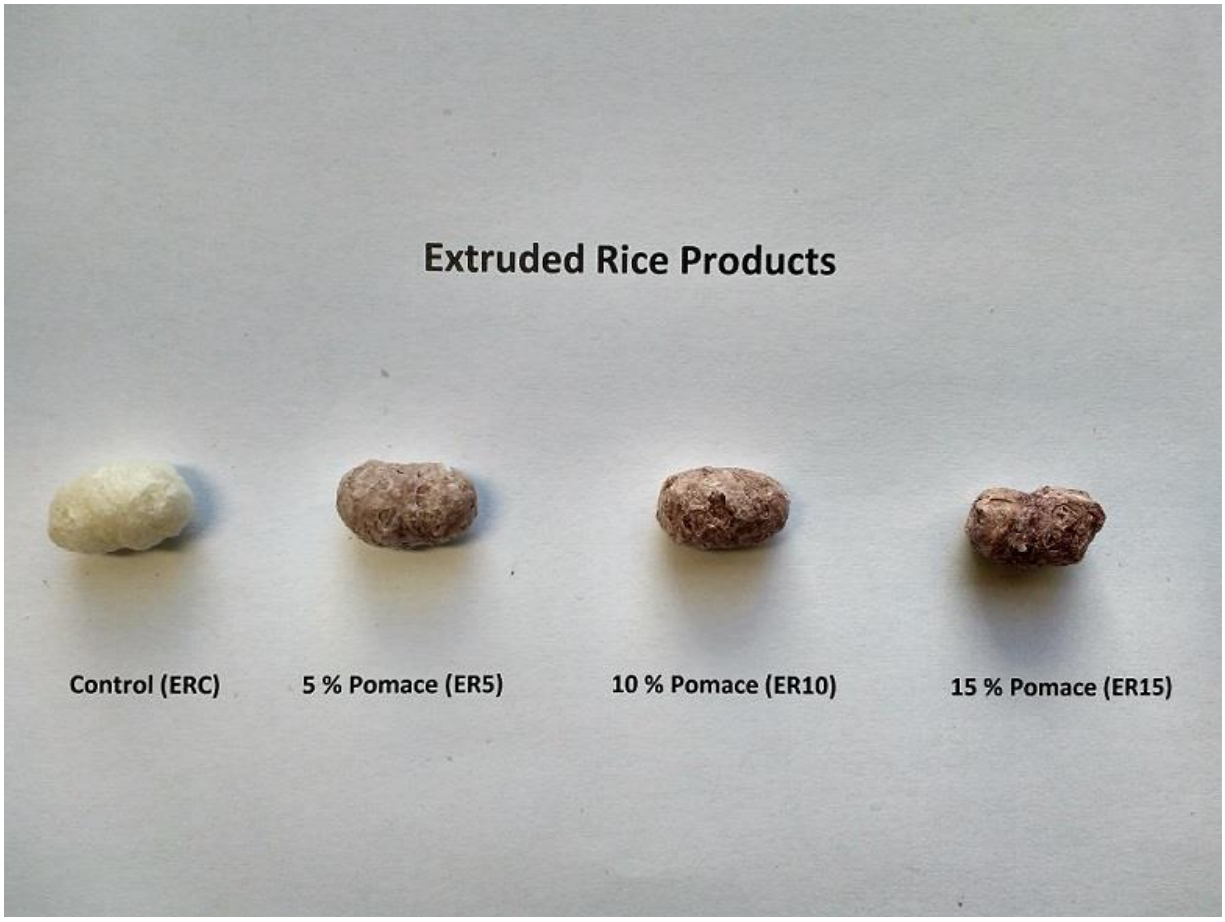


Figure 4.4: Picture of extruded rice products

Extruded rice control (ERC)

Extruded rice+5% pomace (ER5)

Extruded rice+10% pomace (ER10)

Extruded rice+15% pomace (ER15)

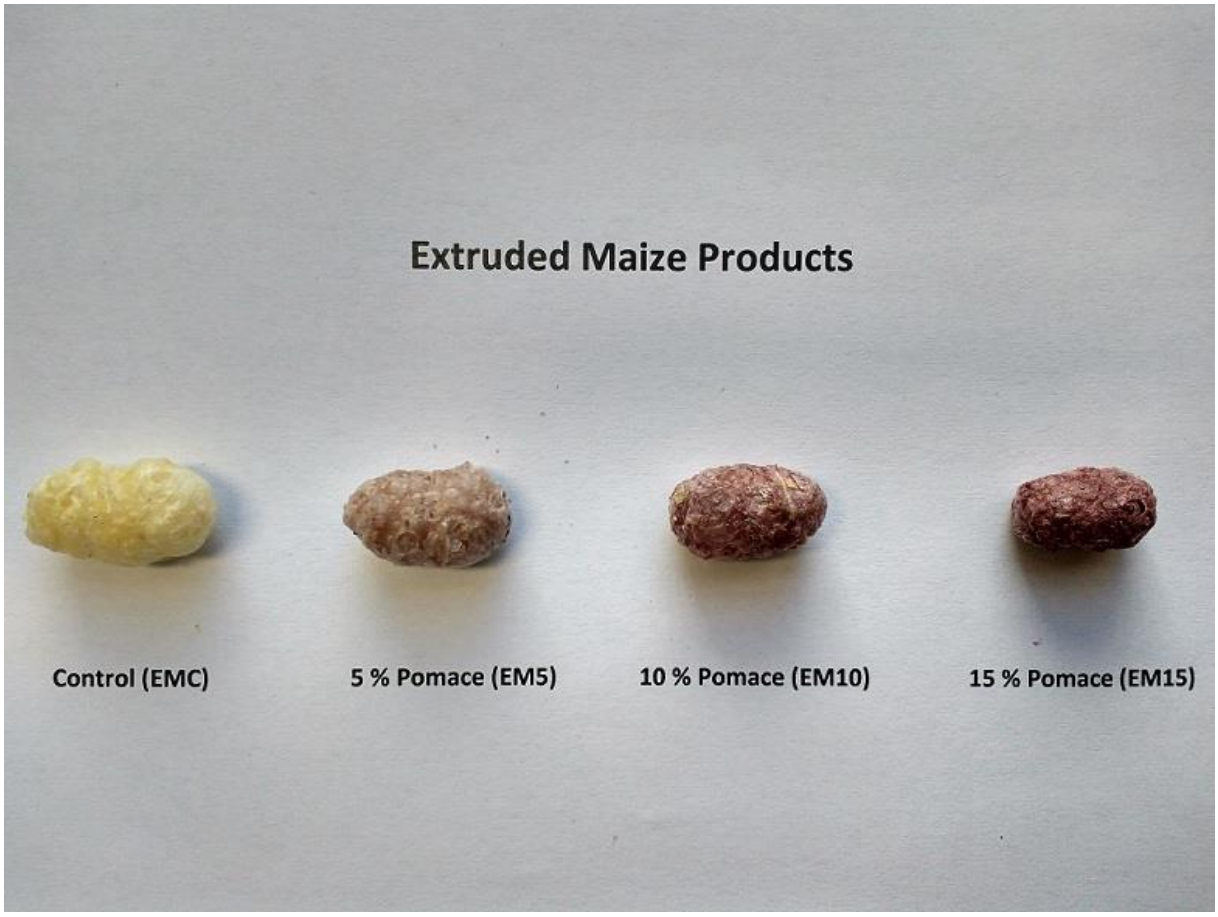


Figure 4.5: Picture of extruded maize products

Extruded maize control (EMC)

Extruded maize+5% pomace (EM5)

Extruded maize+10% pomace (EM10)

Extruded maize+15% pomace (EM15)

Figure 4.6: Picture of boysenberry pomace after drying

Colour is considered an important attribute of the extruded foods. The colour of the extruded product is affected by the factors such as temperature, time of heating and nature of the raw product (Ames, 1998). The colour of the product can provide valuable information about caramelisation and Maillard reaction in an extrusion process (Mesquita, Leonel, & Mischan, 2013). The amount of anthocyanins and polyphenols present in the sample can also be used to correlate with the sample colour (Cardona et al., 2009). The colour of the extrudates was measured in term of CIE colour space L*, a* and b*. L* stands for brightness, on the scale of 0 - 100, values near to 0 stands for darkness or blackish, whereas values near to 100 stands for brightness or whiteness. Positive (+) a* values stands for redness whereas negative (-) a* values stands for greenish, similarly a positive (+) b* values is yellowness and negative (-) b* stands for blueness.

The value of L* for the extruded products ranges from 96.98 to 84.13. There was little difference between the raw mix and the extruded products for the control samples. The L* values ranged between 96.35 and 96.98 appearing whitish in nature. Whereas, the samples with pomace showed a significant difference between the raw mix and extruded products. This result was similar with the result discussed by Sacchetti, Pinnavaia, Guidolin, and Rosa (2004). The introduction of pomace to the samples increased the available reducing sugar which changes the colour of the products due to Maillard reaction. The L* value for the products with pomaces decreased significantly with respects to its raw mix, resulting less whitish in appearance. This is due to the browning effect or Mailliard reaction. L* value for the milled pomace powder was 19.55.

The b* value decreased significantly for the extruded products due to the extrusion process (figure 4.7). This result is similar to the result presented by Durge, Sarkar, and Singhal (2013) where b* value of anthocyanin coloured extruded decreased by the extrusion process.
CIE Lab values were used to calculate the hue angles and chroma for the extruded products and raw feed mix and the values are shown in the table (4.2) below.

Table 4.2: CIE colour reading Chroma and ° Hue angle

Samples	Chroma	° Hue
Maize control	43.23 ± 0.2 [b]	110.499 ± 0.02 [d]
Maize+5% pomace	38.03 ± 0.46 [c]	98.621 ± 0.5 [f]
Maize+10% pomace	33.85 ± 0.21 [d]	92.97 ± 0.46 [i]
Maize+15% pomace	31.07 ± 0.1 [f]	89.563 ± 0.2 [j]
Extruded maize control	38.41 ± 0.59 [c]	111.29 ± 0.22 [cd]
Extruded maize+5% pomace	32.69 ± 0.14 [e]	95.223 ± 0.2 [g]
Extruded maize+10% pomace	29.88 ± 0.12 [g]	89.151 ± 0.55 [j]
Extruded maize+15% pomace	28.03 ± 0.08 [h]	87.9144 ± 0.1 [k]
Rice control	26.50 ± 0.16 [i]	112.15 ± 0.17 [bc]
Rice+5% pomace	29.96 ± 0.06 [g]	98.624 ± 0.362 [f]
Rice+10% pomace	28.45 ± 0.07 [h]	93.062 ± 0.22 [hi]
Rice+15% pomace	27.65 ± 0.06 [h]	89.509 ± 0.192 [j]
Extruded rice control	21.72 ± 0.64 [j]	112.473 ± 0.05 [b]
Extruded rice+5% pomace	32.46 ± 0.12 [e]	101.921 ± 0.47 [e]
Extruded rice+10% pomace	29.64 ± 0.07 [g]	95.77 ± 0.2 [g]
Extruded rice+15% pomace	28.36 ± 0.097 [h]	93.902 ± 0.18 [h]
Boysenberry pomace	47.14 ± 0.80[a]	329.74 ± 0.61[a]

Values that do not share a letter in a column are significantly different. P-value <0.05

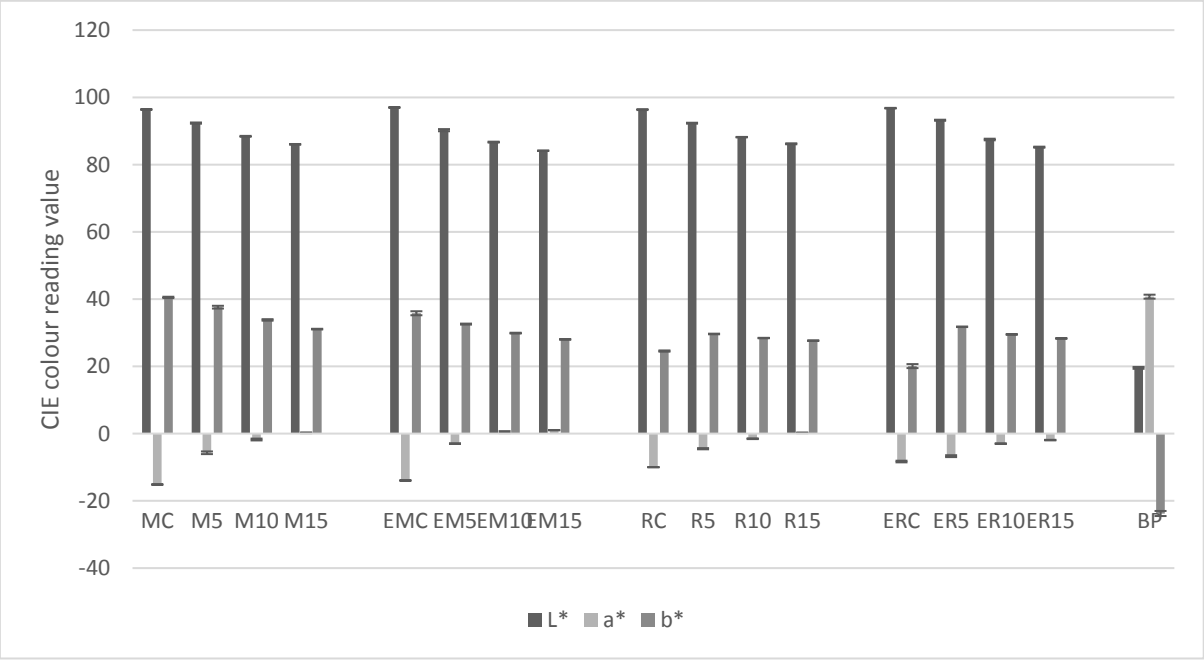


Figure 4.7: CIE colour reading (L*, a* and b*) of sample feed mix and extruded products.

Maize control (MC), Maize+5% pomace (M5), Maize+10% pomace (M10), Maize+15% pomace (M15), Extruded maize control (EMC), Extruded maize+5% pomace (EM5), Extruded maize+10% pomace (EM10), Extruded maize+15% pomace (EM15), Rice control (RC), Rice+5% pomace (R5), Rice+10% pomace (R10), Rice+15% pomace (R15), Extruded rice control (ERC), Extruded rice+5% pomace (ER5), Extruded rice+10% pomace (ER10), Extruded rice+15% pomace (ER15), Boysenberry pomace (BP)
*Stastistical information for the figure see appendix table A.2

Table 4.3: Correlation between moisture, crispiness and expansion for both rice + maize products

	Moisture v/s Crispiness	Crispiness v/s Expansion	° Hue v/s Expansion
Pearson correlation	-0.818	0.8	0.754
p-value *	0.013	0.017	0.031

*($p < 0.05$) correlation is significant

The moisture content had a significant effect on the crispiness of the extruded product. Moisture of the product showed strong negative correlation with the crispiness (table 4.3).

The crispiness of the product showed strong positive correlation with the expansion of the product (table 4.3). Previous reports have suggested that the higher the expansion value, the larger the bubble growth and formation of larger cell cavity in an extruded products (Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011). On the other hand, crispiness of the extruded products represent individual fracture events occurring during the compression test, which is breakage of matrix consisting of the cell cavity and air bubble. Hence, crispiness and expansion of the products are correlated to each other, as increase in the expansion results in increase in the crispiness of the products. This observation was in similar with the result observed by Obatolu et al. (2006) for extruded puffed snacks.

As discussed earlier, the expansion of the extruded products showed significant decreases with increasing pomace content. The pomace replacement created a decrease in the starch availability by participating the Maillard and caramelization reaction during the extrusion process. The colour of the extruded products depends on the base material, pomace content and the Maillard reaction. The percentage expansion and the colour of the products (changes in º Hue) showed positive correlation.

4.1 Important observations:

- An increase in the pomace content in the extruded product decreased the expansion and crispiness.

- The moisture content and the hardness of the extrudates tended to increase with an increase in the pomace content.

- The extrusion process and pomace content showed significant changes to the CIE colour reading of the extruded products. The increasing pomace content resulted in a darker colour of the products.

- The moisture and the crispiness of the extruded products showed negative correlation.

- The expansion of the products showed positive correlation to the crispiness and the CIE colour reading (º Hue) of the extruded product.

Chapter 5

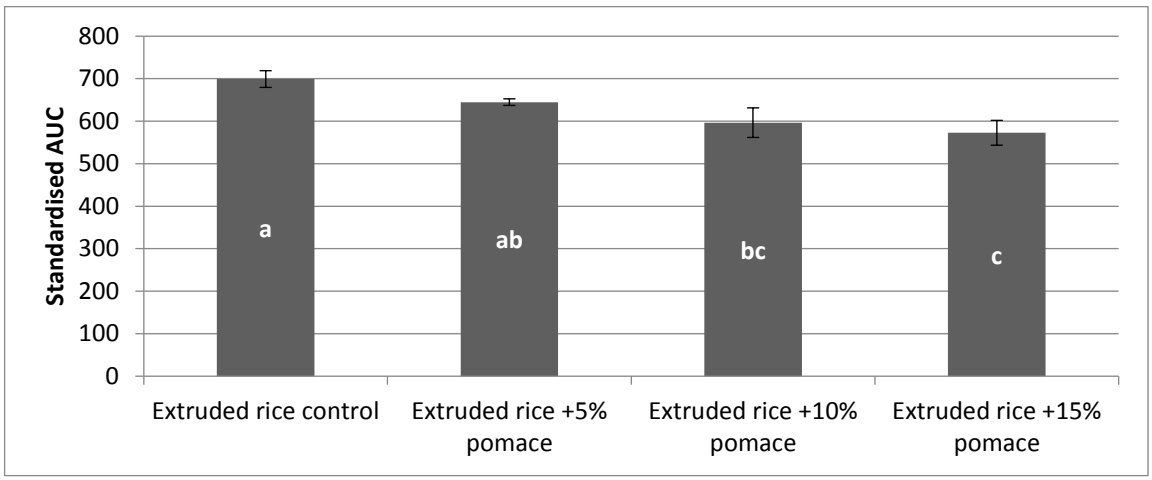
In vitro starch hydrolysis

In vitro enzymatic digestion was carried out on the extruded products to study their nutritional attributes such as starch digestibility and predictive glycaemic response. The *in vitro* enzymatic digestion was designed in such a way that it would mimic the human digestive track (Brennan, Merts, Monro, Woolnough, & Brennan, 2008). The inclusion of pomace at three different levels (5 %, 10 % and 15 %) (w/w) to the maize and rice based extruded products had a significant impact on the available starch of the product. The area under the curve and degree of starch digestion for the time period of 0 min to 120 min during the enzymatic digestion is shown in the figure (5.1- 5.4), rice for based and maize based extruded products. The amount of reducing sugars released reduced significantly after the inclusion of the pomace, and this reduction was observed to increase with increasing pomace content. This observation was consistent with result observed by Leoro, Clerici, Chang, and Steel (2010), where the inclusion of passion fruit fibre in a extruded breakfast cereal showed a reduced glycaemic index compared to control diet.

Previous results observed by Shirani and Ganesharanee (2009) for a chickpea-rice based extruded product, showed that where a control was replaced with fenugreek flour at 2 %, 5 % and 10 % (w/w) level the high dietary fibre content of fenugreek flour was related to a reduction in the glycaemic index of samples. Dietary fibres have been shown to have the ability to trap the starch granules within the cell wall matrix or by its viscous gel matrix and reduce the starch digestibility (Wolever, 1990). Food with lower GI index consist of stronger cellulose and hemicellulose matrix which inhibits starch digestion. Reyes-Pérez, Salazar-García, Romero-Baranzini, Islas-Rubio, and Ramírez-Wong (2013) also reported a decrease in the glycaemic index of cookies by introducing wheat bran with high dietary fibre content.

As discussed before, a decreased amounts of available reducing sugar, during the *in-vitro* digestion could be the result of increased dietary fibre content in the extruded product, when pomace was introduced. However, boysenberry pomace could also contain compounds which inhibit the alpha-glucosidase activity. For instance, Hogan et al. (2010) reported alpha-amylase inhibiting compounds in the grape and apple pomace. These compounds were able to decrease the starch and sugar digestibility reducing the postprandial hyperglycaemic.

Extrusion cooking increases the degree of starch digestibility as extrusion cooking process applies high temperature, high pressure and shear force on the raw food mix, which changes the initial feed molecular, textural and chemical properties (Moscicki & van Zuilichem, 2011). It also breaks down polysaccharides of the raw food products and increases the mono- and oligosaccharides content on the extruded products. Significant increases in the mono- and oligosaccharides such as fructose, glucose, maltotriose and maltose were observed after an extrusion process on the wheat flour samples (Chiang & Johnson, 1977). Similarly, the extrusion process also affects the pomace constituent, it enhances the pomace monomer content especially procyanidins (which is a subclass of flavonoids, consisting monomer, oligomer or polymer of catechin and epicatechins) from 18 % to 80 % in an extruded products (Khanal et al., 2009). The degree of breakdown has been shown to be dependent on the extrusion parameter such as temperature and screw speed. Higher breakage was observed with 180/150 (temperature ^{o}C / rpm) in compared with 160/150, 160/200 and 180/200.



Values that do not share a letter are significantly different. P-value <0.05

Figure 5.1: Area under the curve of rice samples

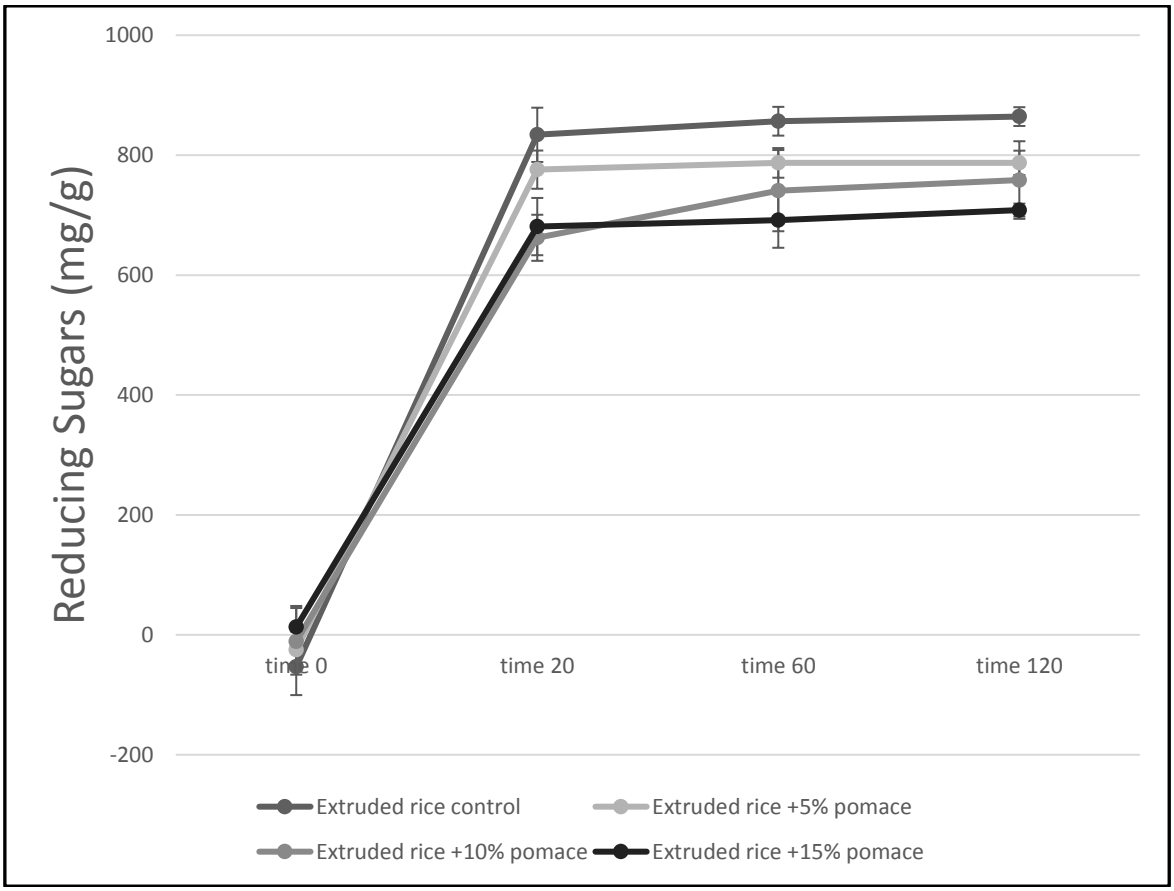


Figure 5.2: Starch hydrolysis of extruded rice sample

Extruded rice control (ERC), Extruded rice+5% pomace (ER5), Extruded rice+10% pomace (ER10), Extruded rice+15% pomace (ER15) *statistical information see appendix table A.6

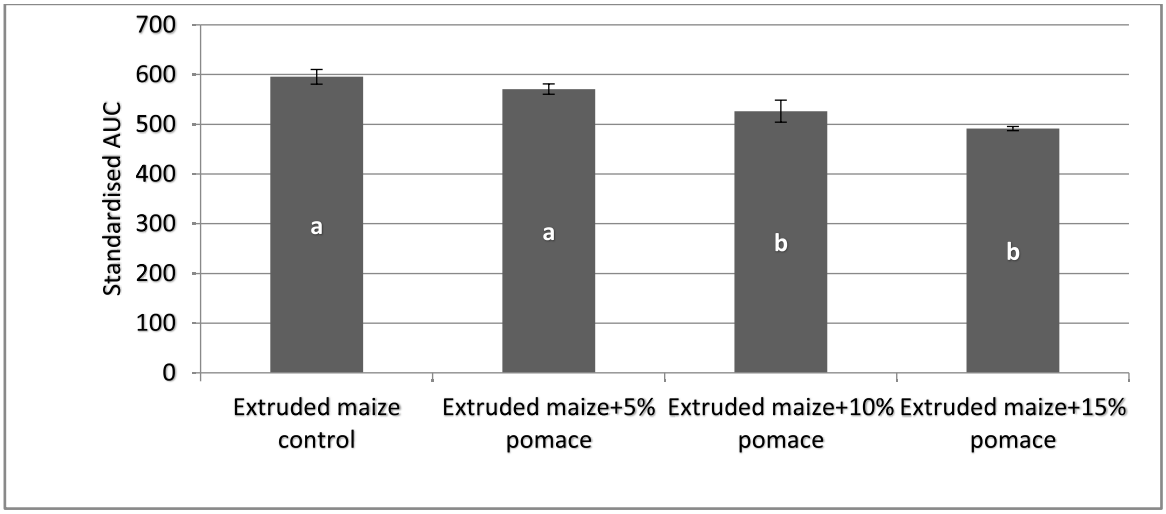
Fresh boysenberry consists of (4-7) % total sugar, therefore the pomace also has sugar in it. When pomace is introduced in the extrudates, it introduce sugar, dietary fibre and antioxidants. While, it also replace the reducing sugars content from the extrudates base. Suppose if we introduce 10 % pomace to the extruded rice base, the 10 % rice base will be replaced with pomace. The result we have observed acknowledges the introduction of sugar by the pomace. The figure 5.1 shows the total AUC (area under the curve) of the products.

The degree of starch digestion during the *in-vitro* digestion of the rice based extruded samples are shown in the figure (5.2). The comparison between the control and the product with pomace shows that the sugar content was more available for digestion in control samples. Throughout the digestion time interval from 0 min to 120 min (figure 5.2), there was a significant decrease in the release of the reducing sugar for rice samples with pomace when compared with the control samples (ERC). The reducing sugar released for the control (ERC) increased with time to reach a value of 864 mg/g sample at 120 min, whereas when the pomace was introduced the value decreased significantly to 787, 758 and 708 mg/g at 120 min for ER5, ER10 and ER15 respectively. The reducing sugar content at 120min was reduced by 8 %, 12 % and 18 % for ER5, ER10 and ER15 respectively compared to control samples. The reducing sugar content for the time 0 min has negative values which are within the bounds of normal variation.

There was a significant decrease in the available reducing sugar as the reaction proceeded from 20 min to 120 min. For instance, the extruded rice sample with 5 % pomace substitution (ER5) showed a 6.9 %, 8.1 % and 8.92 % reduction in the available reducing sugar at time 20 min, 60 min and 120 min respectively with compared to similar time points for the control sample. This result was in similar to the result reported by Brennan et al. (2012) for an extruded snack product incorporating mushroom coproduct material. However the reduction exhibited by extruded snacks with 5 % mushroom coproduct material showed 22-25 % reduction of available reducing sugar, this amount of reduction is much higher when compared with our data of 6-9 % reduction. This could be the result of sugar being present in the pomace content. The total sugar content in a fresh boysenberry is around 7% (Porter, 1988).

As expected the extruded rice sample with 10 % pomace substitution (ER10) showed a decreased reducing sugar content by 20 %, 13 % and 12 % for time 20 min, 60 min and 120

min respectively in comparison with the same time points for the control samples. This indicated that pomace in the sample restricted the amount of starch digestion. The higher inclusion level of the pomace lead to an increased reduction of reducing sugar content at all levels and at all times with respect to control. This fact is also shown in the figure 5.1, which shows the total AUC (area under the curve) of the products. The effect of pomace inclusion at 5 %, 10 % and 15 % level can further be observed with standardised AUC value. The AUC value decreased with increased pomace substitution level, the response being nonlinear. The AUC value decreased by 18 % for ER15 sample when compared with control samples. This shows a significant decrease in the available of reducing sugar for ER15 samples during the *in vitro* digestion. For samples ER5 and ER10 the AUC value decreased by 7 % and 14 % respectively when compared to the control samples.



Values that do not share a letter are significantly different. P-value <0.05

Figure 5.3: Area under the curve of maize samples

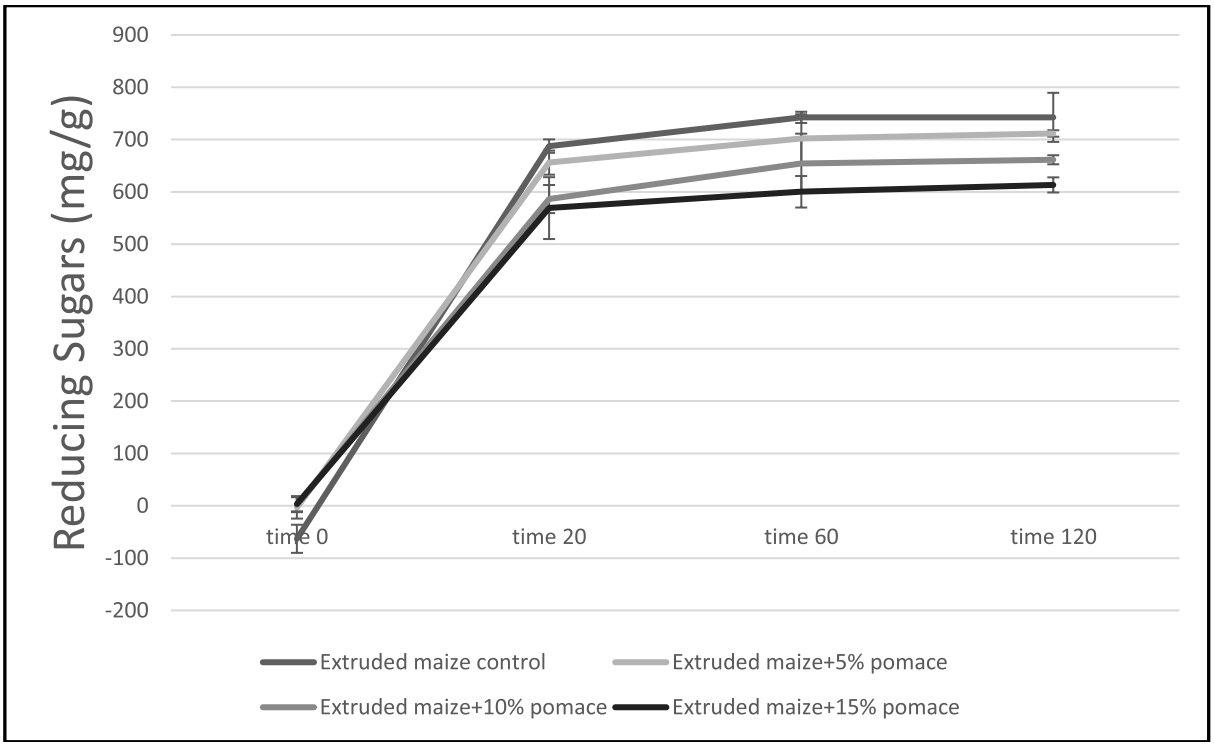


Figure 5.4: Starch hydrolysis of extruded maize samples

Extruded maize control (EMC), Extruded maize+5% pomace (EM5), Extruded maize+10% pomace (EM10), Extruded maize+15% pomace (EM15) *statistical information see appendix table A.7

The degree of starch digestion during the *in-vitro* digestion of the maize based extruded samples is shown in the figure (5.4). The maize-based extruded products also showed similar observation as seen earlier with the rice-based extruded products, the comparison between the control and the product with pomace shows that the starch content was more available for digestion in control samples. Throughout the digestion time interval from 0 min to 120 min (figure 5.4), there was a significant decrease in the release of the reducing sugar for samples extruded maize + 5 % pomace (EM5), extruded maize + 10 % pomace (EM10) and extruded maize + 15 % pomace (EM15) when compared with the extruded maize control (EMC) sample. The reducing sugar release for the control EMC increased with time to reach a value of 742 mg/g sample at 120 min, whereas the value was significantly lower (711, 661 and 613 mg/g) for ER5, ER10 and ER15 respectively. The reducing sugar content at time 120 min was reduced by 4 %, 10 % and 17 % for EM5, EM10 and EM15 respectively in comparison to control sample.

The observed value followed the similar pattern as we have seen above with extruded rice samples. There was a significant decrease in the available reducing sugar as the reaction proceeded from 20 min to 120 min. For instance, the extruded maize sample with 5 % pomace substitution (ER5) showed a 14 %, 11 % and 10 % reduction in the available reducing sugar at time 20 min, 60 min and 120 min respectively when compared to similar time points for the control sample. AUC value decreased by 17 % for EM15 sample in compared with control samples. For samples EM5 and EM10 the AUC value decreased by 4 % and 11 % respectively in compared with the control.

Though the pattern was similar with the rice extruded samples the reduction of starch degradation was slightly less in the maize extruded products. The difference in the reduction percentage could be explained by difference in the chemical constituents of the rice and maize vary. This is illustrated further by the total AUC (area under the curve) value of the products. The AUC value for the extruded maize control samples (EMC) was 595.44 ± 14.78 which is less when compared to 699.27 ± 19.65 value for extruded rice control sample (ERC). This indicates that more starch is available for digestion in the rice extruded samples than in maize extruded products. Compared between rice and maize based extruded samples at different time interval (figure 5.2 and figure 5.4), the amount of reducing sugar was always higher in the rice based extruded samples. This trend was also consistent between products with the same level

of pomace substitution. The reducing sugar release reached a highest concentration of 864.28 ± 15.65 mg/g samples for the extruded rice control (ERC) at time 120 min, while the highest concentration of 742.48 ± 46.9 at time 120 min was observed for the extruded maize control (EMC).

The reduction in the available reducing sugar due to the addition of pomace showed a similar effect in both rice and maize based extruded samples. The reduction percentage increased with increase in the pomace substitution level when compared with the control sample. In comparison with the control samples, the average reduction percentage was 8 %, 15 % and 18 % for the sample ER5, ER10 and ER15 respectively. Similarly, it was 4 %, 12 % and 17 % for the samples EM5, EM10 and EM15 respectively.

Table 5.1: Correlation between AUC and moisture, crispiness and expansion

	AUC v/s Moisture	**AUC v/s Crispiness**	**AUC v/s Expansion**
Pearson correlation	-0.837	0.749	0.811
p-value*	0.01	0.032	0.015

*($p < 0.05$) correlation is significant

The AUC value observed showed a significant positive correlation with the crispiness and expansion attribute of the extruded products, whereas it showed a negative correlation with the moisture content of the extruded products.

The correlation between AUC value and expansion attribute of the product could be explained with the degree of starch gelatinisation. The increases in the AUC value of the product have been linked with increasing gelatinisation of the starch (Alminger, Eklund-Jonsson, Kidman, & Langton, 2012). Whereas, Potter et al. (2013) reported an interruption in the starch gelatinisation process resulted in the less expanded extruded products. The constituent in the pomace, most likely the dietary fibre, could be the reason behind the interruption of starch gelatinisation and a less expanded product.

5.1 Important observations:

- The inclusion of pomace at three different levels (5 %, 10 % and 15 %) (w/w) to the maize and rice based extruded products had a significant impact on the degree of starch digestion.

- The addition of pomace reduce the area under the curve, which means less starch is available for digestion.
- The highest reduction in the starch digestion was 18 % for the rice extruded with 15 % pomace. Whereas for the sample with maize with 15 % pomace, the reduction was by 17 %.
- The AUC showed a negative correlation with the moisture of the product.
- The AUC showed a positive correlation with the crispiness and expansion attribute of the extruded products.

Chapter 6

Total phenolics and antioxidant activity

In this study, boysenberry pomace was incorporated into the extruded products and was analysed for its antioxidant property. The initial hypothesis was made that the incorporation of the pomace would increase the antioxidant activity of the extruded product (table 6.1). The results observed were able to justify the hypothesis. In this study, three different assays (total phenolic, ORAC assay and DPPH) were carried out to study the phenolic and antioxidant attribute of the extruded products and the raw mix.

Table 6.1: Total Antioxidant assay

Samples	**total phenol Gallic acid equivalent (mg) / 100 gram of sample**	**DPPH µM Trolox / gram of sample**	**ORAC µM Trolox / gram of sample**
Maize control	63.44 ± 0.34 ^{m}	17.27 ± .022 ^{g}	58.33 ± 3.04 ^{hi}
Maize+5% pomace	90.08 ± 0.30 ^{j}	25.55 ± 0.45 ^{e}	96.28 ± 4.65 ^{f}
Maize+10% pomace	136.71 ± 1.37 ^{f}	37.45 ± 0.25 ^{d}	127.81 ± 3.74 ^{cd}
Maize+15% pomace	177.65 ± 1.26 ^{b}	45.64 ± 0.22 ^{b}	148.16 ± 5.38 ^{b}
Extruded maize control	72.06 ± 0.47 ^{l}	14.29 ± 0.0 ^{h}	50.11 ± 4.13 ^{ij}
Extruded maize+5% pomace	88.18 ± 0.53 ^{j}	23.75 ± 1.37 ^{f}	72.99 ± 0.371 ^{g}
Extruded maize+10% pomace	123.62 ± 0.74 ^{g}	30.51 ± 0.22 ^{e}	116.89 ± 4.81 ^{de}
Extruded maize+15% pomace	157.96 ± 1.39 ^{d}	39.34 ± 1.01 ^{d}	137.68 ± 2.54 ^{bc}
Rice control	43.98 ± 0.79 ^{n}	14.29 ± 0.0 ^{g}	54.17 ± 4.64 ^{hij}
Rice+5% pomace	84.69 ± 0.56 ^{k}	25.91 ± 0.58 ^{f}	93 ± 3.5 ^{f}
Rice+10% pomace	108.56 ± 0.58 ^{h}	32.40 ± 0.44 ^{e}	127.29 ± 5.65 ^{cd}
Rice+15% pomace	170.60 ± 2.05 ^{c}	42.58 ± 0.83 ^{c}	146 ± 3.4 ^{b}
Extruded rice control	44.40 ± 0.17 ^{n}	8.17 ± 0.83 ^{i}	44.88 ± 3.82 ^{j}
Extruded rice+5% pomace	72.89 ± 0.30 ^{l}	23.75 ± 1.3 ^{f}	65.36 ± 3.97 ^{gh}
Extruded rice+10% pomace	105.22 ± 0.63 ^{i}	30.51 ± 0.22 ^{e}	109.16 ± 4.02 ^{e}
Extruded rice+15% pomace	141.80 ± 0.65 ^{e}	39.34 ± 1.01 ^{d}	131.56 ± 1.23 ^{c}
Boysenberry pomace	1618.57 ± 2.73 ^{a}	95 ± 0.53 ^{a}	732.6 ± 20.02 ^{a}

Tukey Method and 95% Confidence
Means that do not share a letter in the same column are significantly different.

The total phenolic content (TPC) of the boysenberry pomace was 1618.58 ± 2.73 mg GAE/100 g pomace DW. The antioxidant activity for the boysenberry pomace was 95 ± 0.53 and 732.60 ± 20.02 µM TEAC / g pomace DW for DPPH and ORAC assay.

The phenolic content and antioxidant activity of the sample varied with the method of extraction, choice of the solvents and the process opted (Garzón, Riedl, & Schwartz, 2009; Grunovaitė, Pukalskienė, Pukalskas, & Venskutonis, 2016; White, Howard, & Prior, 2009).

The total phenolic value for the boysenberry pomace was higher than the TPC value of 170 mg GAE / 100 g pomace DW for apple pomace reported by García, Valles, and Picinelli Lobo (2009). However, the TPC value was less when compared with that of 2285.6 mg/100 g for black currant (Sójka & Król, 2009).

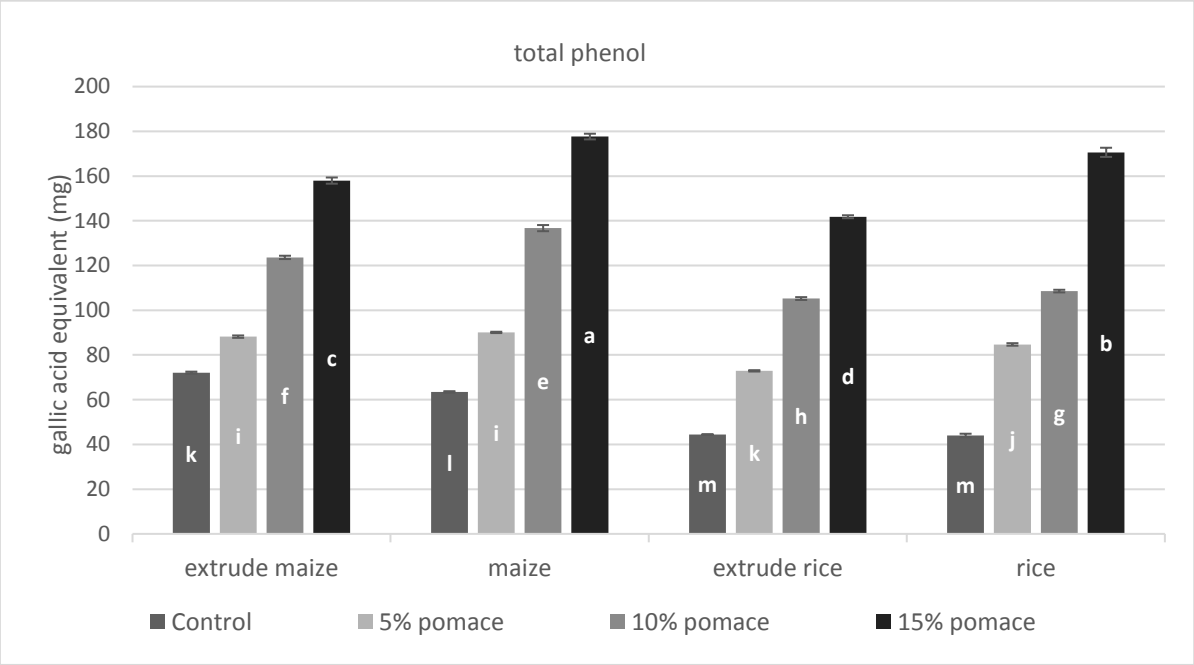
Moreno-Montoro, Olalla-Herrera, Gimenez-Martinez, Navarro-Alarcon, and Rufián-Henares (2015) listed the TPC for a range of fruits reported by previous studies. TCP values of juice from orange, apple, pineapple, pomegranate and strawberry ranged between 544-755, 289-1020, 314-358,732 and 536-2571 mg GAE / L respectively. The values from the juice were lower than the values observed from the boysenberry pomace.

Previously, TCP values for grape juice and wine have been observed to be between 150-1654 and 218-3172 mg GEA / L respectively (Moreno-Montoro et al., 2015). TCP values (196.2 mg GAE / g) was reported for grape pomace by Antoniolli, Fontana, Piccoli, and Bottini (2015). The difference in the TCP content between the grape pomace and juice may be because pomace contains seeds and skin and these fractions is where most the phenolics are found. During the production of wine and juice small fraction migrates from the skin and seed to the wine and juice. Besides factors such as different variety and extraction process also counts. The effect of extraction solvents on the phenolics profile is depicted very well by Kähkönen, Hopia, and Heinonen (2001), where they illustrated the significant difference in phenolic composition of the same sample using aqueous methanol and aqueous acetone as an extraction solvent.

Marinova, Ribarova, and Atanassova (2005) have reported TCP value for a range of fruits and vegetables. The TPC values for the berry fruits such as blueberry, raspberry, strawberry, blackberry and dogwood berry were reported to be 670.9, 178.6, 244.1, 355.3 and 432 mg GAE/ 100g FW respectively. These TPC values were higher than other non-berry fruits and vegetables such as apple, pear, peach, carrots and tomato, which were 125.4, 124.7, 50.9, 96 and 76.9 mg GAE/ 100g FW respectively. The observed values for the boysenberry pomace in our research was similar to the berry fruits and higher in compare with the other vegetables. From this evaluation, we can conclude that berry fruits have high total phenolic content.

The antioxidant activity of the boysenberry pomace obtained by DPPH assay was 95 ± 0.535 µM TEAC / g, which was less than of black currant 126.5 ± 23.8 µM TEAC / g reported by Sójka and Król (2009) .

ORAC values observed for the pomace in this research were in a similar range to those reported by earlier papers for the cranberry and grapes pomace (Harrison, Oomah, Diarra, & Ibarra-Alvarado, 2013; Jara-Palacios et al., 2013). This validates our observed data of antioxidant activity for the pomace. The reported ORAC value of 636 µM TEAC / g (Jara-Palacios et al., 2013) for grape pomace were less than that recorded for our boysenberry pomace. However, a high ORAC value (1183 µM TEAC / g) was reported by Harrison et al. (2013) for the cranberry pomace. This supports the idea that berries are a rich source of polyphenol and possess high antioxidant activity when compared with other fruits.



Values that do not share a letter are significantly different. P-value <0.05

Figure 6.1: Total phenol of the sample mix and extruded products

The boysenberry pomace had a high total phenolic content in comparison with the maize and rice. There was a significant change in the TPC of the raw mix (unextruded) and extruded products, when the pomace was introduced (figure 6.1). In most cases extruded samples contained less total phenol compared against their unextruded counterparts. This illustrated the effect of thermal processing in reducing phenol composition. The TPC increased

significantly with an increase in the pomace contents. The inclusion of pomace at 5 % (w/w), 10 % (w/w) and 15 % (w/w) levels increased the TPC value (43.98 mg GAE / 100g) of control extruded rice sample to 84, 108 and 170 mg GAE / 100g respectively. When compared with the control samples, the percentage increase in the TPC value for the samples with 15 % pomace were 119 %, 180 %, 219 % and 287 % for extruded maize, raw maize mix, extruded rice and raw rice mix respectively.

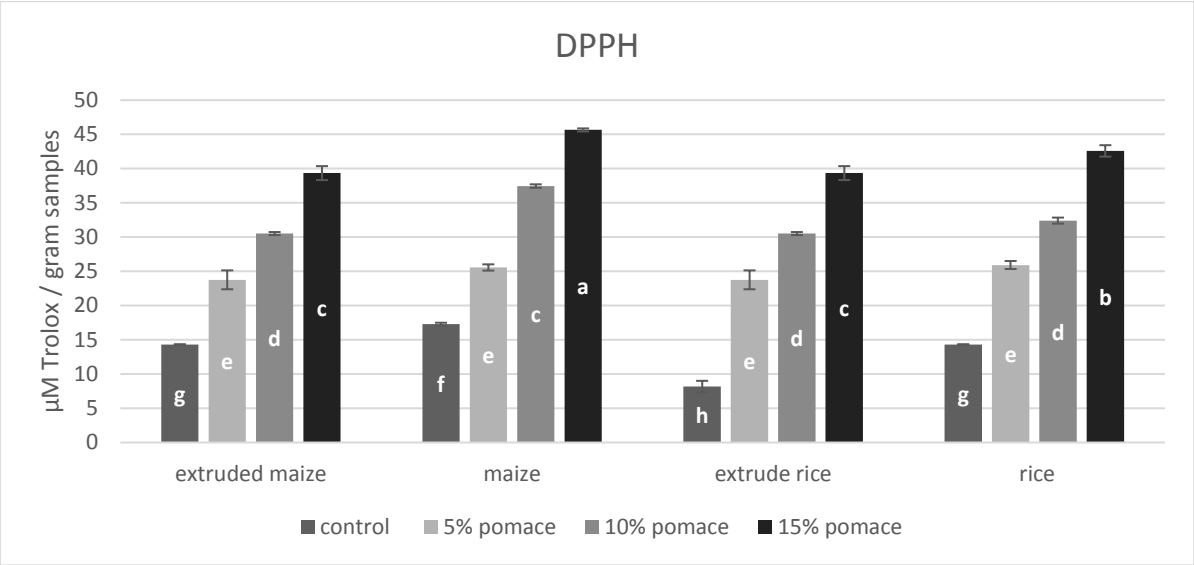
As mentioned previously a significant loss in the TPC was observed in the extruded product due to the extrusion process. The highest loss percentage was 16 % recorded for the extruded rice sample with 15 % pomace level. Similar results have been reported by Anton et al. (2009) where the TPC decreased in the extruded products compared with the raw mix for the corn-based extruded snacks with a common bean.

The observation shows that the antioxidant activity decreased in the extruded products, in comparison with the respective raw mix is similar to observations which have been reported with the extruded cranberry pomace (White et al., 2010). In that study the authors reported that the reduction in the antioxidant activity was dependent on the barrel temperature of the extruder. The higher barrel temperature degrades the anthocyanin and was around 45 % loss at 150 ^{o}C. In an another study reported by Khanal et al. (2009) the total anthocyanin content of the extruded blueberry pomace decreased to 1023 mg/kg from 1757 mg/kg in compared to un-extruded blueberry pomace.

The antioxidant activity of the extruded products in this research ranged between (8 – 39) µM TEAC / gram of sample, lowest for the extruded rice control and highest for the extruded maize with 15 % pomace sample. The observed antioxidant activity was in similar to the antioxidant activity shown by extruded cornmeal products containing fruit powder reported by Camire, Dougherty, and Briggs (2007). Where the mean antioxidant activity of the extruded products containing blueberry, concord grape, cranberry and red raspberry were 24, 19, 27 and 21 respectively.

There are various methods to determine the antioxidant activity of the products and each method creates a variation in the antioxidant activity of the same products leading to an inconsistency in the understanding of exact antioxidant activity of samples (Anton et al., 2009). This is partly why for this research we have used three methods of analysis (DPPH and ORAC assay methods) so that direct comparisons from other manuscripts could be achieved. The reaction mechanism in the ORAC involves the transfer of the hydrogen atom whereas in

the case of DPPH a single electron is transferred (Prior, Wu, & Schaich, 2005). DPPH assay shows low antioxidant activity value in comparison with the ORAC assay values for the same sample. The lower DPPH assay values have been linked with the (515 nm) wavelength used by DPPH methods, other pigments such as carotenoids and anthocyanins interfere as they also absorb at the same wavelength (Anton et al., 2009). The comparison between DPPH and ORAC value observed in this study showed that ORAC values were 3-4 times higher than DPPH values, which is in agreement to the earlier report by Awika, Rooney, Wu, Prior, and Cisneros-Zevallos (2003).

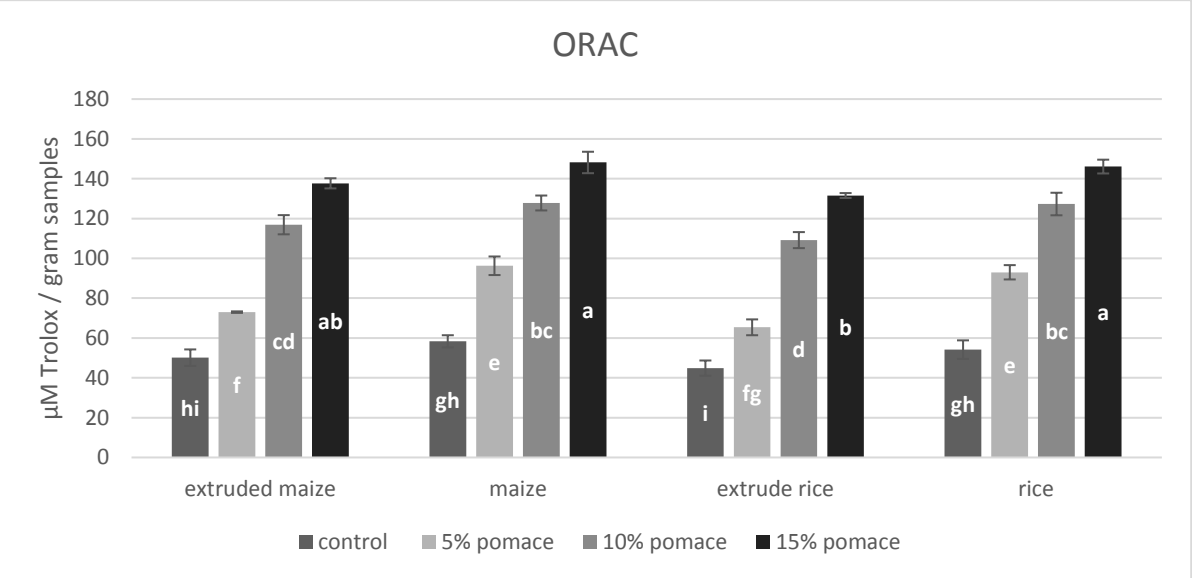


Values that do not share a letter are significantly different. P-value <0.05

Figure 6.2: DPPH result of the raw sample mix and extruded products

The antioxidant activity of the extruded products and raw sample mix observed by DPPH assay is shown in figure 6.2. The inclusion of pomace to the sample increased the antioxidant activity significantly. The incrementing pattern looks similar to the pattern observed for the TPC as discussed above. The antioxidant activity of the extruded products observed by DPPH assay was in accordance to the values reported by Camire et al. (2007). The inclusion of pomace at 5 % (w/w), 10 % (w/w) and 15 % (w/w) levels increased the antioxidant value (8.17 µM TEAC / g) of control extruded rice sample to 23, 30 and 39 µM TEAC / g respectively. In comparison to the control samples, the percentage increase in the TPC value for the samples with 15 % pomace were 175 %, 164 %, 381 % and 197 % for extruded maize, raw maize mix, extruded rice and raw rice mix respectively.

A significant loss in the antioxidant activity was observed in the extruded products due to the extrusion process. The highest loss percentage was 42 % recorded for the extruded rice control sample. However, for other samples with pomace, the loss percentage was much lower and ranged between 5 and 18 %. The observed value indicates that extruded products with pomace content were still able to retain a relatively good amount of antioxidant activity. In contrast to the above result, a 65 % loss in antioxidant activity was observed previously by researchers using DPPH methods in extruded made from corn starch with small red bean flour (Anton et al., 2009). However, in the same paper, the author reported a 22 % loss in antioxidant activity for extruded product made from corn starch with navy bean; this result is similar to the observed loss percentage for our extruded product.



Values that do not share a letter are significantly different. P-value <0.05

Figure 6.3: Orac assay of the sample mix and extruded products

The antioxidant activity of the extruded products and raw sample mix observed by ORAC assay is shown in figure 6.3. The inclusion of pomace to the sample increased the antioxidant activity significantly. The incrementing pattern looks similar to the pattern observed for the TPC and antioxidant activity observed by DPPH assay as discussed previously. The inclusion of pomace at 5 % (w/w), 10 % (w/w) and 15 % (w/w) levels increased the antioxidant value (44 µM TEAC / g) of control extruded rice sample to 65, 109 and 131 µM TEAC / g respectively (table 6.1). In comparison to the control samples, the percentage increase in the TPC value for the

samples with 15 % pomace were 174 %, 153 %, 193 % and 169 % for extruded maize, raw maize mix, extruded rice and raw rice mix respectively.

As observed for the DPPH analysis, a significant loss in the antioxidant activity of the samples was observed in the extruded products due to the extrusion process. The highest loss percentage was 29 % recorded for the extruded rice sample with 5 % pomace and ranged from 7 % to 24 % loss for other extruded samples. The observed loss in antioxidant activity by ORAC assay was in similar to the value observed in DPPH assay (above), which indicated that extruded products with pomace content were able to retain a relatively good amount of antioxidant activity.

Table 6.2: Correlations between ORAC, DPPH, AUC, ºHue and Expansion

	Expansion	Total phenol	ORAC	DPPH
Total phenol	-0.927 *			
	0.001 **			
ORAC	-0.844	0.971		
	0.008	0		
DPPH	-0.889	0.956	0.96	
	0.003	0	0	
AUC	0.811	-0.878	-0.782	-0.773
	0.015	0.004	0.022	0.025
o Hue	0.754	-0.884	-0.896	-0.906
	0.031	0.004	0.003	0.002

Cell Contents: * Pearson correlation (above), ** P-value (below), ($p < 0.05$) correlation is significant

The total phenol content (TPC) showed a strong positive correlation with the antioxidant assay ORAC and DPPH. This observation agrees with the earlier reports that the phenolic content of products showed a linear relation with the antioxidant activity with a correlation value of 0.85 between ORAC and TPC (Prior et al., 1998). Table 6.2 illustrates that in the current research the correlation between ORAC vs TPC was 0.97.

Additionally, ORAC and DPPH showed a strong positive correlation. The observed correlation was 0.96, very much similar to reported correlation value of 0.97 between ORAC and DPPH by Awika et al. (2003).

The TPC, ORAC and DPPH showed a strong negative correlation with the expansion characteristic of the products. This observation was to be expected and could be linked to the earlier observed pattern of expansion, where the increasing pomace content decreased the expansion of the extruded products. This may possibly be linked to the phenolic compounds being associated with the cell wall materials of the pomace and these non-starch polysaccharides restricting expansion of the products. The antioxidant activity and TPC relate to the amount of pomace. Hence, it can be concluded that extruded product with increasing pomace content increased the TPC, and antioxidant activity of the product but decreased the expansion characteristic of the product.

The negative correlation found between hue angle and antioxidant activity could be interpreted by the colour of the pomace and its amount in the extruded products. The pomace colour is darkish purple while the maize and rice are lighter in appearance (figure B.3 in the appendix). The increasing pomace content adds a darker colour to the products and at the same time increases the antioxidant activity of the product (refer figure B.1 and B.2 in the appendix). The calculated hue angle of the product tends to decrease (table 4.2) with the increase in pomace. The available data may not be able to justify the link between the colour and antioxidant activity because it would fit more correctly to correlate the change in colour with varying pomace content. However, the relation between the physical appearance of the food with the antioxidant activity and TPC have been demonstrated in several papers. Wang and Lin (2000) reported blackberry, raspberry and strawberry at green stage showed high antioxidant activity whereas in the pink stage showed low antioxidant activity. In another paper, Walkowiak-Tomczak, Reguła, and Łysiak (2008) reported high antioxidant activity possessed by darker coloured plums.

The TPC, ORAC and DPPH showed a strong negative correlation with the AUC value. The reason behind could be decreased in the starch content of product when pomace was introduced. Further, the introduction of pomace increased the dietary fibre, which could trap the starch molecule and reduce the starch digestibility (Wolever, 1990). This observation could serve the initial hypothesis made in this research that the extruded product with lower glycaemic index and enriched with antioxidant can be produced using the boysenberry pomace.

6.1 Important observations:

- The total phenolic content (TPC) of the boysenberry pomace was 1618.57± 2.73 mg GAE/100 g pomace DW. The antioxidant activity for the boysenberry pomace was 95 ± 0.53 and 732.60 ± 20.02 µM TEAC / g pomace DW for DPPH and ORAC assay.

- The inclusion of pomace increased the antioxidant activity of the extruded product significantly.

- The loss in the antioxidant activity was observed between raw feed mix and extruded product due to the extrusion process.

- The percentage loss of antioxidant activity ranged between 5 % and 24 %, which is relatively less in compared with the previous manuscript.

- The TPC, ORAC and DPPH showed a strong negative correlation with the AUC value showing the possibility for the production of nutritionally valued extruded product enriched with antioxidant and lower glycaemic index.

Chapter 7

Conclusion

To recap, the observation of the previous chapters were:

- An increase in the pomace content in the extruded product decreased the expansion and crispiness.
- The moisture content and the hardness of the extrudates tended to increase with an increase in the pomace content.
- The extrusion process and pomace content showed significant changes to the CIE colour reading of the extruded products. The increasing pomace content resulted in a darker colour of the products.
- The moisture and the crispiness of the extruded products showed negative correlation.
- The expansion of the products showed positive correlation to the crispiness and the CIE colour reading (º Hue) of the extruded product.
- The inclusion of pomace at three different levels (5 %, 10 % and 15 %) (w/w) to the maize and rice based extruded products had a significant impact on the degree of starch digestion.
- The addition of pomace reduce the area under the curve, which means less starch is available for digestion.
- The highest reduction in the starch digestion was 18 % for the rice extruded with 15 % pomace. Whereas for the sample with maize with 15 % pomace, the reduction was by 17 %.
- The AUC showed a positive correlation with the crispiness and expansion attribute of the extruded products whereas showed a negative correlation with the moisture of the product.

- The total phenolic content (TPC) of the boysenberry pomace was 1618.57± 2.73 mg GAE/100 g pomace DW. The antioxidant activity for the boysenberry pomace was 95 ± 0.53 and 732.60 ± 20.02 µM TEAC / g pomace DW for DPPH and ORAC assay.

- The inclusion of pomace increased the antioxidant activity of the extruded product significantly.

- The loss in the antioxidant activity was observed between raw feed mix and extruded product due to the extrusion process.

- The percentage loss of antioxidant activity ranged between 5 % and 24 %, which is relatively less in compared with the previous manuscript.

- The TPC, ORAC and DPPH showed a strong negative correlation with the AUC value showing the possibility for the production of nutritionally valued extruded product enriched with antioxidant and lower glycaemic index.

In this research project, an extruded ready-to-eat product from rice and maize was successfully developed by incorporating the boysenberry pomace, which fulfils the initial objective of the research. The nutritional qualities of these snacks foods were greatly enhanced as the boysenberry pomace was found to be a good source of polyphenols and antioxidant. The TPC, DPPH and ORAC values for the pomace was 1618.57± 2.73 mg GAE/100 g pomace DW, 95 ± 0.53 and 732.60 ± 20.02 µM TEAC / g pomace DW respectively. The various changes in physiochemical properties of extruded products after the addition of pomace appear to have been related to its dietary fibre content. These observed data supports the earlier reports on berry fruits variety being a rich source of antioxidant, polyphenol and dietary fibres. However, the actual dietary fibre content in the pomace was not analysed in this study. Thus further work is required to correlate the relationship of the fibre content of pomace to enhanced nutritional quality and product characteristic which may have been altered.

The inclusion of pomace at three different levels (5 %, 10 % and 15 %) (w/w) to the maize and rice based extruded products had a significant impact on its physiochemical characteristics and nutritional value. Generally, the higher the level of pomace in an extruded products, the higher was the changes in physiochemical characteristics and nutritional value.

The increase in the pomace content in the extruded product decreased the expansion and crispiness whereas increased the moisture content and hardness of the extruded products. The extrusion process and pomace content showed significant changes to the CIE colour reading of the extruded products. The increasing pomace content added a darker colour to the products. This observation supports the initial hypothesis of expected alteration to the physical attribute of the product with the addition of pomace.

The inclusion of pomace increased the antioxidant activity of the extruded product significantly. The percentage increase in the antioxidant activity of the extruded products observed by ORAC assay ranges from 45 % to 193 % and by DPPH assay it ranged from 66 % to 381 %. The percentage increase in the TPC ranged from 22 % to 219 %. The higher the level of pomace in an extruded products, the higher was its antioxidant activity and TPC value.

The ingredients in the pomace responsible for its antioxidant properties survived the extrusion process. There was a significant loss in the antioxidant activity of the products due to the extrusion process. However, the loss observed in this research was much lower than that reported by other researchers (Anton et al., 2009). The percentage loss in the antioxidant activity due to extrusion cooking observed by ORAC assay ranged from 7 % to 24 % and by DPPH assay it ranges from 5 % to 18 %. This observation is a valuable outcome of the research as it adds data for the stability of antioxidant in an extrusion cooking. Besides it also proves the initial hypothesis made in the research.

The antioxidant activity of the product showed a positive correlation with the colour changes of the product however, the available data may not be enough to justify the link between the colour and antioxidant activity because it would fit more correctly to correlate the change in colour with varying pomace content.

The inclusion of pomace at three different levels (5 %, 10 % and 15 %) (w/w) to the maize and rice based extruded products had a significant impact on the degree of starch digestion. The increasing pomace level decreased the degree of starch digestion. The pomace appears to have increased the dietary fibres in the products and decreased the degree of starch digestion. The observed highest reduction in the starch digestion was 18 % and 17 % for the rice and maize extruded product with 15 % pomace level respectively.

The moisture of the extruded products showed a negative correlation with the crispiness and AUC value. Whereas, the AUC showed a positive correlation with the crispiness and expansion attribute of the extruded products. Both the rice and maize extruded control had the highest crispiness attribute, expansion ratio and a higher degree of starch digestion.

The total phenol content showed a strong positive correlation with the antioxidant assay ORAC and DPPH. Additionally, the TPC, ORAC and DPPH showed a strong negative correlation with the AUC value and expansion characteristic of the products. Hence the product manufactured showed potential nutritional benefits with high antioxidant activity with a lower degree of starch digestion. However, the sensory analysis for the product with varying level of pomace content needs to be carried out to understand its acceptability within a consumers market. Such a study involving consumer sensory analysis would provide valuable information on the commercial opportunity of producing berry enriched ready to eat snack products form potential berry processing waste stream.

The observed results of this research provide and add valuable information and data to the manufacturers of extruded products. The successful manufacture of an extruded product with lower glycaemic index and enriched with antioxidant could be created using the boysenberry pomace.

Recommendation for future work

The extrusion cooking was carried out with a single-screw extruder at a constant parameter such as temperature, feed rate. The structural properties such as expansion, moisture, crispiness are depend on temperature and feed rate. Further studies can be carried out with extrusion cooking by the use of twin-screw extruder at variable temperature and residence time.

The research was conducted with the frozen boysenberry available from the local market. The previous papers have reported the fluctuation on the antioxidant activity of the berries with the change in the cultivar, different stage of maturity, growing season and processing technology (Sójka & Król, 2009). The fluctuation in the amount of extracted polyphenol just by the used of various extraction process have been reported earlier by various authors (Cardona et al., 2009; Harrison et al., 2013). Additional research should be carried out for boysenberry pomace obtained from various cultivar and time of maturity.

Further research can be carried out to investigate the shelf life of the extruded products. The extruded products were stored in an air-tight plastic bags. With the passage of time, the colour of some extruded product samples started to fade away. Colour of the products were strongly correlated with the antioxidant activity of the products. Hence, more study needs to be carried out to understand the stability of the antioxidant activity of the extruded products with the passage of time.

The physical and texture appearance of the products is the important aspect looked by the consumer. Sensory analysis by a consumer panel needs to be carried out to see how much pomace content will be acceptable by the consumer market.

Determination of dietary fibre composition of the berry pomace is required in order to establish structure and function relationships between berry pomace utilisation and production of low glycaemic index high antioxidant snack products for tomorrow consumers. This would be highly relevant for export markets and commercialisation.

Appendix A

Raw Data

A.1 Raw Data for colour measurement

Table A.1: Raw data for the colour measurement of raw mix and extruded product samples

Sample	L	a	b
Maize control	96.49	-15.09	40.33
Maize control	96.23	-15.13	40.44
Maize control	96.44	-15.2	40.72
Maize+5% pomace	92.15	-5.25	37.13
Maize+5% pomace	92.5	-5.87	37.79
Maize+5% pomace	92.38	-5.99	37.88
Maize+10% pomace	88.43	-1.45	33.96
Maize+10% pomace	88.53	-1.98	33.9
Maize+10% pomace	88.26	-1.83	33.55
Maize+15% pomace	86.09	0.34	31.15
Maize+15% pomace	85.88	0.25	30.95
Maize+15% pomace	86.07	0.12	31.11
Extruded maize control	97.09	-14.1	36.43
Extruded maize control	96.95	-13.92	35.25
Extruded maize control	96.92	-13.82	35.69
Extruded maize+5% pomace	90.54	-3.09	32.58
Extruded maize+5% pomace	90.26	-2.97	32.41
Extruded maize+5% pomace	89.91	-2.87	32.7
Extruded maize+10% pomace	86.59	0.57	29.95
Extruded maize+10% pomace	86.84	0.11	29.74
Extruded maize+10% pomace	86.62	0.65	29.94
Extruded maize+15% pomace	84.04	1.02	27.92
Extruded maize+15% pomace	84.2	0.97	28.08
Extruded maize+15% pomace	84.16	1.07	28.04
Rice control	96.23	-10.01	24.35
Rice control	96.5	-9.97	24.6
Rice control	96.33	-10	24.7
Rice+5% pomace	92.36	-4.29	29.73
Rice+5% pomace	92.42	-4.63	29.6
Rice+5% pomace	92.16	-4.56	29.56
Rice+10% pomace	88.21	-1.59	28.47
Rice+10% pomace	88.18	-1.58	28.43
Rice+10% pomace	88.07	-1.39	28.34
Rice+15% pomace	86.13	0.34	27.67
Rice+15% pomace	86.08	0.21	27.58
Rice+15% pomace	86.35	0.16	27.7

Sample	L	a	b
Extruded rice control	96.74	-8.04	19.42
Extruded rice control	96.7	-8.34	20.22
Extruded rice control	96.88	-8.53	20.58
Extruded rice+5% pomace	93.15	-6.5	31.79
Extruded rice+5% pomace	93.36	-7.03	31.83
Extruded rice+5% pomace	93.05	-6.59	31.68
Extruded rice+10% pomace	87.25	-2.86	29.44
Extruded rice+10% pomace	87.72	-3.07	29.57
Extruded rice+10% pomace	87.42	-3.02	29.46
Extruded rice+15% pomace	85.36	-1.83	28.42
Extruded rice+15% pomace	85.07	-1.98	28.24
Extruded rice+15% pomace	85.11	-1.98	28.24

(***L**** = 0 yields black and ***L**** = 100 indicates diffuse white)
(***a****, negative values indicate green while positive values indicate magenta)
(***b****, negative values indicate blue and positive values indicate yellow)

Table A.2: CIE colour reading (L*, a* and b*) of sample feed mix and extruded products

Samples	L	a	b
Maize control	96.3867 ± 0.1380 b	-15.1400 ± 0.0557 k	40.497 ± 0.201 a
Maize+5% pomace	92.343 ± 0.178 d	-5.703 ± 0.397 f	37.600 ± 0.410 b
Maize+10% pomace	88.4067 ± 0.1365 f	-1.753 ± 0.273 c	33.803 ± 0.221 d
Maize+15% pomace	86.0133 ± 0.1159 i	0.2367 ± 0.1106 b	31.0700 ± 0.1058 f
Extruded maize control	96.9867 ± 0.0907 a	-13.9467 ± 0.1419 j	35.790 ± 0.59 c
Extruded maize+5% pomace	90.237 ± 0.316 e	-2.9767 ± 0.1102 d	32.5633 ± 0.1457 e
Extruded maize+10% pomace	86.6833 ± 0.1365 h	0.443 ± 0.29 ab	29.8767 ± 0.1185 g
Extruded maize+15% pomace	84.1333 ± 0.0833 k	1.0200 ± 0.0500 a	28.0133 ± 0.0833 h
Rice control	96.3533 ± 0.1365 b	-9.9933 ± 0.0208 i	24.550 ± 0.180 i
Rice+5% pomace	92.3133 ± 0.1361 d	-4.493 ± 0.180 e	29.6300 ± 0.088 g
Rice+10% pomace	88.1533 ± 0.0737 f	-1.5200 ± 0.1127 c	28.4133 ± 0.0666 h
Rice+15% pomace	86.1867 ± 0.1436 i	0.2367 ± 0.0929 b	27.6500 ± 0.0624 h
Extruded rice control	96.3533 ± 0.136 ab	-8.303 ± 0.247 h	20.073 ± 0.594 j
Extruded rice+5% pomace	93.1867 ± 0.1582 c	-6.707 ± 0.284 g	31.7667 ± 0.0777 f
Extruded rice+10% pomace	87.463 ± 0.238 g	-2.9833 ± 0.1097 d	29.4900 ± 0.0700 g
Extruded rice+15% pomace	85.1800 ± 0.1572 j	-1.9300 ± 0.0866 c	28.3000 ± 0.1039 h
Boysenberry pomace	19.55 ± 0.280	40.72 ± 0.5853	-23.76 ± 0.7517

A.2 Raw Data for texture analysis

Table A.3: Raw data fo crispiness and hardness for extruded products

Samples	Repeat	Count Peaks+ F (g) 1:2	Force 1 (N)
		Crispiness	hardness
Extruded maize control	1	39	27.15205
Extruded maize control	2	33	27.40091
Extruded maize control	3	40	32.87983
Extruded maize control	4	36	30.00862
Extruded maize control	5	37	29.48164
Extruded maize control	6	37	32.06531
Extruded maize control	7	43	29.28089
Extruded maize control	8	38	36.0825
Extruded maize control	9	33	31.34699
Extruded maize control	10	39	29.50883
Extruded maize+5% pomace	1	38	28.02095
Extruded maize+5% pomace	2	36	29.09686
Extruded maize+5% pomace	3	29	28.09205
Extruded maize+5% pomace	4	38	29.16587
Extruded maize+5% pomace	5	26	27.21584
Extruded maize+5% pomace	6	31	30.83674
Extruded maize+5% pomace	7	29	31.50069
Extruded maize+5% pomace	8	26	28.87415
Extruded maize+5% pomace	9	30	27.74282
Extruded maize+5% pomace	10	37	31.84678
Extruded maize+10% pomace	1	51	30.16756
Extruded maize+10% pomace	2	48	37.5777
Extruded maize+10% pomace	3	34	33.14855
Extruded maize+10% pomace	4	40	31.78718
Extruded maize+10% pomace	5	34	28.22379
Extruded maize+10% pomace	6	52	31.8792
Extruded maize+10% pomace	7	40	28.47683
Extruded maize+10% pomace	8	38	39.04363
Extruded maize+10% pomace	9	34	27.34131
Extruded maize+10% pomace	10	38	30.15919
Extruded maize+15% pomace	1	34	27.8526
Extruded maize+15% pomace	2	33	30.91725
Extruded maize+15% pomace	3	35	28.91284
Extruded maize+15% pomace	4	38	28.57302
Extruded maize+15% pomace	5	37	35.66949
Extruded maize+15% pomace	6	35	32.36226
Extruded maize+15% pomace	7	26	33.24161
Extruded maize+15% pomace	8	28	32.23679
Extruded maize+15% pomace	9	27	36.98798
Extruded maize+15% pomace	10	37	30.48855
Extruded rice control	1	53	22.01817
Extruded rice control	2	54	20.69131
Extruded rice control	3	49	19.79315
Extruded rice control	4	48	22.13842
Extruded rice control	5	49	23.47155
Extruded rice control	6	58	23.75177

Samples	Repeat	Count Peaks+ F (g) 1:2	Force 1 (N)
Extruded rice control	7	38	21.47656
Extruded rice control	8	54	22.01504
Extruded rice control	9	47	25.83774
Extruded rice control	10	43	25.06504
Extruded rice+5% pomace	1	53	26.95862
Extruded rice+5% pomace	2	48	30.9413
Extruded rice+5% pomace	3	32	31.29471
Extruded rice+5% pomace	4	40	33.88256
Extruded rice+5% pomace	5	45	31.19956
Extruded rice+5% pomace	6	31	29.44923
Extruded rice+5% pomace	7	36	27.68217
Extruded rice+5% pomace	8	46	30.04104
Extruded rice+5% pomace	9	42	24.17838
Extruded rice+5% pomace	10	47	28.72463
Extruded rice+10% pomace	1	45	38.84392
Extruded rice+10% pomace	2	43	42.37071
Extruded rice+10% pomace	3	48	39.70863
Extruded rice+10% pomace	4	50	38.38804
Extruded rice+10% pomace	5	46	38.94534
Extruded rice+10% pomace	6	44	31.85515
Extruded rice+10% pomace	7	41	37.86524
Extruded rice+10% pomace	8	30	36.98171
Extruded rice+10% pomace	9	45	38.2887
Extruded rice+10% pomace	10	38	39.7034
Extruded rice+15% pomace	1	41	29.5747
Extruded rice+15% pomace	2	41	26.83419
Extruded rice+15% pomace	3	33	40.64025
Extruded rice+15% pomace	4	28	36.38886
Extruded rice+15% pomace	5	36	25.77396
Extruded rice+15% pomace	6	36	27.80764
Extruded rice+15% pomace	7	36	25.65894
Extruded rice+15% pomace	8	33	30.01176
Extruded rice+15% pomace	9	36	33.18201
Extruded rice+15% pomace	10	29	37.53588

A.3 Raw data for antioxidant assay

Table A.4: Raw data for Total phenol, ORAC and DPPH

Samples	Rep	Total Phenol	ORAC	DPPH
Maize control	1	63.10756972	167.2099	17.54054
Maize control	2	63.4490609	185.0698	17.27027
Maize control	3	63.79055208	172.7622	17
Maize+5% pomace	1	90.19920319	290.6258	25.37838
Maize+5% pomace	2	90.31303358	274.061	25.10811
Maize+5% pomace	3	89.74388162	301.8434	26.18919
Maize+10% pomace	1	135.2760387	373.345	37.27027
Maize+10% pomace	2	138.0079681	395.5558	37.27027
Maize+10% pomace	3	136.8696642	381.4504	37.81081
Maize+15% pomace	1	176.7103017	426.0249	45.91892
Maize+15% pomace	2	177.1656232	451.4955	45.37838
Maize+15% pomace	3	179.1007399	456.0003	45.64865
Extruded maize control	1	72.44166192	141.8199	14.2973
Extruded maize control	2	72.21400114	144.6284	14.2973
Extruded maize control	3	71.53101878	164.5972	14.2973
Extruded maize+5% pomace	1	87.58110415	218.7214	21.86486
Extruded maize+5% pomace	2	88.3779169	220.2149	24.2973
Extruded maize+5% pomace	3	88.60557769	218.0343	25.10811
Extruded maize+10% pomace	1	123.6653386	361.9287	30.51351
Extruded maize+10% pomace	2	124.348321	334.4091	30.78378
Extruded maize+10% pomace	3	122.8685259	355.7291	30.24324
Extruded maize+15% pomace	1	159.1804212	421.6589	38.62162
Extruded maize+15% pomace	2	158.269778	407.0471	38.62162
Extruded maize+15% pomace	3	156.4484917	410.4703	40.78378
Rice control	1	43.64257257	173.2762	14.2973
Rice control	2	44.89470689	146.7778	14.2973
Rice control	3	43.41491178	167.527	14.2973
Rice+5% pomace	1	85.07683552	289.2905	25.37838
Rice+5% pomace	2	84.96300512	267.7657	26.72973
Rice+5% pomace	3	84.05236198	280.0281	25.64865
Rice+10% pomace	1	108.412066	362.3946	32.94595
Rice+10% pomace	2	108.0705748	393.335	32.40541
Rice+10% pomace	3	109.2088788	389.927	31.86486
Rice+15% pomace	1	168.6283438	432.12	42.13514
Rice+15% pomace	2	172.7262379	450.3143	43.75676
Rice+15% pomace	3	170.4496301	432.5371	41.86486
Extruded rice control	1	44.43938532	125.1801	8.621622

Samples	Rep	Total Phenol	ORAC	DPPH
Extruded rice control	2	44.55321571	147.3895	7
Extruded rice control	3	44.21172453	131.3661	8.891892
Extruded rice+5% pomace	1	72.66932271	201.8586	21.86486
Extruded rice+5% pomace	2	73.23847467	182.3822	24.2973
Extruded rice+5% pomace	3	72.7831531	204.0687	25.10811
Extruded rice+10% pomace	1	105.3386454	315.4321	30.51351
Extruded rice+10% pomace	2	105.793967	339.5712	30.78378
Extruded rice+10% pomace	3	104.5418327	327.5016	30.24324
Extruded rice+15% pomace	1	141.4228799	398.3992	38.62162
Extruded rice+15% pomace	2	141.4228799	391.0046	38.62162
Extruded rice+15% pomace	3	142.5611838	394.7019	40.78378

Total phenol : Gallic acid equivalent (mg) / 100 gram of sample; DPPH : µM Trolox / gram of sample; ORAC: µM Trolox / gram of sample

A.4 Raw data of starch content

Table A.5: Raw data for starch content of the extruded samples

Sample	Frequency	Starch (g/100 g) "dwb"
Extruded rice control	1	71.01928
Extruded rice control	2	69.88597
Extruded rice control	3	76.0939
Extruded rice+5% pomace	1	69.01088
Extruded rice+5% pomace	2	66.9361
Extruded rice+5% pomace	3	69.97997
Extruded rice+10% pomace	1	70.41061
Extruded rice+10% pomace	2	64.84203
Extruded rice+10% pomace	3	60.67768
Extruded rice+15% pomace	1	65.42154
Extruded rice+15% pomace	2	61.47435
Extruded rice+15% pomace	3	60.97313
Extruded maize control	1	68.13289
Extruded maize control	2	60.24584
Extruded maize control	3	61.05964
Extruded maize+5% pomace	1	61.0549
Extruded maize+5% pomace	2	55.45455
Extruded maize+5% pomace	3	60.52655
Extruded maize+10% pomace	1	51.54864
Extruded maize+10% pomace	2	54.61365
Extruded maize+10% pomace	3	56.20385
Extruded maize+15% pomace	1	51.24774
Extruded maize+15% pomace	2	52.00122
Extruded maize+15% pomace	3	51.35504

A.5 Starch hydrolysis data

Table A.6: Starch hydrolysis of extruded rice samples

Samples	Reducing sugar (mg/g)			
	0 min	**20 min**	**60 min**	**120min**
Extruded rice control	-53.37 ± 47.07 [a]	833.85 ± 45.24 [a]	856.52 ± 24 [a]	864.28 ± 15.65 [a]
Extruded rice +5% pomace	-24.76 ± 30.30 [a]	775.76 ± 31.88 [ab]	787.04 ± 24.75 [ab]	787.13 ± 20.3 [ab]
Extruded rice +10% pomace	-10.80 ± 55.55 [a]	662.15 ± 38.41 [c]	740.78 ± 67.72 [ab]	758.42 ± 64.72 [b]
Extruded rice +15% pomace	12.82 ± 35.33 [a]	680.84 ± 47.75 [bc]	691.57 ± 46.06 [b]	708.36 ± 10.81 [b]

Means that do not share a letter along a column are significantly different. P-value < 0.05

Table A.7: Starch hydrolysis of extruded maize samples

	Reducing sugar (mg/g)			
Samples	**0 min**	**20 min**	**60 min**	**120min**
Extruded maize control	-63.18 ± 26.89 [b]	687.56 ± 12.83 [a]	742.46 ± 10.71 [a]	742.48 ± 46.9 [a]
Extruded maize+5% pomace	-3.13 ± 21.39 [a]	655.91 ± 22.98 [ab]	702.17 ± 46.21 [ab]	711.65 ± 61 [ab]
Extruded maize+10% pomace	2.07 ± 14.31 [a]	586.32 ± 26.83 [b]	654.55 ± 56.7 [ab]	661.40 ± 8.6 [bc]
Extruded maize+15% pomace	3.53 ± 14.16 [a]	569.01 ± 59.12 [b]	600.20 ± 30.1 [b]	613.30 ± 14.45 [c]

Means that do not share a letter along a column are significantly different. P-value < 0.05

A.6 Correlation data

Table A.8: Correlation between the various parameter observed in this study

	Moisture	Crispiness	Hardness	Expansion	Total phenol	Orac	Dpph	AUC	o Hue
Crispiness	-0.818								
	0.013								
Hardness	0.324	-0.3							
	0.434	0.47							
Expansion	-0.65	0.8	-0.425						
	0.081	0.017	0.294						
Total phenol	0.635	-0.623	0.566	-0.927					
	0.091	0.099	0.143	0.001					
Orac	0.524	-0.455	0.609	-0.844	0.971				
	0.182	0.257	0.109	0.008	0				
Dpph	0.522	-0.585	0.641	-0.889	0.956	0.96			
	0.184	0.127	0.087	0.003	0	0			
AUC	-0.837	0.749	-0.542	0.811	-0.878	-0.782	-0.773		
	0.01	0.032	0.165	0.015	0.004	0.022	0.025		
o Hue	-0.669	0.555	-0.568	0.754	-0.884	-0.896	-0.906	0.838	
	0.069	0.153	0.142	0.031	0.004	0.003	0.002	0.009	
Chroma	0.187	-0.475	0.366	-0.1	-0.048	-0.196	-0.035	-0.252	0.071
	0.658	0.234	0.373	0.814	0.911	0.642	0.935	0.548	0.868

Upper cell indicate Pearson coefficient; lower cell indicate p-value, ($p < 0.05$) correlation is significant

Appendix B

Picture of raw products

Figure B.1: Picture of budget long brown rice used in this research

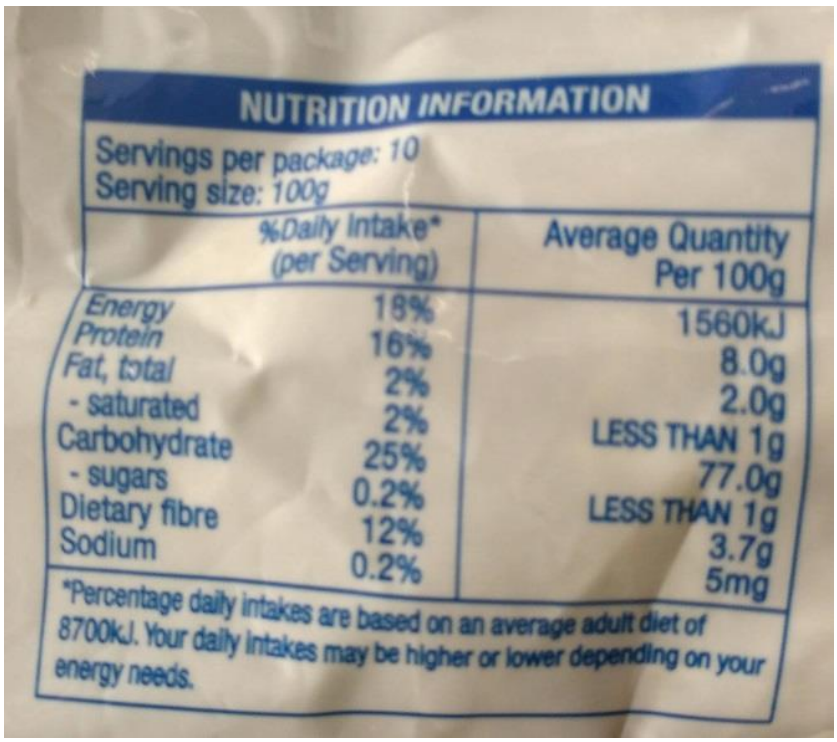


Figure B.2: Picture showing nutrition information of the long brown rice

Figure B.3: Picture of popping corn (maize) used in this research

Figure B.4: Picture showing nutrition information of the popping corn (maize)

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