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UNIVERSITY COLLEGE, CORK

DEPARTMENT OF FOOD AND NUTRITIONAL SCIENCES



Characterisation and application of fruit by-products as novel ingredients in gluten-free products.

Thesis presented by

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Carried out at Teagasc Food Research Centre, Ashtown, Dublin & in the Department of Food and Nutritional Sciences, National University of Ireland, University College, Cork.

Under the direction of

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And the supervision of

Dr. Eimear Gallagher,

Teagasc Food Research Centre, Ashtown, Dublin.

For the degree of

Doctor of Philosophy - (PhD in Food Science and Technology)

Head of School

Prof. Yrjo Roos - January 2014

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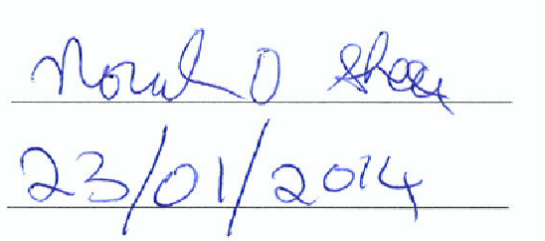
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Declaration by Candidate:

I hereby certify that the work on this, my thesis is based on my own independent work, except where I have received help as stated in the acknowledgements and text.

All quotations and summary of the work of others have been acknowledged where appropriate.

Signature of candidate: 

Date: 23/01/2014

Abstract:

Literature has revealed that "waste" left from the processing of fruit can still contain a substantial quantity of macro and minor nutrients. The aim of this thesis was to ascertain the nutritional and structural properties and potential uses of two fruit by-products [apple pomace (*Malus domestica* Cv. "Karmijn de Sonnaville") and orange pomace (*Citrus sinensis* L. Cv. "Valencia")] in gluten-free bread and extruded snack formulations.

The physicochemical and nutritional properties of the fruit by-products were initially studied. Apple pomace contained a high level of fibre and pectin. The isolated pectin was demonstrated to have a high level of methylation which developed viscous pastes. Orange pomace also had high levels of fibre and pectin, and it was an abundant source of minerals such as potassium and magnesium. Orange pomace had a poor gelling ability.

The flour obtained after milling dried orange pomace was used in the formulation of gluten-free bread with the aid of a response surface design. Due to the fibrous properties of orange pomace flour, proofing and water addition were also studied. When added at levels greater than 6%, the loaf volume decreased. The number of cells per slice also decreased with increasing orange pomace addition. Inclusion of orange pomace at levels of up to 4% increased crumb softness. An optimised formulation and proofing time was derived using the optimisation tool; these consisted of 5.5% orange pomace, 94.6% water inclusion and with 49 minutes proofing. These optimised parameters doubled the total dietary fibre content of the bread compared to the original control.

The pasting properties, rheology, microstructure and sensory characteristics of the optimised formulation (batter and bread) were investigated. Pasting results showed how orange pomace inclusions reduced the final viscosity of the batter, hence reducing the occurrence of starch gelatinisation. Rheological properties such as the storage modulus (G') and complex modulus (G*) increased in the orange pomace batter compared to the control batter. This demonstrates how the orange pomace as an ingredient improved the robustness of the formulation. Sensory panellists scored the orange pomace bread comparably to the control bread.

Milled apple pomace was studied as a potential novel ingredient in an extruded snack. As extrusion requires the trialling of a number of extruder parameters, a response surface design was again used to develop an optimised snack. The parameters studied were apple pomace addition, die head temperature and screw speed. Screw speed had the most significant impact on extrudate characteristics. As screw speed increased the favourable extrudate characteristics such as radical expansion ratio, porosity and specific volume decreased. The inclusion of apple pomace had a negative effect on extrudate characteristics at levels greater than 8% addition. Including apple pomace reduced the hardness and increased the crispiness of the snack. Using the optimisation tool, the optimised and validated formulation and extrusion process contained the following parameters: 7.7% apple pomace, 150^{o}C die head temperature and a screw speed of 69 rpm.

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1. Introduction:

The fruit processing industry generates tonnes of fruit "waste" every year. Depending on the processing method e.g. juice pressing to develop final products; some create more "waste" or by-product than others. This "waste" or by-product can be described as the pips, kernel, skin or peel of the fruit. Presently, there are no functional applications for these by-products. Processors can offer some types of by-products e.g. apple pomace, to farmers for animal food but the remaining fruit waste streams are incinerated or dumped in landfills, at a cost to the processor.

Previous research groups have illustrated fruit by-products to still contain an abundant quantity of macro and minor nutrients e.g. dietary fibre and minerals. As these fruit by-products are derived from the exterior of the fruit, they tend to contain a substantial quantity of fibre e.g. cellulose, hemicelluloses and pectin. Not only does fibre offer significant health benefits, but it has also been demonstrated to contain a high functionality which can be utilized as an ingredient in food products. It has such functional properties as water holding, binding, swelling and the ability to form viscous solutions. Consumers of late have become more conscious as to the source of the ingredients which make up the products they consume. In particular, they have become more aware of, and dislike the inclusion of synthetic ingredients in food products. As fruit by-product flours are from a natural source and have not undergone any chemical changes they may be considered to be natural ingredients. They are also free of gluten and lactose, which makes them ideal candidates for gluten and lactose-free products.

The first experimental chapter describes the preparation, composition and physicochemical properties of apple and orange pomace from the cultivars "Karmijn de Sonnaville" and "Valencia". The gelling and viscosity created by these by-products was also studied to help decipher the type of end product to which they would be suited.

Coeliac disease is an autoimmune disease which is triggered by the digestion of the protein components of gluten (gliadins and glutenins). When gluten is ingested it results in the inflammation of the small intestinal mucosa. The main cause of inflammation in the small intestine is the passing over of incomplete ingested gluten proteins across the epithelium and entering the lamina propria of the small intestine. This inflammation can cause the malabsorption of essential nutrients leading to nutritional deficiencies, gastrointestinal cancers and autoimmune diseases such as anaemia and osteoporosis. Cereal products in the diet of a coeliac mainly consist of refined flours and starches. Unlike their gluten counterparts, these flours and starches are not enriched or fortified with vitamins or minerals. Therefore, the diets of coeliac sufferers are neglected in some of the most important nutrients needed to sustain a balanced healthy diet.

The quality of these gluten-free products is another issue. Bread formulations created from these starches tend to lack flavour, mouth feel and have a very short shelf-life. Cereal technologists are endeavouring to develop gluten-free products which are similar to their wheat equivalents. To develop a greater understanding of gluten-free formulations, cereal technologists investigate all aspects of the formulation from the raw materials (and their functionality) to the batter

development and the physical characteristics of the end product.

The second experimental chapter investigated the inclusion of orange pomace as a value added ingredient in a gluten-free bread formulation. This was carried with the aid of a response surface design. Analysis was performed on all bread quality characteristics.

Once the bread formulation was optimised, its structure and rheological properties were studied in the third experimental chapter. This study demonstrated the cooked starch properties, rheological characteristics and the microstructure of the orange pomace-enhanced formulation.

The final experiential chapter illustrates how apple pomace can be used as an ingredient by incorporating it into an extruded puffed snack without compromising on product quality.

Chapter 1

Literature Review: Part A

Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products.

Published in Innovative Food Science and Emerging Technologies (2012), 16, 1-10.

1.1 Part A: Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products.

1.1.1 Abstract:

Presently, producers are striving to create products which contain a value added factor, such as dietary fibre or in more recent times, phytochemicals. The production and addition of such nutrients can be quite costly for the producer. In the fruit and vegetable industry, the preparation and processing procedures can lead to one third of the product being discarded. This can be costly for the manufacturer and also may have a negative impact on the environment. Research has shown that these by-products can have a high nutritional value. It has also been suggested, that they could be used as a food ingredient due to their functional abilities such as gelling and water binding. The focus of this review is on the nutritional and functional properties of the by-products of food processing and their potential applications as nutritional new ingredients in foods.

1.1.2 Introduction:

Dietary guidelines advise a diet rich in fruit and vegetables for a healthy lifestyle. At present up to one third of fruit and vegetables in the form of peels, pips and skins can be discarded during preparation and processing, therefore creating a 'waste', while also decreasing the maximum nutritional potential of the fruit or vegetable. Researchers are discovering new alternative uses for such 'waste' as potential value added ingredients.

Currently, consumers are becoming increasingly interested in maintaining a healthy diet and lifestyle. Schieber et al. (2001) carried out a review on by-products, focusing on the major functional compounds such as carotenoids, polyphenols, tocopherols and others. This review highlighted the excess availability of untapped natural sources of micronutrients and their potential to be readily capitalised as a healthy ingredient. One of the main nutrients included in such diets is dietary fibre. Over the years, dietary fibre has received much positive attention with regards to its potential as a pharmafood, due to its ability to reduce cholesterol, diabetes and coronary heart disease and ease constipation (Telrandhe et al., 2012).

In recent times, fibre has also been described as having use as an ingredient with specific functions in food production. Due to the nature of fibre having both insoluble and soluble properties, it has a range of technological attributes such as water binding, gelling, structure building and use as a fat replacer.

Larrauri (1999) described the "perfect fibre" as having the following characteristics:

- It must not contain any components that are nutritionally offensive.
- To maximise its use, it must be of high concentrate in a small quantity.
- It should have no taste and no negative odour, colour or texture effects.
- It should contain a balance between soluble and insoluble fibre with an acceptable presence of bioactive compounds.
- Its addition must not affect the food it is being added to, but it must also have a long shelf life.
- It should work harmoniously with food processing.
- It should have a positive consumer image.
- It should contain the expected physiological effects.
- It should be an adequate price (Larrauri 1999; Kunzek, Müller, Vetter & Godeck, 2002).

Typically, fibres such as wheat, corn and rice have been used in food production in the past, both for their health attributes and technical functions. However, very recently, novel sources of fibre have been discovered and utilised. One of these sources is the by-product fraction from different types of food processing. In particular, the by-products obtained from fruit and vegetable processing (e.g. juices, drinks etc) are gaining attention as novel and economic sources of a healthy functional ingredients (Ayala-Zavala et al., 2011). Such by-products can be described as the remnants after the manufacturing of fruit and vegetable-based

products; these remnants include peel, pips, skins, stems and cores. Currently these by-products are disposed of, usually at a cost to the producer via animal feed, landfill or incineration; thus potentially creating negative effects on the environment (Angulo et al., 2012; Leroy, Bommele, Reheul, Moens & De Neve, 2007).

To follow, some of the most recent documented sources of dietary fibre from food processing by-products are discussed:

1.1.3 Fruit sources:

1.1.3.1 Apple (*Malus domestica*):

The major product from apple processing is apple juice. The entire fruit is usually pressed in a cold press to extract the juice from the fruit. This can result in much waste, which is termed apple pomace. Apple pomace is thought to consist of approximately 25 % of fresh apple weight. In general, producers usually discard tonnes of pomace at a cost to themselves. Alternatively, it is used as animal feed. Unlike cereals, there is a higher percentage of the soluble fibre fraction in apple fibre, thus giving rise to the availability of the polymer pectin. Pectin has such characteristics as gelling, thickening and can be used as a stabilizer in foods. It is also a health-enhancing polymer, thought to lower cholesterol and delay gastric emptying (Hwang, Kim & Kim, 1998; Royer, Madieta, Symoneaux & Jourjon, 2006; Rha et al., 2011).

Gorinstein et al. (2001a) investigated the dietary fibre levels of a whole apple, its pulp and its peel. Interestingly, they found that the majority of the total fibre was located in the peel of the apple (0.91 % fresh weight [FW]). The percentage of insoluble (0.46 % FW) to soluble fibre (0.43 % FW) was found to be well balanced in terms of receiving a health benefit.

These authors also found apple peels to contain significant levels of calcium and magnesium. Higher levels of zinc, iron and copper were present in the peel, than those found in the flesh of the apple. Minerals such as iron, copper and manganese have been proven to work in synergy, as effective catalysts in the prevention of certain diseases such as atherosclerosis.

Garcia et al. (2009) studied the phytochemicals present in apple pomace which was created during cider processing. The phytochemicals the authors found were phenolic acids such as chlorogenic, protocatechuic and caffeic acid and polyphenols such as flavonoids e.g. flavanols and flavonols. Schieber et al. (2003a) also investigated the phenolic compound content of apple pomace. These authors reported similar findings to Garcia et al. (2009); chlorogenic acid, phloridzin and a number of quercetin glycosides were present, with quercetin 3-galactoside being the predominant flavonol. Derivatives of flavanols i.e. catechins and procyanidin were also available in considerable amounts. The phenolic compound content of the apple seeds were also research by Schieber et al. (2003a). Phloridzin was shown to be the most plentiful compound present, while chlorogenic acid was also available. Procyanidins and flavonol glycosides were either seen in low amounts or absent altogether.

The phytochemicals present in apples have been associated with many health-enhancing benefits e.g. cancer cell proliferation, decrease lipid oxidation and lower cholesterol. In turn these beneficial phytochemicals have external effects on reducing chronic diseases present in the western world e.g. heart disease, obesity and cancer. The author's findings highlighted the availability of phenolic acids e.g. chlorogenic acid which has a high alkyl peroxyl radical scavenging activity therefore apples has a protective effect against cancer. Procyanidins have a high antioxidant activity and inhibit low density lipoprotein oxidation (Boyer & Liu 2004). Quercetin is one of the main flavonoids found in apple, predominantly apple peel. It has been linked with reduced incidences of breast cancer and leukemia (Rice-Evens, Miller & Paganga, 1997; Boyer & Liu 2004).

This information illustrates the availability of phytochemicals in apple pomace. It also suggests that the inclusion of apple pomace as an ingredient in food products could dramatically improve the nutritive properties of such products and perhaps the health of the consumer.

Due to its functional characteristics (water holding, gelling, thickening and stabilizing abilities), nutrients and phytochemcials, apple pomace has been used by researchers in a variety of food products such as sausages, jams and baked goods (Henriquez et al., 2010).

An apple skin powder (ASP) was added to muffins to improve their phenolic content. It was found to enhance the flavour while at the same time increasing the phenolic and antioxidant content (Rupasinghe, Wang, Huber & Pitts, 2008). Rupasinghe et al. (2009) concluded ASP from the cultivar 'Idared' could be used as a replacement for wheat flour in muffins. The replacement of wheat with 16 % (weight basis [w/w]) ASP still received favourable sensory scores. Apple pomace (AP) was also added to cakes; however, the authors found that as the pomace level increased beyond a particular level, the volume of the cake decreased. Further work revealed, that due to the water binding capability of AP, additional water was required to fully hydrate the dough. Authors also noticed the colour of the cake became darker as the level of AP decreased. The addition of AP conferred some favourable attributes such as a fruit aroma and taste, thus allowing the level of sugar added to be reduced (Masoodi, Sharma & Chauhan, 2002; Sudha, Baskaran & Leelavathi, 2007).

1.1.3.2 Grape (*Vitis vinifera* L.):

Grape pomace consists of seeds, skins and stems, and in some cases this by-product is used to extract grape seed oil. It is also used in the production of citric acid, methanol, ethanol and xanthan gum as a result of fermentation (Deng, Penner & Zhao, 2011). The nutritional and compositional characteristics of grape pomace are known to vary, depending on grape cultivar, growth climates and processing conditions (Deng, Penner & Zhao, 2011).

Grape pomace has been shown to be a rich source of dietary fibre; its components mainly comprise of cellulose, small porporations of pectins and hemi-celluloses (Kammerer, Schieber & Carle, 2005a). Gonzalez-Centeno et al. (2010) studied the dietary fibre content of ten varieties of grape and their by-products (stems and pomace). 'Tempranillo' red grape cultivar contained the highest amount of dietary fibre in the grape (5.1 g/100g FW), stem (34.8 g/100g FW) and pomace (36.9 g/100g FW).

Llobera & Cañellas (2007) studied the dietary fibre content of 'Manto Negro' red grape pomace. They found the total fibre to be 77.2 % dry matter [DM]; of this the insoluble fibre (73.5 % DM) was greater than the soluble fibre (3.77 % DM). In red grape pomace the total dietary fibre was similar to that of white grape pomace ('Prensal Blanc' cultivar 71.56 % DM). Of the total dietary fibre, the insoluble fraction (61.26 % DM) of the white grape pomace was found again to exceed the soluble fraction (10.33 % DM) (Llobera & Canellas 2008).

Ruberto et al. (2007) carried out a study on the polyphenol content of Sicilian red grape pomace. Anthocyanins, flavonols and the phenolic acid, gallic acid, where the main polyphenols present, accounting for 49.33 mg/g MeOH extract in

the 'Nerello Cappuccio' grape cultivar. The authors found cyanidin, peonidin, delphinidin, petunidin and malvidin derivatives to be the main anthocyanins in the red grape pomace; quercetin was the main flavonol available. The anthocyanin contents of red grape pomace ('Cabernet Mitos') were researched in depth by Kammerer et al. (2005b). These authors reported results which were in agreement with Ruberto et al. (2007), where cyanidin, petunidin, peonidin, malvidin and 3-0-monoglucosides of delphinidin. 3-O- monoglucosides were seen to be the main compounds present, representing 46.5 to 50.0 % of the anthocyanins available. A Rockenbach et al. (2011) on red grape pomace revealed quercetin (56.65 mg/100g DW 'Merlot' cultivar) to be the main flavonol and gallic acid (18.68 mg/100g DW 'Bordeaux' cultivar) to be the main phenolic acid present. Anthocyanins (496.61 mg/100 g DW 'Isabel' cultivar) represented a large proportion of the phenolic substances. A detailed study of phenolic antioxidants present in red and white grape seed varieties was carried out by Maier et al. (2009). Gallic acid was the main phenolic acid present. Its amount varied among varieties, however it was present in highest (1116.5 mg/kg DM) levels in the 'Samtrot' red grape seed cultivar, in comparison to the 'Müller-Thurgau' white grape seed cultivar which contained the lowest levels (188.7 mg/kg DM). The authors found epicatechin, flavan-3-ols catechin, epicatechin gallate and dimeric procyanidins B1 and B2 as the dominant flavonoids present in the 'Spätburgunder' red grape seed cultivar. The 'Kerner' white grape seed cultivar contained the lowest quantities of flavonoids (Maier, Schieber, Kammerer & Carle, 2009).

The actions of polyphenols such as anthocyanins and flavonoids have been

linked with a reduction in cardiovascular disease (CVD) and some types of cancers. This occurs by increasing the plasma antioxidant capacity; therefore inhibiting oxidation of LDL. They have also been shown to reduce the systolic pressure and level of plasma. As discussed, grape pomace is an excellent source of these polyphenols (Sehm, Treutter, Lindermayer, Meyer & Pfaffl, 2011).

Polyphenols such as flavonoids can react with superoxide anions, hydroxyl radicals and lipid peroxyl radicals, which are known to cause lipid oxidation. Lipid oxidation is one of the main causes of products having a short shelf-live; therefore this positive benefit gives an extra health dimension to grape pomace and promotion of its use (Arvanitoyannis, Ladas & Mavromatis, 2006).

The phytochemicals in grape pomace have proven to confer many promising health attributes. Ruberto et al. (2007) and Maier et al. (2009) clearly demonstrate the availability of flavonoids and phenolic acids in grape pomace. These have been linked with reducing CVD, a heart disease which is beginning to become rampant in the western world.

1.1.3.3 Lemon (*Citrus limon*):

Once the juice and essential oil have been extracted from lemons, the remaining by-product constitutes approx 50 % of the original fruit. This 'waste product' consists of peels (albedo and flavedo), seeds and fruit pulp. Usually this is discarded for animal feed or, not as commonly, pectin is extracted (Lario et al., 2004).

However, some research studies have highlighted the potentially healthy

attributes of the by-product. Gorinstein et al. (2001b) found levels of dietary fibre to be 14 g/100 g DM in the peels of lemons, in comparison to 7.34 g/100 g DM in the peeled fruit itself. The total dietary fibre contained insoluble fibre of 9.04 g/100g DM and 4.93 g/100g DM soluble fibre. They also found the peel of the lemon to be a good source of iron.

Lemon, like most citrus fruits, has been reported to have a high content of antioxidants and polyphenols. Marin et al. (2002) found lemon juice to contain high levels of ascorbic acid. Ascorbic acid has been considered to aid in the absorption of iron, hormones and cell oxidoreduction processes. They also found high levels of flavonoids present, in particular flavanones and flavones. Another phytochemical present was carotenoid; although it was not as abundant as the flavonoids presence. One flavonoid in particular, hesperidin has been seen to work as a treatment for rheumatoid arthritis. This by-product has been shown to have potential as a health enhancing ingredient but currently lacks a sufficient quantity of literature to draw final conclusions (González-Molina, Domínguez-Perles, Moreno & García-Viguera, 2010).

1.1.3.4 Mango (*Mangifera indica*):

The 'waste' created after mango processing can range from 35 % to 60 % of the total fruit weight, and usually includes the mango peel and the kernel. Mango is mainly produced in India; however it is also now being produced in Spain and the Canary Islands. It is mainly processed to be used as slices in a syrup sauce or for purees. Much of the waste is disposed of. (Larrauri, Ruperez, Borroto & Saura-Calixto, 1996).

Mango peel has also been reported to contain a high level of dietary fibre. Ajila et al. (2008, 2009) reported levels of 51.2 % DM total dietary fibre. This comprised 19 % DM soluble fibre and 32 % DM insoluble fibre. Vergara-Valencia et al. (2007) also investigated the dietary fibre content of the mango fruit ('Tommy Atkins' cultivar) and found it to contain 28.05 % DM of dietary fibre (13.80 % DM insoluble fibre and 14.25 % DM soluble).

Researchers have recently analysed the composition of mango 'waste' as a potential functional ingredient. The ripe and unripe peels of the mango are a good source of polyphenols. Ajila et al. (2007a) studied the polyphenol content of ripe and unripe mango varieties. Their results showed the total phenolic content to be 109.7 mg/100g DM in un-ripened ('Raspuri' cultivar) mango peels. Ripened mango peels contained 436µg/g DM of carotenoid ('Raspuri' cultivar) and 565 mg/ 100 g DM of anthocyanins ('Badami' cultivar). Pott et al. (2003) also analysed the carotenoid content of mango ('Kent' cultivar). The authors reported β-carotene to be the prominent carotenoid available. Flavonoids, a group of polyphenols, have been widely explored by Schieber et al. (2003b), their findings show mango peel ('Tommy Atkins', in particular) to be a rich source of flavonol glycosides. Beradini et al. (2005) analysed the anthocyanin contents of red coloured mango cultivars. They also found the cultivar 'Tommy Atkins' to contain the highest levels of anthocyanins (3719 µg/kg DM). Mango peel is also a good source of antioxidants such as vitamin C and vitamin E, and in particular in ripened peels (Ajila, Bhat & Prasada Rao, 2007b). Phytochemicals such as these discussed above have been proven to prevent DNA oxidative damage, prevent inhibition of cell communication and scavenge for free radicals. Free

radicals have been seen to drain immune system antioxidants, catalyse abnormal proteins which are known to start degenerative disease and aging and change gene expression (Masibo & He 2008).

The addition of mango peel to a food product can improve the nutritional contents of the product by increasing the dietary fibre and phytochemcial levels without negating the quality of the product.

1.1.3.5 Orange (*Citrus sinensis*):

An orange is an attractive and nutritional piece of fruit. It has a unique colour, smell and taste. Nutritionally, it is one of the most plentiful sources of vitamin C amongst fruit and vegetables. However, vitamin C alone is not its only nutritional benefit; it is also a good source of carotenoids, flavonoids, essential oils, sugar, fibre and some minerals (Niu et al., 2008). Oranges are generally eaten either on their own or in the form of freshly squeezed orange juice, juice from concentrate or pasteurized. 85 % of oranges are processed into some form of orange juice, leaving behind tonnes of by-product after production. Usually this 'waste' is fed to animals or disposed of at a cost to the producer (Topuz, Topakci, Canakci, Akinci & Ozdemir, 2005).

Chau & Huang (2003) investigated the dietary fibre content of the peel of orange ('Liucheng' cultivar). They found the peel to contain 57 % DW total dietary fibre; of this 47.6 % DW was the insoluble fraction and 9.41 % DW the soluble fraction. The insoluble fraction is the dominant fraction thus providing health benefits such as intestinal regulation and increased stool volume. It was also

determined that pectic polysaccharides and cellulose where the main constituents of the fibre.

Figuerola et al. (2005) revealed values of 54 % DM in the orange peel ('Valencia' cultivar) they studied, and Grigelmo-Miguel & Martin Belloso (1999a) presented values of 37.8 g/100 g DM. Both authors were in agreement that the primarily fraction of dietary fibre in orange is the insoluble fraction.

Oranges have been found to contain many phytochemicals such as flavanones, in particular hesperidin similar to lemon it accounts for 50 % of the total phenolic compounds, flavones such as neodiosmin and hydroxycinnamic acids such as ferulic acid (Fernandez-Lopez et al., 2009). Fernandez-Lopez et al. (2009) suggested that oranges are a rich source of flavonoids, with flavanones encompassing 50-80 % of the total flavonoids content of oranges. Roussos (2011) identified hesperidin (236.4 mgL^{-1}) as the main flavonoid found in orange followed by (71.4 mgL^{-1}) and only trace amounts of β-carotene (0.43 mgL^{-1}) was found in oranges. Orange pomace, like lemon pomace is a good source of flavonoids particularly hesperidin, however its direct association with health benefits needs to be further researched before solid conclusions can be drawn.

As a result of the functional and nutritional characteristics of orange peel, it may be considered to be a viable ingredient for a wide variety of products such as meat pastes, baked goods and yogurt. Fernandez-Lopez et al. (2008) added orange fibre to "salchichon", a dry fermented sausage. They found no negative effects on flavour; its presence promoted the growth of micrococcus, thus decreasing nitrite levels and it was shown to possibly have a protective role

against rancidity. Viuda-Martos et al. (2010a, 2010b) followed on from Fernandez-Lopez's study, proving that orange fibre has a positive effect with regards to retarding oxidation and reducing the microbial growth of unwanted microbes, therefore increasing the shelf-life of the sausage. They also successfully maintained the levels of polyphenolic compounds in the sausage, thereby conferring a health advantage to the consumer.

Larrea et al. (2005) investigated the effects of incorporating extruded orange pulp on the quality of cookies. They successfully increased the dietary fibre content of the biscuit (11.25 % DM) compared to the control (2.10 % DM). The quality of the biscuit, however, was found to be quite hard, but it was reported that this issue could be improved with the addition of the correct levels of water as fibre generally has high water absorption abilities. The gelling and thickening abilities of orange fibre was explored in an enriched yogurt. Sendra et al. (2010) found that orange fibre increased the viscosity of the yogurt. At low concentrations the authors proved the presence of fibre to have a disruptive effect on the structure of the yogurt. Consequently, after pasteurisation at higher levels of fibre (greater than 6 %) it was found to strengthen the gel.

1.1.3.6 Peach (*Prunus persica*):

The fruit, peach has been widely used around the world in the form of peach slices in syrup or just eaten as a dessert. The remnants from peach processing usually include the kernel and the peel. Over the years, these remnants have been used for their pectin as a thickener in jams; nowadays they are used commercially as a general thickener in foods. The use of peach fibre in

muffins has also been researched. Results showed, that it had a positive effect on muffins in terms of flavour and texture, and it has the potential to be used a fat or flour replacer (Grigelmo-Miguel, Carreras-Boladeras & Martin-Belloso, 1999b).

Grigelmo-Miguel et al. (1999c) investigated the dietary composition of the peach ('Sudanell' cultivar). They found it to consist of 30.7-36.1 % DM total dietary fibre. This comprised 23.8 % DM insoluble fibre and 12.3 % DM soluble fibre.

Kurz et al. (2008) characterized the cell wall polysaccharides of peaches. Their findings illustrate that the main polysaccharides found were in the form of pectin. In particular, the unpeeled ripening peach ("Royal Glory" cultivar) was shown to contain 30.2 g/100g AIR HCl-soluble pectin and 30.1 g/100g AIR NaOH/EDTA-soluble pectin. A marginal difference was noticed between peeled and unpeeled peaches in the form of lignan and cellulose (C) and hemicellulose (HC). The C and HC were seen to be present in higher quantities in the peeled peach (17.0 g/100g AIR and 13.1 g/100g AIR) then the unpeeled peach (16.4 g/100g AIR and 12.9 g/100g AIR) (Kurz, Carle & Schieber, 2008).

Adil et al. (2007) found peaches to contain a total phenolic content that was intermediate in value e.g. 84.07 mg gallic acid (equivalent of /100g of fresh fruit). It was established that peaches contain antioxidants such as vitamin C; its phenolic content was found to be higher in the peels than the flesh of the peach.

Peaches contain high levels of carotenoids in particular β-carotene (430 µg kg FW) and β-cryptoxanthin (70 µg kg FW). Low levels of α-carotene have also been recorded (Gil, Tomás-Barberán, Hess-Pierce & Kader, 2002). The author acknowledges that the literature available at present on peach pomace and its

effects on products is lacking but what is available suggests its addition to food products would be beneficial due to its high content of antioxidants.

1.1.4 Vegetable sources:

1.1.4.1 Carrot (*Daucus carota* L.):

Generally, carrots are a stable vegetable included in most diets today. As carrots are seasonal in nature they are processed in many forms, such as chopped, frozen or canned. As a fresh vegetable, carrots are also utilized in many cooking recipes such as soups, sauces and dinner meals. All of these uses initially begin with the peeling and removal of the top and bottom of the carrot. Like the carrot itself, these peelings are a rich source of phenolic compounds and dietary fibre. Unlike other vegetables, phenolic compounds contribute to some physical characteristics of the carrot. For instance, anthocyanins and carotenoids are responsible for the colour, aroma and bitterness of carrots (Gonçalves, Pinheiro, Abreu, Brandão & Silva, 2010).

Chau et al. (2004) investigated the dietary fibre content of carrot pomace (the by-product produced after carrot juice extraction). The total dietary fibre content of the carrot pomace was found to be 63.6 % DM, with 50.1 % DM being the insoluble fraction and 13.5 % DM the soluble fraction.

Chantaro et al. (2008) studied the dietary fibre of fresh carrot peels and the effect of blanching on these results. Total dietary fibre content was 45.45 % DM. After the peels had been blanched the total dietary fibre increased significantly (73.32 % DM). This author proposed that this was due to the loss of low molecular constituents into the blanching water.

Carrots have been shown to contain numerous phytochemicals such as carotenoids and flavonoids. The type and percentage of the phenolic compound

present greatly depend on the cultivar and colour of the carrot. Sun et al. (2009) studied the phenolic compounds of different coloured carrots. The main phenolic acid types present were chlorogenic acid, caffeic acid, p-OH-benzoic acid, ferulic acid and cinnamic acid isomers. Chlorogenic acid and caffeic acid where found in abundance in all of the carrots, but in particular chlorogenic acid was found in the purple yellow varieties. With regards to the carotenoid content, lutein, lycopene, α-carotene and β-carotene were present in the highest quantity. As expected, the carotenoids appeared in the dark orange and orange varieties. Anthocyanins where only identified in purple- orange and purple yellow carrot varieties.

Surles et al. (2004) carried out a similar study; however they concentrated predominately on the carotenoid contents of carrots of various colours. Their results are in agreement with the above authors, in that a significant level of β-carotene (0.185 % FW) was found in high-βC orange varieties. As anticipated, lycopene (0.061 % FW) was found mostly in the red cultivar of carrot.

Stoll et al. (2003a) investigated the utilization of a previously concentrated carrot hydrolyzate (Stoll, Schweiggert, Schieber & Carle, 2003b) recovered from carrot pomace ('Karotan' cultivar) as an ingredient in beverages. The authors revealed positive results, and concluded that carrot pomace can be successfully added to carrot juice. After bottling, the cloud stability of the juice was found to be satisfactory during storage. Addition of carrot pomace to the juice also naturally increased the polyphenol content.

Durrani et al. (2011) investigated the potential of carrot as the main ingredient in

a honey based candy. The authors found the product received positive sensory scores and acceptable physico-chemical and microbiological results. Due to the nature of the production of the product, it was found that it could be safely preserved for 6 months at 25-30 ºC.

As shown, carrots have a high content of carotenoids; they have been shown to be good for eyesight. Carrots also contain phenolic acids which have be proven to have strong antioxidant potential; anthocyanins like discussed earlier have been shown to reduce inflammation and lipid oxidation thus reducing cardiovascular heart disease (Arscott & Tanumihardjo 2010). Unlike fruit which may contain kernals and seeds, the pomace received from carrots can easily be added to a product without introducing negative functional or flavour issues while still retaining a lot of its phytochemicals (Chantaro, Devahastin & Chiewchan, 2008). Therefore, this by-product can be used as an ideal ingredient for addition into food.

1.1.4.2 Cauliflower (*Brassica oleracea* L. *var. botrytis*):

Cauliflower is mostly included in dishes such as soups and stews or on its own as an accompanying dish with a main meal. In preparation of this vegetable for use, only 40% of it is utilized; the rest is usually discarded (Femenia, Robertson, Waldron & Selvendran, 1998). The characteristics of this vegetable make it appealing as a potential functional ingredient which could be added to improve the nutritional content of a product. Encouraging characteristics such as its pale colour, bland taste and high nutritional content make it an attractive novel

ingredient (Stojceska, Ainsworth, Plunkett, Ibanoglu & Ibanoglu, 2008).

Femenia et al. (1997, 1998) analysed the non-starch polysaccharides (NSP) in the floret and stem (i.e. the by-product fractions) of the cauliflower. They found the stem to contain 3.11 % fresh weight (FW) compared to the floret, which contained a lower amount of NSP 2.31 % FW. In both by-products, the insoluble fraction was found to be present in significant higher quantities then the soluble fraction. Pectic polysaccharides where found to be the main NSP present in both the floret and the stem.

Llorach et al. (2003) analysed the antioxidant capacity of cauliflower by-products. They established that flavonoids and hydroxycinnamic acids where the main phenolics present. In addition, the authors found that kaempferol and quercetin were the main flavonols detected, and caffeic acid and sinapic acid where the major hydroxycinnamic acids available. They concluded that the edible part of the cauliflower was quite low in phenolic compounds, where only trace amounts of hydroxycinnamic acid were found. Cabello-Hurtado et al. (2012) inspected the glucosinolates content of by-product of cauliflower (consisting of leaves and non-edible part of the cauliflower). The authors found sinigrin (34.46 %), glucoiberin (32.45 %), glucobrassicin (12.81 %) to be the main glucosinolates present and 4-OH-glucobrassicin was present in small amounts (0.29 %). Kushad et al. (1999) found similar results in the seeds of cauliflower, where sinigrin was the prominent glucosinolate. They obtained a higher result for 4-hydroxy glucobrassicin but similar results for glucobrassicin.

Abul-Fadl (2012) explored the utilization of the leaf midribs, upper stem and

stalks of cauliflower as a fat substitute in the production of beef sausages. The cauliflower flour was contained 727 mg/100g DM glucosinolates in the upper stem portion and 495 mg/100g DM in the leaf midribs portion. No significant difference was found between the control (taste: 8.5 [hedonic test, ten point scale]) and the inclusion of the cauliflower flour in the beef sausages (taste: 8.8) at levels of up to 7.5 % addition.

The above authors have proven cauliflower to be a good source of glucosinolates, flavonoids and phenolic acid. Glucosinolates can aid in reducing the incidence of tumours in the reproductive organs and the growth of breast cancer cells (Jahangir, Kim, Choi & Verpoorte, 2009). Similar to some of the fruit and vegetables already discussed, cauliflower by-products (such as the stem) have been shown to contain a significant amount of phytochemicals. Health benefits associated with the consumption of phytochemicals have also been detailed in the manuscript.

1.1.4.3 Onion (*Allium cepa* L.):

Unlike broccoli or carrots, onions are often under-recognised in terms of their health benefits. In Europe, it is mainly the white/yellow flesh with yellow/brown skin onion used in culinary practice. However, red/purple onions are now beginning to become popular in sandwiches and salads. The yellow/brown skin onions are popular in cooking, fast food and restaurant food due to their strong flavour and long shelf life (Griffiths, Trueman, Crowther, Thomas & Smith, 2002). As a result of its popularity in culinary processes, a need for the onion to be received in a more convenient way has arisen; thus creating a niche for

manufactures to produce prepared onions i.e. pre-chopped and peeled, even in a powder or flake form (Griffiths, Trueman, Crowther, Thomas & Smith, 2002). Consequently 500,000 tonnes of onion by-product is being produced in the EU annually (Benítez et al., 2011). This 'waste' mainly consists of onion skins, roots, two outer fleshy scales of the onion and undersized or malformed, diseased or damaged onions. Unfortunately, onion waste is not ideal for the usual outlets for vegetable waste. Its aromatic characteristics are too pungent for animal feed and it cannot be used as a fertilizer for land due to its phytopathogenic agents (Benítez et al., 2011). Therefore researchers are investigating its use as a potential food ingredient, due to its nutritional properties, nutrients such as dietary fibre and the presence of nutraceuticals (Griffiths et al., 2002).

Dietary fibre is present in different quantities throughout the different layers of the onion. Jaime et al. (2002) analysed the dietary fibre of the entire onion from the skin to the layers in three different varieties of onion. They found that highest levels of total dietary fibre were present in the skin of the onion (68.3 % DM 'Grano de Oro' cultivar), and lowest levels were found in the inner part of the onion (11.6 % DM). Also, the highest level of insoluble fibre was present in the skin of the onion (66.6 % DM 'Grano de Oro' cultivar) with lowest levels again located in the inner part of the onion. The authors suggested that this was due to the constituents and location of the layers. For example, each layer is made up of differing amounts of inner, outer and skin tissue, therefore the outer skin layer largely contains skin tissue compared to the inner layer, which mainly consists of inner tissue. This decreases as the layers build outward to the skin layer. The soluble fibre content of the onion was found to be considerably lower

than the insoluble fibre content of the onion. No significant difference was found in quantity or location of the soluble fibre (10.2 % DM ('Grano de Oro' cultivar) in the bottom layer of the onion).

Finally, the ratio of soluble to insoluble fibre was investigated in the onion. For an onion to be a viable food ingredient, the ratio of IDF: SDF should be close to 1:2. Unlike other vegetables, it is harder to give an overall ratio value for the onion, as each part has a different insoluble: soluble ratio. Thus they concluded that the ratio decreased from the inner to the outer tissues. The inner tissue would be more suited for use as a food ingredient; however the outer and skin tissue consisted of the greatest amount of dietary fibre and had the most potential as a fibre ingredient. Benitez et al. (2011) also characterized the dietary fibre content of the onion cultivars ("Recas and Figueres") and its by-products; their conclusions were in agreement with the above author's findings.

The phytonutrient properties of onions have been recognised over the past number of years; in particular phytochemicals such as flavonoids and phenolic acids. Ng et al. (2000) explored the phenolic composition of onion tissue ('Sturon' cultivar). Phenolic acids and flavonoids where found to be the main phytochemicals present. Protocatechuic acid was in abundance in the papery scales of the onion but was not present in any other layers. Ferulic acid was found in significant quantities in both the papery scales and fleshy scales of the onion. Finally, vanillic acid was present mainly in the papery scales of the onion. Up to 50 flavonoids have been recognised in the scales of an onion. In particular, flavonoids such as flavonols (which give the yellow pigment to onions), anthocyanins (found mainly in red onions) and dihydroflavonols have

been characterised in onions (Slimestad, Fossen & Vågen, 2007). Rattanachaikunsopon & Phumkhachorn (2009) explored the diallyl sulphide content of shallots. Diallyl disulphide (1228 μg/g) was the main diallyl sulphide present followed by diallyl trisulfide (800 μg/g). The total sulphide content was 4982 μg/g).

For some time, flavonoids have been of great interest, owing to their potential use in reducing inflammation, coronary heart disease and cancer; they have also been shown to contain anti-HIV properties. This review has demonstrated the high proportion of sulphides present in onion. These organosulfur compounds have also been found to have pharmacological properties, which demonstrates onion by-product as an under-utilized source (Lanzotti 2006).

A study was carried out to investigate the potential of onion by-products as food ingredients. Roldan et al. (2008) developed a paste from onion by-products (mixture of 'Figueres' and 'Recas'varieties); they found it was best to use a pasteurisation process to produce the paste. This created a number of benefits such as preserving the bio-active compounds, it also maintained a significant antioxidant potential and, functionally, from a producer's point of view, it did not allow browning to occur.

1.1.4.4 Potato (*Solanum tuberosum* L.):

In countries such as Ireland, potatoes are a staple food in the diet. As lifestyles are becoming busier, a demand for prepared vegetables has arisen. Therefore producers are manufacturing potatoes which are pre peeled, washed, chopped

and even frozen. Potatoes have also been manufactured into processed foods such as potato cakes, croquettes and even prepared potato mash. Nutritionally, potatoes contain significant dietary fibre, carbohydrates, minerals and phenolic substances (Abu-Ghannam & Crowley 2006). As a result of preparation and processing potatoes, tonnes of potato peel and pulp is generated; therefore creating a potential for new ways of disposing of and using this by-product.

Due to the constituents of potatoes such as starch and fibre, technologically the addition of this vegetable as a novel food ingredient could create functional benefits such as gelling, and additional health benefits to products; for example in baked products and fresh meat pastes (Kaack, Pedersen, Laerke & Meyer, 2006).

Liu et al. (2007) analysed the composition of three varieties of potatoes and potato peel. They found no significant difference in total fibre content (5.6 %, Karnico cultivar). They found the potato to contain 72.4 % DM total starch.

Before potatoes are consumed they are usually cooked. Thed & Phillips (1995) investigated the effects of cooking on total dietary fibre content. They concentrated on boiling, microwaving, baking and deep frying. The authors found deep-fat frying and microwave heating significantly increased the total fibre content from 7.60 % DM (control) to 8.92 % DM (deep-fat frying) and 9.08 % DM (microwave heating). They propose that this increase was from the formation of lignin substances and chemically modified indigestible starch.

In terms of phenolic substances, potatoes contain chlorogenic acid in abundance in the soluble fraction and caffeic acid is present in insoluble form. Mattila &

Hellstrom (2007) found chlorogenic acid and its derivatives were the most abundant soluble phenolic acid in potato peels. Caffeic acid was present as a bound insoluble phenolic acid in potato peel ('Van Gogh' cultivar) varying from 26 mg/100g DM. Im et al. (2008) found similar results, in concurrence with the above authors. Kim et al. (2012) investigated the anthocyanin content of Korean purple fleshed potato ('Shinzami' cultivar). The authors reported the potato to contain 1342 DW total anthocyanin.

The health benefits arising from the consumption of phenolic substances found in potatoes e.g. chlorogenic acid discussed above have been shown to reduce tumours, possess antioxidant properties, help in lowering glycaemic index, thus making potatoes acceptable for diabetic patients to consume. As discussed earlier in this review, anthocyanins have been shown to prevent diseases such as cancer, diabetes and cardiovascular heart disease. Coloured potato varieties such as the cultivar ('Korean') discussed above has been shown to suppress prostate cancer (Ezekiel, Singh, Sharma & Kaur, 2013). As previously discussed, potato pomace or potato peels are generated on a regular basis, and their functional benefits are regularly expressed in the literature. The link between phytochemicals in potato peel and subsequent health benefits is now becoming more acknowledged.

Kaack et al. (2006) studied the effects of potato peel incorporation in a wheat bread formulation. Their results show solubilised potato peel fibre decreased hardness and gumminess of the bread. A sensory trial was also carried out; at 12% levels of addition the solubilised potato peel fibre received favourable sensory scores. The dietary fibre content of the bread was also increased from

10.8 % in the control to 17.5 % in the potato peel enriched bread.

1.1.4.5 Tomato (*Solanum lycopersicum* L.):

There are many products, which are manufactured from tomatoes e.g. ketcup, sauces, pasta dishes and juices. The by-product that remains after processing can still contain many nutrients and phytochemicals. Tomato, like other fruits has been found to contribute to the prevention of some cancers. Lycopene is a compound from the carotenoid family and is present in high values in tomatoes. (Chang, Lin, Chang & Liu, 2006).

Also, tomato pomace mainly consists of fibre; it can represent up to 50 % of the by-product on a dry weight basis (Del Valle, Camara & Torija, 2006).

Garcia Herrera et al. (2010) created a tomato fibre (TF) from dried and ground tomato peels received after tomato processing. They studied the content of dietary fibre present in the TF. The total fibre content was 82.7 % FW; of this the insoluble fraction was higher than the soluble fraction in a ratio of 10:1, illustrating the fibre type to be more similar to fibres found in cereals then those present in fruit.

Lycopene is partially responsible for the red colour in tomatoes. Knoblich et al. (2005) extracted lycopene from tomato pomace and found its levels to be 864 µg g^{-1} DM. These authors also found the tomato pomace to contain lower levels of other carotenoids such as lutein, zeaxanthin, α-carotene, β-carotene and cis–β-carotene.

Ilahy et al. (2011) investigated the phytochemical composition of six high lycopene tomato cultivars compared to one ordinary tomato cultivar 'Donald cultivar'. Cultivar 'HLY 02' was found to have the highest phytochemical content of 394.5 mg GAE/kg FW, cultivar 'HLY 13' was seen to have the highest flavonoids content of 511.9 mg RE/kg FW both cultivars were found to be significantly higher than the cultivar 'Donald cultivar'. Lycopene was seen to be 232.9 mg/kg FW in the cultivar 'HLY 18'compared to 'Donald' 96.9 mg/kg FW.

As discussed tomatoes are a rich source of lycopene; it has been reported that lycopene can aid in decreasing the risk of cancers, such as prostate, pancreas and stomach cancer (Chang, Lin, Chang & Liu, 2006). Lycopene has been proven to be a cancer fighting phytochemical. As it is the most abundant carotenoid present in tomato and particularly its peel, it can be concluded that as a by-product ingredient, the health advantages associated with tomato peel is very valuable.

Food researchers have recently examined the potential of using tomato pomace as a novel ingredient in different types of foods. Faranhnaky et al. (2008) added tomato pulp (TP) as a potential thickener to tomato ketchup. The authors found that the TP could be used to replace hydrocolloids, to improve and maintain the colour and texture of the product.

Calvo et al. (2008) incorporated tomato powder (from tomato peel) into fermented sausages. It was found, that this addition significantly increased the level of carotene in the diet. Further trials are needed to look at its potential to

reduce lipid oxidation as a result of adding an antioxidant such as lycopene to the sausage recipe.

Tomato peel was successfully added to hamburgers to improve its nutritional content via the presence of lycopene. The only negative effect noticed was the change in the colour of the hamburgers, thus turning the meat orange in colour. This is as a result of high levels of the pigment carotene in the hamburgers (Luisa García, Calvo & Selgas, 2009).

Altan et al. (2008) researched the addition of tomato pomace to a barley-based extruded snack. It was found that the end product greatly depended on temperature of the extruding process and pomace level added. The tomato pomace predominately affected the texture of the end-product. Levels of 2 % and 10 % of tomato pomace extruded at 160 ºC and 200 rpm were found to be optimal, whereby an acceptable product with good textural and sensory properties was formulated (Altan, McCarthy & Maskan, 2008).

1.1.5 Pre-treatments of fruit and vegetable by-products:

This is a crucial step required in the initial stages of converting the fruit and vegetable 'waste' to a potential by-product ingredient. It is a vital step, as creating an ingredient which is both microbially stable, while minimizing bio-active losses (i.e. polyphenols, phenolic acids, flavonoids and carotenoids) helps develop an ingredient which has a wide variety of health enhancing benefits (Larrauri 1999). Many methods have been involved in the development of these by-product ingredients. For example, practices such as wet milling (milling a wet pomace to a certain particle size), washing (washing of pomaces to remove unwanted substances), drying (a number of methods can be used here such as the sun, oven drying and freeze drying) and dry milling (to mill the dried pomace to a certain particle size) have been demonstrated and documented (Larrauri 1999). Depending on the available nutrients and metabolites of the by-product, one or a combination of the above treatments can be used to produce a nutrient dense food ingredient.

The phenolic content of the apple seeds were research by Schieber et al. (2003a). The authors suggest that apple seed oil can be extracted from apple seeds via cold-pressed processes, at a feasible cost, therefore creating a phenolic enriched low-cost ingredient. Wolfe & Liu (2003) analysed blanched and freeze-dried apple peels for their phytochemical content. The authors found, that despite the high/low temperatures used in the blanching/freeze drying processes, the presence of phenolic acids and polyphenols were still found, while at the same time a more shelf-stable stable ingredient was created. Rupasinghe et al. (2009) and Masoodi et al. (2002) carried out similar pre-treatments which included a

drying step via convection oven or cabinet oven and milling of the dried pomace to a certain particle size. These pre-treatments worked favourably for the inclusion of the by-product into muffins and cakes.

Due to the method by which the grapes are processed (usually not exposed to light or heat), they still contain a relatively high amount of phenolic compounds (Arvanitoyannis, Ladas & Mavromatis, 2006). Therefore researchers can easily carry out such methods as freeze drying followed by frozen storage at -20 ^{o}C to maintain a stable product e.g. Ruberto et al. (2007) carried out this method successfully to store the pomace before extraction of polyphenols.

For the safe preparation and storage of onion by-products, authors have favoured the emersion of the onion by-product into liquid nitrogen followed by freeze drying and storage at -20 ^{o}C Jaime et al. (2002) and Benitez et al. (2011). These authors effectively continued on to extract dietary fibre and phenolic compounds from their pomace sample.

Tomato pomace has the potential to be used in a variety of new products in the form of tomato paste and tomato powders. Researchers are becoming aware of this and are endeavouring to perfect processes to produce an ingredient that is functional yet possess nutritional benefits such as lycopene and dietary fibre. Dermesonlouoglou et al. (2007) demonstrated that pre-treating tomato slices with an osmotic treatment at 35 ^{o}C in a solution of glucose, high dextrose equivalent maltodextrin (HDEM) and oligofructose improved the sensory scores of the tomatoes after freezing. It also created a protective effect on the colour and vitamin C content of the tomatoes. Thus, the ingredient created after this

treatment could be used in food products e.g. pizzas and ready to eat frozen meals. Davoodi et al. (2007) studied the effects of another type of pre-treatment (calcium chloride and potassium metabisulphite) before drying. They found that calcium chloride had a retarding effect on the colour of the tomato powder, and potassium metabisulphite created a protective effect for the lycopene content. It was also found that tunnel drying was the most significant method of drying for retaining the lycopene content. As a result, pre-treated tunnel dried tomato powder stored for six months presented comparable quality results when compared with fresh tomato powder.

In conclusion, the most favourable pre-treatments were observed to be in the form of drying, being it convection drying (Femenia, Lefebvre, Thebaudin, Robertson & Bourgeois, 1997) or freeze drying, followed by milling and frozen storage at -20 ^{o}C.

1.1.6 Conclusion:

As producers are continuously looking for more affordable ingredients with an added value, the by-products of fruit and vegetable processing present a possible solution. This paper illustrates the diversity of fruits and vegetables being studied by food researchers both for their nutritional and functional capabilities. The results from this review clearly demonstrate the high nutritional value that many by-products possess.

Dietary fibre has been widely accepted as an important nutrient in the human diet. Extensive research has shown fruit and vegetable by-products to be a high source of dietary fibre. Also, their use can impart such functional benefits as gelling, thickening and water binding. These properties are advantageous and may be utilised in many fields such as bakery products, meat products, snacks and diabetic beverages.

The by-products can also possess phytochemicals such as flavonoids, gallic acid and anthocyanins. Phytochemicals have also been shown to be present in higher levels in the pomace of the fruit than in the actual flesh of the fruit. For example lycopene has been found at peak levels in the peel of a tomato, phenols have been reported in great levels in the skin of the peach and researchers have found phenolic acids and antioxidant activity to be four times greater in the peel of apples then in the flesh. This unique nutritional benefit adds more value to fruit by-products (Remorini et al., 2008).

Researchers have proven a positive link between the consumption of phytochemicals and health benefits such as anti–carcinogenic and scavenging of

free radicals.

Favourable results from recent studies have shown the enrichment of food products with these by-products is possible. Studies are ongoing in this area which is continuing to gain interest from both the food scientist and food processor.

1.1.7 References:

Abul-Fadl, M.M. (2012). Nutritional and chemical evaluation of white cauliflower by-products flour and the effect of its addition on beef sausage quality. *Journal of Applied Sciences Research*, 8(2), 693-704.

Abu-Ghannam, N., & Crowley, H. (2006). The effect of low temperature blanching on the texture of whole processed new potatoes. *Journal of Food Engineering*, 74(3), 335-344.

Adil, İ.H., Cetin, H.İ., & Bayindirli, A. (2007). Subcritical (carbon dioxide & ethanol) extraction of polyphenols from apple and peach pomaces, and determination of the antioxidant activities of the extracts. *The Journal of Supercritical Fluids*, 43(1), 55-63.

Ajila, C.M., Naidu, K.A., Bhat, S.G., & Prasada Rao, U.J.S. (2007a). Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry*, 105(3), 982-988.

Ajila, C.M., Bhat, S.G., & Prasada Rao, U.J.S. (2007b). Valuable components of raw and ripe peels from two Indian mango varieties. *Food Chemistry*, 102(4), 1006-1011.

Ajila, C.M., Leelavathi, K., & Prasada Rao, U.J.S. (2008). Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. *Journal of Cereal Science*, 48(2), 319-326.

Ajila, C.M., Aalami, M., & Prasada Rao, U.J.S. (2009). Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations.

Innovative Food Science & Emerging Technologies, 11(1), 219-224.

Altan, A., McCarthy, K.L., & Maskan, M. (2008). Evaluation of snack foods from barley-tomato pomace blends by extrusion processing. *Journal of Food Engineering*, 84(2), 231-242.

Angulo, J., Mahecha, L., Yepes, S.A., Yepes, A.M., Bustamante, G., Jaramillo, H., Valencia, E., Villamil, T. & Gallo, J. (2012). Nutritional evaluation of fruit and vegetable waste as feedstuff for diets of lactating Holstein cows. *Journal of Environmental Management*, 95, Supplement (0), S210-S214.

Arscott, S.A., & Tanumihardjo, S.A. (2010). Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. *Comprehensive Reviews in Food Science and Food Safety*, 9(2), 223-239.

Arvanitoyannis, I.S., Ladas, D., & Mavromatis, A. (2006). Potential uses and applications of treated wine waste: a review. *International Journal of Food Science & Technology*, 41(5), 475-487.

Ayala-Zavala, J.F., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J.A., Siddiqui, M.W., & González-Aguilar, G.A. (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International*, 44(7), 1866-1874.

Berardini, N., Fezer, R., Conrad, J., Beifuss, U., Carle, R., & Schieber, A. (2005). Screening of mango (Mangifera indica L.) Cultivars for their contents of flavonol O- and xanthone C-glycosides, anthocyanins, and pectin. *Journal of Agricultural and Food Chemistry*, 53(5), 1563-1570.

Benítez, V., Mollá, E., Martín-Cabrejas, M.A., Aquilera, A., López-Andréu, F. J., Cools, K., Terry, L.A., & Esteban, R.M. (2011). Characterization of industrial onion wastes (Allium cepa L.): dietary fibre and bioactive compounds. *Plant Foods for Human Nutrition* (Formerly Qualitas Plantarum), 66(1), 48-57.

Boyer, J., & Liu, R. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal*, 3(1), 5.

Cabello-Hurtado, F., Gicquel, M., & Esnault, M.-A. (2012). Evaluation of the antioxidant potential of cauliflower (Brassica oleracea) from a glucosinolate content perspective. *Food Chemistry*, 132(2), 1003-1009.

Calvo, M.M., García, M.L., & Selgas, M.D. (2008). Dry fermented sausages enriched with lycopene from tomato peel. *Meat Science*, 80(2), 167-172.

Chang, C.-H., Lin, H.-Y., Chang, C.-Y., & Liu, Y.-C. (2006). Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. *Journal of Food Engineering*, 77(3), 478-485.

Chantaro, P., Devahastin, S., & Chiewchan, N. (2008). Production of antioxidant high dietary fiber powder from carrot peels. *Lwt-Food Science and Technology*, 41(10), 1987-1994.

Chau, C.F., & Huang, Y.L. (2003). Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of Citrus sinensis L. Cv. Liucheng. *Journal of Agricultural and Food Chemistry*, 51(9), 2615-2618.

Chau, C.-F., Chen, C.-H., & Lee, M.-H. (2004). Comparison of the characteristics, functional properties, and in vitro hypoglycemic effects of various carrot insoluble fiber-rich fractions. *Lwt-Food Science and Technology*, 37(2), 155-160.

Davoodi, M.G., Vijayanand, P., Kulkarni, S.G., & Ramana, K.V.R. (2007). Effect of different pre-treatments and dehydration methods on quality characteristics and storage stability of tomato powder. *Lwt-Food Science and Technology*, 40(10), 1832-1840.

Del Valle, M., Camara, M., & Torija, M.E. (2006). Chemical characterization of tomato pomace. *Journal of the Science of Food and Agriculture*, 86(8), 1232 - 1236.

Deng, Q., Penner, M.H., & Zhao, Y. (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Research International*, 44(9), 2711-2719.

Dermesonlouoglou, E.K., Giannakourou, M.C., & Taoukis, P. (2007). Stability of dehydrofrozen tomatoes pretreated with alternative osmotic solutes. *Journal of Food Engineering*, 78(1), 272-280.

Durrani, A., Srivastava, P., & Verma, S. (2011). Development and quality evaluation of honey based carrot candy. *Journal of Food Science and Technology*, 48(4), 502-505.

Ezekiel, R., Singh, N., Sharma, S., & Kaur, A. (2013). Beneficial phytochemicals in potato — a review. *Food Research International, 50*(2), (487-

496).

Farahnaky, A., Abbasi, A., Jamalian, J., & Mesbahi, G. (2008). The use of tomato pulp powder as a thickening agent in the formulation of tomato ketchup. *Journal of Texture Studies*, 39(2), 169-182.

Femenia, A., Lefebvre, A.C., Thebaudin, J.-Y., Roberston, J. A., & Bourgeois, C. -M. (1997). Physical and sensory properties of model foods supplemented with cauliflower fiber. *Journal of Food Science*, 62(4), 635-639.

Femenia, A., Robertson, J.A., Waldron, K.W., & Selvendran, R.R. (1998). Cauliflower (Brassica oleracea L), globe artichoke (Cynara scolymus) and chicory witloof (Cichorium intybus) processing by-products as sources of dietary fibre. *Journal of the Science of Food and Agriculture*, 77(4), 511-518.

Fernández-López, J., Sendra, J., Sayas-Barberá, E., Navarro, C., & Pérez-Alvarez, J.A. (2008). Physico-chemical and microbiological profiles of "salchichón" (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Science*, 80(2), 410-417.

Fernandez-Lopez, J., Sendra-Nadal, E., Navarro, C., Sayas, E., Viuda- Martos, M., & Perez-Alvarez, J.A. (2009). Storage stability of a high dietary fibre powder from orange by-products. *International Journal of Food Science and Technology*, 44(4), 748-756.

Figuerola, F., Hurtado, M.L., Estévez, A.M., Chiffelle, I., & Asenjo, F. (2005). Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food Chemistry*, 91(3), 395-401.

Garcia, Y.D., Valles, B.S., & Lobo, A.P. (2009). Phenolic and antioxidant composition of by-products from the cider industry: Apple pomace. *Food Chemistry*, 117(4), 731-738.

García Herrera, P., Sánchez-Mata, M.C., & Cámara, M. (2010). Nutritional characterization of tomato fiber as a useful ingredient for food industry. *Innovative Food Science & Emerging Technologies*, 11(4), 707-711.

Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., & Kadar, A.A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *Journal of Agricultural and Food Chemistry*, 50(17), 4976-4982.

Gonçalves, E.M., Pinheiro, J., Abreu, M., Brandao, T.R.S., & Silva, C.L.M. (2010). Carrot (Daucus carota L.) peroxidase inactivation, phenolic content and physical changes kinetics due to blanching. *Journal of Food Engineering*, 97(4), 574-581.

González-Centeno, M.R., Rosselló, C., Simal, S., Garau, M.C., Lopez, F., & Femenia, A. (2010). Physico-chemical properties of cell wall materials obtained from ten grape varieties and their byproducts: grape pomaces and stems. *Lwt-Food Science and Technology*, 43(10), 1580-1586.

González-Molina, E., Domínguez-Perles, R., Moreno, D.A., & García-Viguera, C. (2010). Natural bioactive compounds of Citrus limon for food and health. *Journal of Pharmaceutical and Biomedical Analysis*, 51(2), 327-345.

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M.,

Weisz, M., Trakhtenberg, S., & Màrtín-Belloso, O. (2001a). Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. *Journal of Agricultural and Food Chemistry*, 49(2), 952-957.

Gorinstein, S., Martin-Belloso, O., Park, Y.S., Haruenkit, R., Lojek, A., Ciz, M., Caspi, A., Libman, I., & Trakhtenberg, S. (2001b). Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry*, 74(3), 309-315.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., & Smith, B. (2002). Onions—A global benefit to health. *Phytotherapy Research*, 16(7), 603-615.

Grigelmo-Miguel, N., & Martín-Belloso, O. (1999a). Comparison of dietary fibre from by-products of processing fruits and greens and from cereals. *Lwt-Food Science and Technology*, 32(8), 503-508.

Grigelmo-Miguel, N., Carreras-Boladeras, E., & Martín-Belloso, O. (1999b). Development of high-fruit-dietary fibre muffins. *European Food Research and Technology*, 210(2), 123-128.

Grigelmo-Miguel, N., Gorinstein, S., & Martin-Belloso, O. (1999c). Characterisation of peach dietary fibre concentrate as a food ingredient. *Food Chemistry*, 65(2), 175-181.

Henriquez, C., Speisky, H., Chiffelle, I., Valenzuela, T., Araya, M., Simpson, R., & Almonacid, S. (2010). Development of an ingredient containing apple peel, as a source of polyphenols and dietary fiber. *Journal of Food Science*, 75(6), H172-H181.

Hwang, J.-K., Kim, C.–J., & Kim, C.-J. (1998). Extrusion of apple pomace facilitates pectin extraction. *Journal of Food Science*, 63(5), 841-844.

Ilahy, R., Hdider, C., Lenucci, M.S., Tlili, I., & Dalessandro, G. (2011). Phytochemical composition and antioxidant activity of high-lycopene tomato (Solanum lycopersicum L.) cultivars grown in Southern Italy. *Scientia Horticulturae*, 127(3), 255-261.

Im, H.W., Suh, B.–S., Lee, S.-U., Kozukue, N., Ohnisi-Kameyama, M., Levin, C.E., & Friedman, M. (2008). Analysis of phenolic compounds by high-performance liquid chromatography and liquid chromatography/mass spectrometry in potato plant flowers, leaves, stems, and tubers and in home-processed potatoes. *Journal of Agricultural and Food Chemistry*, 56(9), 3341-3349.

Jahangir, M., Kim, H.K., Choi, Y.H., & Verpoorte, R. (2009). Health-affecting compounds in Brassicaceae. *Comprehensive Reviews in Food Science and Food Safety*, 8(2), 31-43.

Jaime, L., Molla, E., Fernández, A., Martín-Cabrejas, M.A., López-Andréu, F.J. & Esteban, R.M. (2002). Structural carbohydrate differences and potential source of dietary fiber of onion (Allium cepa L.) tissues. *Journal of Agricultural and Food Chemistry*, 50(1), 122-128.

Kaack, K., Pedersen, L., Laerke, H.N., & Meyer, A. (2006). New potato fibre for improvement of texture and colour of wheat bread. *European Food Research and Technology*, 224(2), 199-207.

Kammerer, D.R., Schieber, A., & Carle, R. (2005a). Characterization and revovery of phenolic compounds from grape pomace – A review. *Journal of Applied Botany and Food Quality*, 79, 189-196.

Kammerer, D., Claus, A., Schieber, A., & Carle, R. (2005b). A novel process for the recovery of polyphenols from grape (Vitis vinifera L.) pomace. *Journal of Food Science*, 70, 157-163.

Kim, H.W., Kim, J.B., Cho, S.M., Chung, M.N., Lee, Y.M., Chu, S.M., Che, J.H., Kim, S.N., Kim, S.Y., Cho, Y.S., Kim, J.H., Park, H.J., & Lee, D.J. (2012). Anthocyanin changes in the Korean purple-fleshed sweet potato, Shinzami, as affected by steaming and baking. *Food Chemistry*, 130(4), 966-972.

Knoblich, M., Anderson, B., & Latshaw, D. (2005). Analyses of tomato peel and seed byproducts and their use as a source of carotenoids. *Journal of the Science of Food and Agriculture*, 85(7), 1166-1170.

Kunzek, H., Müller, S., Vetter, S., & Godeck, R. (2002). The significance of physico chemical properties of plant cell wall materials for the development of innovative food products. *European Food Research and Technology*, 214(5), 361-376.

Kurz. C., Carle, R., & Schieber, A. (2008). Characterisation of cell wall polysaccharide profiles of apricots (Prunus armeniaca L.), peaches (Prunus persica L.), and pumpkins (Cucurbit asp.) for the evaluation of fruit product authenticity. *Food Chemistry*, 106, 421-430.

Kushad, M.M., Brown, A.F., Kurilich, A.C., Juvik, J.A., Klein, B.P., Wallig,

M.A., & Jeffery, E.H. (1999). Variation of glucosinolates in cegetable crops of Brassica oleracea. *Journal of Agricultural and Food Chemistry*, 47(4), 1541-1548.

Lanzotti, V. (2006). The analysis of onion and garlic. *Journal of Chromatography A*, 1112(1–2), 3-22.

Lario, Y., Sendra, E., Garcia-Perez, J., Fuentes, C., Sayas-Barbera, E., Fernández-López, J., & Pérez-Alvarez, J.A. (2004). Preparation of high dietary fiber powder from lemon juice by-products. *Innovative Food Science & Emerging Technologies*, 5(1), 113-117.

Larrauri, J.A., Rupérez, P., Borroto, B., & Saura-Calixto, F. (1996). Mango peels as a new tropical fibre: preparation and characterization. *Lwt-Food Science and Technology*, 29(8), 729-733.

Larrauri, J.A. (1999). New approaches in the preparation of high dietary fibre powders from fruit by-products. *Trends in Food Science & Technology*, 10(1), 3-8.

Larrea, M.A., Chang, Y.K., & Martinez-Bustos, F. (2005). Some functional properties of extruded orange pulp and its effect on the quality of cookies. *Lwt-Food Science and Technology*, 38(3), 213-220.

Leroy, B.L.M.M., Bommele, L., Reheul, D., Moens, M., & De Neve, S. (2007). The application of vegetable, fruit and garden waste (VFG) compost in addition to cattle slurry in a silage maize monoculture: Effects on soil fauna and yield. European *Journal of Soil Biology*, 43(2), 91-100.

Liu, Q., Tarn, R., Lynch, D., & Skjodt, N.M. (2007). Physicochemical properties of dry matter and starch from potatoes grown in Canada. *Food Chemistry*, 105(3), 897-907.

Llobera, A., & Cañellas, J. (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (Vitis vinifera): pomace and stem. *Food Chemistry*, 101(2), 659-666.

Llobera, A. & Canellas, J. (2008). Antioxidant activity and dietary fibre of Prensal Blanc white grape (Vitis vinifera) by-products. *International Journal of Food Science and Technology*, 43(11), 1953-1959.

Llorach, R., Espín, J.C., Tomás-Barberán, F.A., & Ferreres, F. (2003). Valorization of cauliflower (Brassica oleracea L. var. botrytis) by-products as a source of antioxidant phenolics. *Journal of Agricultural and Food Chemistry*, 51(8), 2181-2187.

Luisa García, M., Calvo, M., & Selgas, M.D. (2009). Beef hamburgers enriched in lycopene using dry tomato peel as an ingredient. *Meat Science*, 83(1), 45-49.

Maier, T., Schieber, A., Kammerer, D.R., & Carle, R. (2009). Residues of grape (Vitis vinífera L.) seed oil production as a valuable source of phenolic antioxidants. *Food Chemistry*, 112, 551-559.

Marín, F.R., Martinez, M., Uribesalgo, T., Castillo, S., & Frutos, M.J. (2002). Changes in nutraceutical composition of lemon juices according to different industrial extraction systems. *Food Chemistry*, 78(3), 319-324.

Masibo, M., & He, Q. (2008). Major mango polyphenols and their potential significance to human health. *Comprehensive Reviews in Food Science and Food Safety*, 7(4), 309-319.

Masoodi, F.A., Sharma, B., & Chauhan, G.S. (2002). Use of apple pomace as a source of dietary fiber in cakes. *Plant Foods for Human Nutrition*, 57(2), 121-128.

Mattila, P., & Hellström, J. (2007). Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis*, 20(3-4), 152-160.

Ng, A., Parker, M.L., Parr, A.J., Saunders, P.K., Smith, A.C., & Waldron, K.W. (2000). Physicochemical characteristics of onion (Allium cepa L.) tissues. *Journal of Agricultural and Food Chemistry*, 48(11), 5612-5617.

Niu, L.-y., Wu, J.-h., Liao, X.-j., Chen, F., Wang, Z.-f., Zhao, G.-h., & Hu, X.-s. (2008). Physicochemical characteristics of orange juice samples from seven cultivars. *Agricultural Sciences in China*, 7(1), 41-47.

Pott, I., Breithaupt, D.E., & Carle, R. (2003). Detection of unusual carotenoid esters in fresh mango (Mangifera indica L. cv. 'Kent'). *Phytochemistry*, 64(4), 825-829.

Rattanachaikunsopon, P., & Phumkhachorn, P. (2009). Shallot (Allium ascalonicum L.) oil: Diallyl sulfide content and antimicrobial activity against food-borne pathogenic bacteria. *African Journal of Microbiology Research*, 3(11), 747-750.

Rha, H.J., Bae, I.Y., Lee, S., Yoo, S.-Y., Chang, P.-S., & Lee, H.G. (2011). Enhancement of anti-radical activity of pectin from apple pomace by hydroxamation. *Food Hydrocolloids*, 25(3), 545-548.

Remorini, D., Tavarini, S., Degl'Innocenti, E., Loreti, F., Massai, R., & Guidi, L. (2008). Effect of rootstocks and harvesting time on the nutritional quality of peel and flesh of peach fruits. *Food Chemistry*, 110(2), 361-367.

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, 2(4), 152-159.

Rockenbach, I.I., Rodrigues, E., Gonzaga, L.V., Caliari, V., Genovese, M.I., Gonçalves, A.E.S.S., & Fett, R. (2011). Phenolic compounds content and antioxidant activity in pomace from selected red grapes (Vitis vinifera L. and Vitis labrusca L.) widely produced in Brazil. *Food Chemistry*, 127(1), 174-179.

Roldán, E., Sánchez-Moreno, C., Ancos, B., & Cano, M.P. (2008). Characterisation of onion (Allium cepa L.) by-products as food ingredients with antioxidant and antibrowning properties. *Food Chemistry*, 108(3), 907-916.

Roussos, P.A. (2011). Phytochemicals and antioxidant capacity of orange (Citrus sinensis (l.) Osbeck cv. Salustiana) juice produced under organic and integrated farming system in Greece. *Scientia Horticulturae*, 129(2), 253-258.

Royer, G., Madieta, E., Symoneaux, R., & Jourjon, F. (2006). Preliminary study of the production of apple pomace and quince jelly. *Lwt-Food Science and Technology*, 39(9), 1022-1025.

Ruberto, G., Renda, A., Daquino, C., Amico, V., Spatafora, C., Tringali, C., & De Tommasi, N. (2007). Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape cultivars. *Food Chemistry*, 100(1), 203-210.

Rupasinghe, H.P.V., Wang, L.X., Huber, G.M., & Pitts, N.L. (2008). Effect of baking on dietary fibre and phenolics of muffins incorporated with apple skin powder. *Food Chemistry*, 107(3), 1217-1224.

Rupasinghe, H.P.V., Wang, L.X., Pitts, N.L., & Astatkie, T. (2009). Baking and sensory characteristics of muffins incorporated with apple skin powder. *Journal of Food Quality*, 32(6), 685-694.

Schieber, A., Stintzing, F.C., & Carle, R. (2001). By-products of plant food processing as a source of functional compounds – recent developments. *Trends in Food Science & Technology*, 12, 401-413.

Schieber, A., Hilt, P., Streker, P., Endress, H., Rentschler, C., & Carle R. (2003a). A new process for the combined recovery of pectin and phenolic compounds from apple pomace. *Innovative Food Science & Emerging Technologies*, 4, 99-107.

Schieber, A., Berardini, N., & Carle, R. (2003b). Identification of flavonol and xanthone glycosides from mango (Mangifera indica L. Cv. "Tommy Atkins") peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry*, 51(17), 5006-5011.

Sehm, J., Treutter, D., Lindermayer, H., Meyer, H.H. D., & Pfaffl, M.W. (2011).

The influence of apple- or red-grape pomace enriched piglet diet on blood parameters, bacterial colonisation, and marker gene expression in piglet white blood cells. *Food and Nutrition Sciences*, 2, 366-376.

Sendra, E., Kuri, V., Fernández-López, J., Sayas-Barberá, E., Navarro, C., & Pérez-Alvarez, J.A. (2010). Viscoelastic properties of orange fiber enriched yogurt as a function of fiber dose, size and thermal treatment. *Lwt-Food Science and Technology*, 43(4), 708-714.

Slimestad, R., Fossen, T., & Vågen, I.M. (2007). Onions: A source of unique dietary flavonoids. *Journal of Agricultural and Food Chemistry*, 55(25), 10067-10080.

Stojceska, V., Ainsworth, P., Plunkett, A., Ibanoglu, E., & Ibanoglu, S. (2008). Cauliflower by-products as a new source of dietary fibre, antioxidants and proteins in cereal based ready-to-eat expanded snacks. *Journal of Food Engineering*, 87(4), 554-563.

Stoll, T., Schweigert, U., Schieber, A., & Carle, R. (2003a). Application of hydrolyzed carrot pomace as a functional food ingredient to beverages. *Journal of food Agriculture & Environment*, 1, 88-92.

Stoll. T., Schweiggert, U., Schieber, A., & Carle, R. (2003b). Process for the recovery of a carotene-rich functional food ingredient from carrot pomace by enzymatic liquefaction. *Innovative Food Science & Emerging Technologies*, 4, 415-423.

Sudha, M.L., Baskaran, V., & Leelavathi, K. (2007). Apple pomace as a source

of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. *Food Chemistry*, 104(2), 686-692.

Sun, T., Simon, P.W., & Tanumihardjo, S.A. (2009). Antioxidant phytochemicals and antioxidant capacity of biofortified carrots (Daucus carota L.) of various colors. *Journal of Agricultural and Food Chemistry*, 57(10), 4142-4147.

Surles, R.L., Weng, N., Simon P.W., & Tanumihardjo, S.A. (2004). Carotenoid profiles and consumer sensory evaluation of specialty carrots (Daucus carota, L.) of various colors. *Journal of Agricultural and Food Chemistry*, 52(11), 3417-3421.

Telrandhe, U.B., Kurmi, R., Uplanchiwar, V., Mansoori, M.H., Raj, V.J., Jain, K., & Jain, S.K. (2012). Nutraceuticals - A phenomenal resource in modern medicine. *International Journal Of Universal Pharmacy And Life Sciences*, 2(1), 179 - 195.

Thed, S.T., & Phillips R.D. (1995). Changes of dietary fiber and starch composition of processed potato products during domestic cooking. *Food Chemistry*, 52(3), 301-304.

Topuz, A., Topakci, M., Canakci, M., Akinci, I., & Ozdemir, F. (2005). Physical and nutritional properties of four orange varieties. *Journal of Food Engineering*, 66(4), 519-523.

Vergara-Valencia, N., Granados-Pérez, E., Agama-Acevedo, E., Tovar, J., Ruales, J., & Bello-Pérez, L.A. (2007). Fibre concentrate from mango fruit:

Characterization, associated antioxidant capacity and application as a bakery product ingredient. *Lwt- Food Science and Technology*, 40(4), 722-729.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J. A. (2010a). Effect of added citrus fibre and spice essential oils on quality characteristics and shelf-life of mortadella. *Meat Science*, 85(3), 568-576.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J.A (2010b). Effect of orange dietary fibre, oregano essential oil and packaging conditions on shelf-life of bologna sausages. *Food Control*, 21(4), 436-443.

Wolfe, K.L., & Liu, R.H. (2003). Apple peels as a value-added food ingredient. *Journal of Agricultural and Food Chemistry*, 51(6), 1676-1683.

Chapter 1

Literature Review: Part B

State of the art in gluten-free research

Accepted by Concise Reviews and Hypotheses in Food Science.

1.2 Part B: State of the art in gluten-free research.

1.2.1 Abstract:

Coeliac Disease (CD) is widespread and is often under diagnosed. It can affect a variety of genetically susceptible people from the young to the old. Presently the only treatment for coeliac patients is life-long avoidance of any food, drink, sauce or dressing containing gluten.

Scientists and technologists continue in their quest to improve the quality of gluten-free products. Their main goal is to create a product of a similar standard to the gluten-containing products, currently on the market. However, the quality of these products still tends to be poor. Bread products have a low volume, pale crust, crumbly texture, bland flavour and a high rate of staling. Other gluten-free products contain minimal nutrition and substandard product characteristics e.g. pasta having an inferior texture, sauces which separate more easily.

The main focus of this review is to discuss the most recent advances in gluten-free research which have arisen between the years 2011 and 2013. In particular the manuscript focuses on ingredients and processing methods which have been documented to develop or improve the processing characteristics and nutritional properties of gluten-free products.

1.2.2 Introduction:

The development or enhancement of gluten-free products continues to grow. In the past, gluten-free batters and doughs have been described as being less cohesive and elastic then wheat doughs, difficult to handle and have poor gas holding retention. The products these batters and doughs create have been portrayed as having a low volume, pale crust, crumbly texture, dense crumb structure, bland starchy flavour, minimal nutrition and have a high rate of staling. Production costs for manufactures have also proven to be problematic, as bakery equipment needs to be guaranteed gluten-free, ingredients tend to be costly and distribution can be difficult due to the higher rate of staling of the products.

Researchers and media have published extensively in relation to the increased incidence of coeliac disease, gluten intolerance, wheat allergies and stomach problems. The use of gluten-free ingredients such as starches, hydrocolloids, proteins, enzymes, fat sources, pseudocereals and sourdough have been documented by food scientists (Gallagher, Gormley & Arendt, 2004; Arendt, Ryan & Dal Bello, 2007; Anton & Artfield 2008; Alvarez-Jubete, Arendt & Gallagher, 2010; Hüttner & Arendt 2010; Houben, Höchstötter & Becker, 2012).

This review concentrates on the most recent advances made in this area (from 2011-2013), in particular focusing on reviewing new ingredients and processing methods in the gluten-free field.

1.2.3 Innovative gluten-free flours:

1.2.3.1 Novel gluten-free flours:

Currently utilized gluten-free flours include maize, potato and rice flour and starches. These are used as base flours due to their bland flavour and neutral effects on baked products. These flours and starches usually tend to be low in nutrition and have very minimal structure-building potential.

Chestnut flour presents itself as potential viable flour in the development of gluten-free products. It has been reported to contain good nutritive properties such as vitamin E & B, iron, folate, essential fatty acids and dietary fibre (nutritional components which gluten-free products are usually lacking) (Sacchetti, Pinnavaia, Guidolin & Rosa, 2004).

Moreira et al. (2011a) investigated the effects of the inclusion of different hydrocolloids (agar gum, guar gum, tragacanth gum, CMC, HPMC and xanthan gum) on the rheological properties of gluten-free doughs containing chestnut flour. From the chemically synthesized and biosynthetic hydrocolloids (CMC, HPMC and xanthan gum), HMPC was found to perform the best. Mixing and pasting studies indicated that HPMC at a 1.5 % inclusion increased the consistency of the dough, while reducing pasting temperatures. Rheological tests demonstrated how HPMC increased the elasticity of the dough, observed from the increased storage modulus (G'). The authors proposed as a result of these findings that HPMC addition could reduce the effects of staling and crumb firmness. From the plant hydrocolloids (agar gum, guar gum and tragacanth gum) guar gum at a 1 % addition was found to perform the best with similar findings to HPMC (Moreira, Chenlo & Torres, 2011a; Moreira, Chenlo &

Torres, 2011b).

To compensate for the lack of protein in chestnut-based doughs, and to help improve structure, the above authors also incorporated chia flour into their formulation. Chia is a rich source of essential fatty acids, protein and fibre. From rheological tests, the authors found that chia flour increased the stability of chestnut-containing doughs; this was further improved with the inclusion of guar gum and HPMC. The combination of 4 % chia flour and 1 % guar gum / 1.5 % HPMC (in a chestnut flour-based batter) reduced apparent viscosity while also increasing dough stability and elasticity (these characteristics are important in producing a stable dough) (Moreira, Chenlo & Torres, 2013).

As outlined by Houben et al. (2012), the best approach to producing a bread of favourable baking characteristics from a highly visco-elastic gluten-free batter, is to use a combination of ingredients. A substantial amount of research was successfully completed by the authors on the effects of chestnut flour and other flours in combination with hydrocolloids. However, before a definitive conclusion can be made a number of bread studies should be completed in order to confirm the observed results.

Doughs formulated with carob germ flour have been described as having similar viscoelastic properties to that of wheat dough. Caroubin proteins are said to function in a similar way to wheat proteins, due to disulfide-bonded high molecular weight proteins. Authors have illustrated how the use of carob germ flour in bread formulations can reduce the rate of staling (Smith, Bean, Herald & Aramouni, 2012). Minarro et al. (2012) investigated the rheological properties of

dough which contained carob germ flour. The authors found that this dough demonstrated greater viscoelastic properties than the corn-starch based control, as evidenced from the increased G'. Carob germ possesses particular attributes which could influence the viscoelastic properties of dough. The authors noted that carob germ flour contained a higher level of total fibre (24 %) compared to other legumes. Gums (galactomannan) remaining from the endosperm may still be present in the carob germ and contribute to an increase in G' values (Miñarro, Albanell, Aguilar, Guamis & Capellas, 2012). Tsatsaragkou et al. (2012) investigated the effects of carob germ flour in a rice based gluten-free bread formulation. At 15 % carob germ addition, a bread of acceptable crumb expansion and low crumb firmness was developed. This bread was also considered as a fibre enriched bread with a large amount of protein. The rheological properties of the dough containing three levels of carob germ (5 %, 10 % and 15 %) were investigated. Results from a frequency sweep and creep recovery demonstrated that as the carob germ increases it elastifies the doughs structure. Addition of excess carob germ in dough was identified as possessing an extreme elastic nature. It created a dough structure which would not develop during proofing, hence creating a small loaf volume (Tsatsaragkou et al., 2012, 2013). Using a response surface design, Smith et al. (2012) were able to optimise a carob germ formulation with the aid of the hydrocolloid HPMC and an optimised water level. The final formulation contained 7 % carob germ flour, 3 % HPMC and 80 % water addition. This formulation produced favourable baking results such as reduced crumb hardness and a high loaf specific volume. The authors also recognised that the dough had similar handling characteristics to a wheat dough formulation. The final bread confirmed this observation, as it had

similar aesthetics of a wheat bread (Figure 1-1).

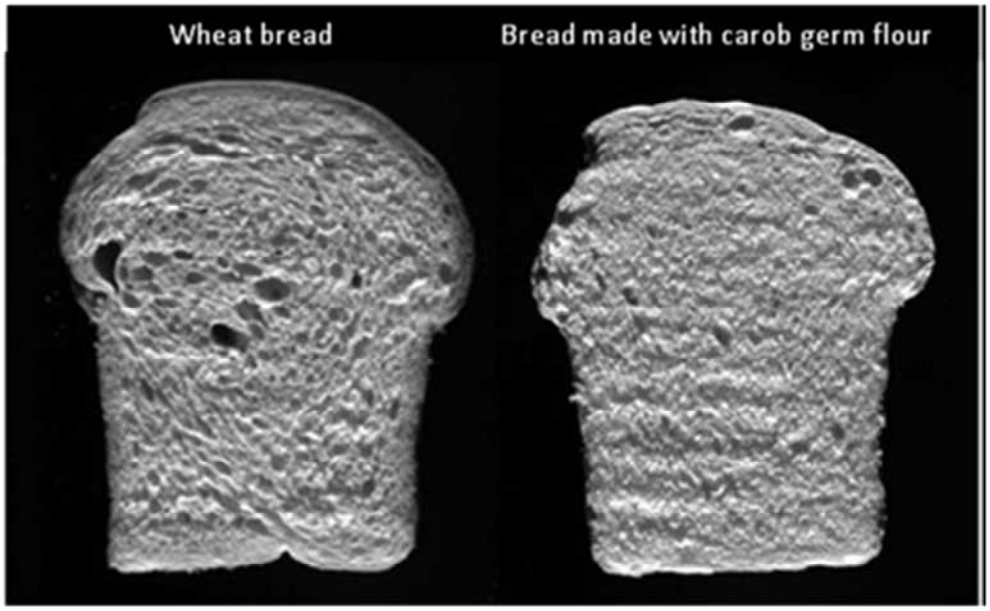


Figure 1-1: Image illustrating similarities in crumb structure between wheat bread and gluten-free bread containing carob germ flour (Smith et al., 2012).

Minarro et al. (2012) and Tsatsaragkou et al. (2012, 2013) reported comprehensive results on both dough findings and bread characteristics. However as demonstrated by Smith et al. (2012), to achieve optimal baking results optimisation of carobgerm addition along with water addition is critical. Finally, in order to fully assess the potential of carobgerm flour, a full sensory analysis program should be completed to evaluate consumer acceptability, of both the flavour and texture characteristics of the optimised bread.

Tigernut (Cypersu esculentus) is an excellent source of minerals (phosphorus,

potassium, iron and calcium), vitamins (vitamins E and C), dietary fibre and fatty acids (myristic acid, oleic and linoleic acid) (Demirkesen, Sumnu & Sahin, 2013). Demirkesen et al. (2013) investigated the effects of using tigernut flour in a gluten-free formulation in comparison to a rice bread control. The authors described how the use of tigernut decreased bake loss, increased specific volume and decreased firmness of the resulting breads when incorporated at the optimal level (10:90 / tigernut:rice). They proposed that the high fibre content of tigernut flour (22.3 %) provided enhanced water holding abilities. Furthermore, the high level of fat present in tigernut (20.5 %) generated a plasticizing effect on the rheological and proofing properties of the dough, resulting in enhanced crumb structure and bread volume. It was also noted by the authors how the inclusion of tigernut flour affected the starch properties. Results obtained from differential scanning calorimetry depicted an increase in onset, peak and final temperature of the dough containing tigernut flour when compared to the control. The authors suggest additional solutes (such as fat, fibre and sugar) are present in higher amounts in the tigernut dough. These additional constituents prevent the starch from being fully hydrated, by attracting the water away from starch, therefore increasing the gelatinisation temperatures.

Padalino et al. (2013) researched a range of vegetable flours (yellow pepper, green pepper, red pepper, tomato, spinach, pumpkin, zucchini, carrot, asparagus, fennel and eggplant flours) in the formulation of gluten-free spaghetti. From initial sensory screening tests, yellow pepper pasta was found to be the most desirable vegetable flour due to its orange colour, homogeneity and pleasant taste. This flour also increased the total dietary fibre of the pasta to 5.4 %

compared to the control 4.1 %. Quality cooking parameters illustrated how the cooking loss of yellow pepper flour pasta increased (12.0 %) compared to the control (7.8 %). Cooking loss is a deciding factor for the quality of pasta; a high cooking loss indicates a high level of solids released in the cooking water. The authors propose the high cooking loss was as a result of a reduction in starch gelatinization, therefore allowing the solids to be discharged into the water. Nevertheless, the yellow pepper pasta decreased the hardness of the pasta compared to the control (Padalino, Mastromatteo, Lecce, Cozzolino & Del Nobile, 2013).

Susanna & Prabhasankar (2013) researched the inclusion of high protein flours for the production of gluten-free pasta. They included soya flour (47.61 % fresh weight (FW) protein), chana flour (18.0 % FW protein) and sorghum flour (9.0 % FW protein). The formulations containing the highest level of soya flour (39.50 %) and chana flour (34.60 %), together with hydrocolloids (xanthan gum, HPMC and guar gum) and whey protein concentrate produced the best cooked pasta characteristics, having the lowest cooking loss, soft texture and pasta of a higher protein content compared to the control (triticum durum flour).

De-bittered lupin seed flour (Lupinus albus L.) is a highly proteinaceous flour (38.71 %); it also contains high levels of macro and minor minerals e.g. calcium (396.0 mg/100g) and manganese (152.89 mg/100) (Levent & Bilgiçli 2011).

Levent & Bilgicli (2011) incorporated de-bittered lupin seed flour into a gluten-free cake formulation and compared it to a buckwheat (Fagopyrum esculentum M.) cake. It was observed that lupin increased the protein content of the gluten-

free cake i.e. at 20 % addition; lupin cakes contained 8.72 % dry matter (DM) protein, compared to 20 % buckwheat cakes contained 6.25 % DM protein. In addition, higher levels of calcium and magnesium were also found in the lupin containing cake. Interestingly, cakes containing lupin at 40 % gave comparable sensory results when compared to cakes that contained 20 % buckwheat. The above research studies are summarized in Table 1-1.

Table 1-1: A summary of research using novel gluten-free flours.

Gluten-free flour	Additional ingredient	Product	Result	Reference
Chestnut Flour	HPMC & guar gum	Gluten-free batter	HPMC @ 1.5% Guar gum @ 1% ↑ batter consistency & G'.	Moreira and others (2011a) Moreira and others (2011b)
Chestnut Flour	Chia flour	Gluten-free batter	↓ apparent viscosity ↑dough stability.	Moreira and others (2013)
Carob germ Flour	-	Gluten-free batter	↑ G', ↑ viscoelastic properties.	Minarro and others (2012)
Carob germ flour	-	Gluten-free batter	↑ elastic nature of the dough.	Tsatsaragkon and others (2013)
Carob germ flour	-	Gluten-free bread	Low crumb firmness Acceptable crumb expansion.	Tsatsaragkon and others (2012)
Carob germ flour	HPMC	Gluten-free bread	↓ crumb hardness, ↑ specific volume.	Smith and others (2012)
Tiger nut flour	-	Gluten-free bread	↓ bake loss, ↑ specific volume, ↓ firmness, ↑ onset, peak, & final temp of dough.	Demirkesen and others (2013)
Yellow pepper flour	-	Gluten-free spaghetti	↑ cooking loss, ↓ spaghetti hardness, ↑dietary fibre content.	Padalino and others (2013)
High protein flours (soya flour, channa flour & sorghum flour)	Xanthan gum, HPMC & guar gum	Gluten-free pasta	↓ cooking loss, ↓ pasta hardness, ↑ protein content.	Susanna and Prabhasankar (2013)
De-bittered seed lupin flour	-	Gluten-free cake	↑ protein content, ↑ calcium & magnesium contents, favourable sensory results.	Levent and Bilgicli (2011)

1.2.3.2 Food by-products as gluten-free flours:

In recent times, food by-products have been highlighted as potential under-utilized ingredient due to their technical and nutritional properties. These by-products can be described as the remnants after the manufacturing of fruit and vegetable-based products. These include the seeds, peel, pips, skins, stems and cores of the product (O'Shea, Arendt & Gallagher, 2012).

Zandonadi et al. (2012) developed gluten-free pasta which contained green banana (GB) flour (a sub-product of the banana industry). This would normally have little or no commercial value. The authors chose GB flour due to its resistant starch and phenolic acid contents. The subsequent pasta was found to have a reduced fat content (0.00 %) and increased carbohydrate (74.10 %) content compared to standard wheat pasta (5.74 %, 59.00 %). They suggested that the increase in carbohydrate content was due to the higher levels of resistant starch present in the green banana flour. GB pasta was also shown to have favourable cooking characteristics; an increased water absorption, and was less firm compared to the control. It was also demonstrated that GB pasta had good sensory characteristics, in particular with respect to aroma, flavour and texture (Zandonadi et al., 2012).

Orange pomace (OP) constitutes 45-60 % of the whole fruit, and is usually discarded at a cost to the producer. This by-product is a source of dietary fibre (up to 40 % dry matter (DM)), bio-actives (e.g. vitamin C and minerals) and is low in fat (O'Shea et al., 2012). Researchers have investigated the benefits and limitations of using OP in a gluten-free bread formulation. As OP is a highly fibrous ingredient, the authors used a response surface design to identify the

optimal OP usage level, water addition and proofing time (variables most affected by the inclusion of fibrous ingredients). Results for loaf specific volume highlighted how the inclusion of OP increased volume up to a certain level of inclusion, crumb texture was shown to be comparable to the control. The optimised formulation contained 5.5 % OP. Sensory results illustrated that panellists scored the texture of optimised bread favourably. The total dietary fibre was shown to increase from 2 % in the control to 4 % in the optimised bread (O'Shea, Rossle, Arendt & Gallagher, 2014).

Other by-products being researched are defatted strawberry seeds (DS-ST) and blackcurrant seeds (DS-BC), which is the material left behind once the oil has been extracted. These by-products have come to the attention of researchers due to their nutritional potential, e.g. protein, fibre and bioactivity (Korus et al., 2012). In a gluten-free bread formulation, the incorporation of DS-ST was found to significantly increase the loaf volume compared to DS-BC-containing breads and the control. The authors hypothesised that the positive results could be attributed to additional sugars present, which could enhance the ability of the yeast to produce CO_2, hence increasing specific volume. At lower levels, the bread was shown to have similar crumb porosity to the control. Both by-products reduced crumb hardness two hours after baking, however only breads containing DS-ST showed significantly reduced crumb hardness compared to the control after 24 hours. The inclusion of DS-ST improved the dietary fibre and protein content of the gluten-free bread (5.43 %, 1.40 % respectively) compared to the control (2.99 %, 1.05 %).

Pumpkin seeds discarded after pre-processing of the fruit were assessed in a

corn-based gluten-free cake formulation and compared to a wheat control. Gorgônio et al. (2011) measured the density of the batter produced following the addition of 40 % pumpkin seed flour (PSF) and found it to be lower than that of the density of the wheat batter. This would suggest that the structure of the final cake would be more aerated than the wheat control. The authors also described how cakes with a comparable volume that of the control were produced and that the crumb structure was more uniform following the inclusion of PSF. Gluten-free cakes containing 40 % PSF contained significantly enhanced levels of protein (12.28 %) and insoluble fibre (10.20 %) (Gorgônio, Pumar & Mothé, 2011).

Apple is consumed in numerous ways, e.g. the whole fruit, processed into slices, as sauces or juiced (O'Shea, et al., 2012). O'Shea et al. (2013) investigated the effects of using freeze-dried apple pomace flour (consisting of the peel, core and pips) in a gluten-free extruded puffed snack. The flour was successfully incorporated at levels up to 7.7 %, creating a puffed snack with comparable expansion characteristics to the control. The by-product was shown to affect the textural and physical characteristics of the snack. At inclusions beyond 7.7 %, the apple pomace decreased the radical expansion ratio and porosity of the product (O'Shea, Arendt & Gallagher, 2013).

The results discussed in this section 1.2.3.2. are summarized in Table 1-2.

Table 1-2: A summary of research using food by-products as gluten-free flours.

By-product flour	Product	Result	Reference
Green banana flour	Gluten-free pasta	↑ water absorption, ↓ firmness, favourable sensory results.	Zandonadi and others (2012)
Orange Pomace flour	Gluten-free bread	↑ specific volume, comparable texture to the control, favourable sensory characteristics, ↑ fibre content.	O'Shea and others (2013a)
Defatted strawberry seeds	Gluten-free bread	↑ loaf volume, similar crumb porosity to control, ↓ crumb firmness @ 2 & 24 hours ↑ dietary fibre and protein content.	Korus and others (2012)
Blackcurrent seeds	Gluten-free bread	↓ loaf volume, ↓ crumb firmness @ 2 hours.	
Pumpkin seeds	Gluten-free cake	↓ batter density, comparable cake volume, more uniform crumb structure, ↑ protein and insoluble fibre.	Gorgônio and others (2011)
Apple Pomace flour	Gluten-free extruded puffed snacks	Comparable expansion properties to the control.	O'Shea and others (2013b)

These recent and ongoing studies have demonstrated how by-products from food processing have the potential to be incorporated into a wide range of gluten-free products. They naturally enhance the flavour, structure and nutrition of the products, and are potentially inexpensive ingredients (reducing formulation costs). It would appear, however, that there are certain limitations to their use i.e. certain by-products may be more suited to specific products. Also, essential pre-processing (e.g. freeze-drying) of the by-products to stabilize them may over-ride the cost saving benefits. Further research in this area is necessary.

1.2.4 New approaches to aid processing and enhance nutrition:

Gluten-free products (especially bread products) have been widely documented, in particular with respect to their poor structure (inability to retain CO_2, appearance of a dense crumb grain) and the lack of nutritional content (Thompson 2000; Zannini, Jones, Renzetti & Arendt, 2012). This section reviews ingredients which have been studied to address these problems.

1.2.4.1 Ingredients to aid processing:

Moreira et al. (2012) assessed the effects of two types of shortening (sunflower oil and olive oil) as a possible means to improve the rheological properties of chestnut flour-based doughs. As expected, adding the above oils to the flour decreased the water absorption of the resulting doughs, while decreasing the stability of the dough. It was also emphasised that these oils decreased the apparent viscosity and storage modulus of the chestnut doughs (Moreira, Chenlo & Torres, 2012).

Zein-based doughs have been described as having similar properties to wheat dough (Erickson, Campanella & Hamaker, 2012). These authors discussed in great detail the improvements made to gluten-free dough, seen via fibrous β-sheet-rich protein networks.

High molecular weight glutenin (HMWG) can contribute to the elasticity of wheat dough. Fevzioglu et al. (2012) demonstrated that zein proteins can behave in a similar manner to gliadin upon the addition of HMWG. To produce a stable visco-elastic structure, rheological methods e.g. large strain (uniaxial and biaxial)

and small strain tests, were utilized to assess the effect of HMWG. HMWG addition was shown to develop dough with improved visco-elastic properties as a result of interactions occurring between the prolamin (zein) and HMWG. The authors proposed that a non-gluten protein with similar properties to HMWG could improve the visco-elastic properties of zein (Fevzioglu, Hamaker & Campanella, 2012).

Meso-structured protein particle systems created from whey is another unique documented method of imitating a gluten structure/network. Van Riemsdijk et al. (2011a) investigated the possible rheological potential of three whey protein (WP) structures (WP aggregates, WP cold set gel (via acidification) and WP particles (via mixing with locust bean and acidification)). The author's findings demonstrated how WP particles at the mesoscopic level yielded similar strain hardening and recovery results to wheat dough. These researchers recommended that the use of additional functional ingredients e.g. shortening could create dough with even more comparable properties to wheat dough. This research was preceded by incorporating the three meso-structured whey protein structures into a gluten-free bread formulation and comparing it to a gluten-containing formulation. All three ingredients produced loaves with similar volumes to the wheat control. A negative result was seen when assessing the crumb structure, where the WP aggregates and WP cold set gel, developed breads with a crumb structure which had enlarged cells and large holes. The WP particle breads had crumb structures comparable to the control; the authors attribute this to the WP particle structures being able to withstand higher strain levels compared to the other WP structures (Van Riemsdijk, Pelgrom, van der Goot, Boom & Hamer,

2011a; Van Riemsdijk, van der Goot, Hamer & Boom, 2011b).

Blanco et al. (2011) investigated the effects of four additives (acetic acid, lactic acid, citric acid and monosodium phosphate) in a rice flour and HPMC-based bread formulation. The authors found that the use of monosodium phosphate increased loaf volume significantly; it was also noted that the inclusion of this additive resulted in the largest cell area compared to the control. These positive effects may be a consequence of hydrogen bonding during the proofing stage between HPMC and monosodium phosphate, preventing the CO_2 from escaping, thus resulting in larger loaf volumes (Blanco, Ronda, Pérez & Pando, 2011).

1.2.4.2 Ingredients to enhance nutrition:

Due to the high prevalence of osteoporosis in coeliacs, and the difficulty with including sources of calcium such as dairy ingredients (due to lactose intolerance), the direct addition of calcium substitutes has been investigated by researchers.

Krupa-Kozak et al. (2011) used two types of calcium supplements (calcium caseinate (CAS)) and calcium citrate (CIT)) and investigated their addition on the baking characteristics of a gluten-free formulation. At 2 % addition, calcium citrate showed the most positive effects on bread characteristics. Its presence increased the specific volume from 2.29 cm^3/g (control sample) to 3.34 cm^3/g. Inclusion of CIT was also found to increase bake loss; the authors suggested this was due to larger cell volume found in the crumb structure which would accelerate the loss of moisture. A 2 % addition of CIT significantly increased the

calcium content from 22.2 mg/100g to 469.3 mg/100g (Krupa-Kozak, Troszyńska, Bączek & Soral-Śmietana, 2011). The same authors illustrated the effects of two organic (calcium citrate (CIT), calcium lactate (LAC)) and two inorganic (calcium chloride (CHL) and calcium carbonate (CAR)) calcium salts in an inulin/corn/potato starch-based gluten-free formulation. Interestingly, the organic salts increased the consistency of the dough, whereas the inorganic salts had the opposite effects. The authors attribute this result to the chemical structure of the salt altering the water absorption properties of the starch, hence affecting the consistency of the dough. CAR and CHL significantly increased the calcium contents of the bread. Overall, the use of CAR produced the most favourable baking characteristics with a specific volume similar to the control, softer crumb, higher level of springiness and positive sensory characteristics (Figure 1-2) (Krupa-Kozak, Altamirano-Fortoul, Wronkowska & Rosell, 2012). The addition of calcium substitutes is not just restricted to bread. Han et al. (2012) included calcium hydroxide in a buckwheat-based noodle formulation. Noodles containing 0.4 % calcium hydroxide showed increased tensile strength and firmness; beyond this level of addition, results for these characteristics began to decrease. Microstructure images indicated how calcium hydroxide at 0.4 % addition had a compact, homogeneous and less porous structure (i.e. high quality) compared to the control (Han, Lu, Hao, Cheng & Li, 2012).

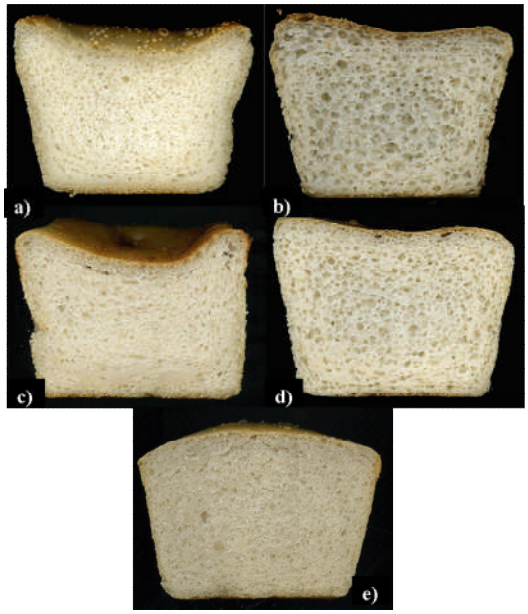


Figure 1-2: a) control, b) calcium carbonate (CAR), c) calcium chloride (CHL), d) calcium citrate (CIT) & e) calcium lactate (LAC). Scale bar 10mm (Krupa-Kozak et al., 2012).

Anaemia is a disease which is also frequently associated with coeliac disease, due to poor iron absorption, or a reduction in the consumption of products which are fortified with iron. Kiskini et al. (2012) analysed the effects of five sources of iron (ferric pyrophosphate, ferric pyrophosphate/emulsifiers, sodium iron EDTA, ferrous sulphate and elemental iron) in a gluten-free bread formulation. Overall, iron addition produced breads with similar features to the control, apart from ferric pyrophosphate/emulsifier and ferrous sulphate, which retained more moisture compared to the control. Breads containing ferric pyrophosphate and sodium iron EDTA had firmer crumb texture. In terms of sensory findings, the authors reported how the use of elemental iron created breads with the least metallic taste (Kiskini, Kapsokefalou, Yanniotis & Mandala, 2012).

Sedej et al. (2011) included soaked flax seed and sesame seed in a buckwheat

cracker recipe and compared the end product to a wheat recipe. The authors found the inclusion of these two ingredients significantly increased the γ-tocopherol (2.31 mg/100g) content compared to the wheat sample (1.52 mg/100g). The enhanced buckwheat cracker also received favourable sensory scores (Sedej et al., 2011).

Continued increasing interest lies with the polysaccharide inulin. This is due to its nutritional properties and functional abilities. To date, it has been widely utilized as a processing ingredient due to its bulking ability, structure forming capacity, water retention and its ability to improve rheological properties and stabilize emulsions and foams (Juszczak et al., 2012). Ziobro et al. (2013) incorporated 3 types of inulin with differing degrees of polymerization (DP): HIS (DP < 10), GR (DP ≥ 10) and HPX (DP > 23) into a gluten-free bread formulation at three levels (4 %, 6 %, and 8 %). The authors found that inulin containing a low degree of polymerization (DP) (DP ≤10) produced the best results irrespective of addition level. Loaf volume increased with inulin level and particularly at 12 % GR inulin addition; it was also shown to reduce hardness over 36 hours. The number of cells present in the crumb structure decreased with increasing HSI and GR addition; the area of each cell increased with HIS and GR addition. This depicts a stronger and robust crumb structure which can withstand proofing and still retain its volume during baking (Ziobro, Korus, Juszczak & Witczak, 2013). The study by Hager et al. (2011) generated conflicting results compared to the previous authors. These researchers found the incorporation of inulin to have an unfavourable effect on crumb hardening, and the rate of staling was accelerated. However, the inulin increased the loaf

specific volume compared to the control and induced a darkening of colour in the crust, which can be viewed as a favourable result, as gluten-free breads are normally quite pale in appearance (Hager et al., 2011).

Capriles & Areas (2013) included inulin-type fructans (ITF) at four levels (4 %, 8 %, 10 % and 12 %) in rice flour and potato starch based gluten-free bread formulation. The authors discussed how levels up to 10 % increased the specific volume; above 10 % inclusions a decline in specific volume occurred. A reduction in crumb firmness was also noted. ITF increased total dietary fibre (18.37 %) and total fructan content (12.50 %) (Capriles & Arêas 2013).

In a study involving cake, Gularte et al. (2012) incorporated inulin at 20 %. When compared to other soluble/insoluble fibres such as oat and oat-guar gum cakes, the inulin-containing cake collapsed at a higher level. Interestingly, although the specific volume of the cake was similar to the control, its texture was firm and non-springy (Gularte, de la Hera, Gómez & Rosell, 2012).

The use of inulin as a fat replacer in a gluten and lactose-free white sauce was investigated (Guardeño, Hernando, Llorca, Hernández-Carrión & Quiles, 2012). The authors illustrated how the inclusion of inulin produced a sauce with good sensory attributes. The colour of the sauce remained constant during storage and no evidence of separation occurred. As demonstrated by the above authors, inulin is a popular ingredient in the formulation of bakery products. Both Ziobro et al. (2013) and Hager et al. (2011) researched the application of inulin in gluten-free bread. Although the addition of inulin generally produced favourable baking characteristics, conflicting crumb texture results were reported by these

two authors. Choosing inulin with the appropriate degree of polymerization may be the key to improving the texture as illustrated by Ziobro et al. (2013). More studies are required to further quantify its affect in bread. Additional research into the organoleptic properties is also required in order to assess its potential utilization as a bakery ingredient.

Beta-glucan ((1→3) (1→4)-β-D-glucan), a soluble fibre found in large quantities in both oats and barley, has been reported to reduce LDL-cholesterol levels in the blood, and reduces the postprandial glycaemic response (Sullivan, O'Flaherty, Brunton, Arendt & Gallagher, 2011). Kim & Yokoyama (2010) discussed how de-branned oat contained 1.28 % beta-glucan, pearled barley contained 3.58 % β-glucan and whole wheat flour only contains 0.17 % β-glucan (Kim & Yokoyama 2010).

Beta-glucan also has the potential to act as a hydrocolloid. Andersson et al. (2011) investigated the effects of β-glucan (from oat bran) in combination with HPMC in a zein and maize starch-based dough. The β-glucan did not perform as well as expected. In preliminary rheological tests it gave promising results however these positive dough effects were not evident during the baking trials. When compared to doughs containing HPMC, the use of β-glucan gave an uneven and large porous crumb structure. This is most likely due to the surface active properties of HPMC i.e. stabilization of the CO_2 developed structure (Andersson, Öhgren, Johansson, Kniola & Stading, 2011).

Beta-glucan enriched oat-bran (containing 22 %) was assessed in a maize-based spaghetti formulation. Padalino et al. (2011) carried out sensory analysis on

spaghetti samples containing 20 % oat bran. Sensory panellists scored this sample lowest in terms of sensory acceptance. Hydrocolloids were then added to improve the sensory perception of the spaghetti. Carboxylmethylcellulose (CMC) and chitosan performed well. Chitosan increased the consistency index of the dough compared to the other doughs. In the cooked spaghetti, CMC and chitosan samples obtained the highest sensory scores. The authors proposed this was due to the CMC slowing down the diffusion of amylose from the inner part of the spaghetti to the surface. They also suggested that chitosan had a higher affinity for pre-gelatinized starch, developing a more continuous starch network and promoting greater viscosity of the spaghetti dough (Padalino, Mastromatteo, Sepielli & Nobile, 2011). The research discussed in this section is summarized in Table 1-3.

Table 1-3: A summary of nutritional ingredients to enhance nutrition.

Gluten-free nutritional ingredient	Product	Result	Reference
Calcium citrate	Gluten-free bread	↑ loaf volume, ↑ bake loss, ↑ calcium content.	Krupa-Kozak and others (2011)
Calcium carbonate	Gluten-free batter & bread	↓ dough consistency, loaf volume similar to the control, ↑ calcium content, ↓ crumb firmness, ↑ springiness, positive sensory results.	Krupa-Kozak and others (2012)
Calcium Hydroxide	Gluten free noodles	↑ tensile strength and firmness.	Han and others (2012)
Ferric pyrophosphate, Ferric pyrophosphate/emulsifiers, Sodium iron EDTA, Ferrous sulphate, Elemental iron.	Gluten-free bread	Firmer crumb texture. Retained moisture compared to control. Firmer crumb texture. Retained moisture compared to control. Best sensory results.	Kiskini and others (2012)
Soaked flax seed and sesame seed	Buckwheat cracker	↑ γ-tocopherol, Favourable sensory scores.	Sedej and others (2011)
Inulin (<10 degree of polymerization)	Gluten-free bread	↑ loaf volume, ↓ crumb firmness	Ziobro and others (2013)
Inulin	Gluten-free bread	↑ loaf volume, darkening in the crust, ↑ crumb firmness	Hager and others (2011)
Inulin-type fructans	Gluten-free bread	↑ loaf volume, ↓ crumb firmness	Capriles and Areas (2013)
Inulin	Gluten-free cake	Similar cake volume to control, ↑ crumb firmness, ↓ springiness	Gularte and others (2012)
Inulin	Lactose-free white sauce	Consistent colour, no sauce separation.	Guardeño and others (2012)
Beta-glucan	Gluten-free bread	Positive rheology results, large porous crumb structure.	Andersson and others (2011)
Beta-glucan	Gluten-free spaghetti	Acceptable sensory scores	Padalino and others (2011)

1.2.5 Novel processing methods: new applications in gluten-free processing.

Most cereal researchers concentrate on developing new gluten-free formulations and incorporating novel blends of ingredients in an effort to produce an end product with similarities to wheat-based products. A downside to such approaches is the cost incurred due to the price of these extra or unique ingredients. For this reason, researchers are beginning to study parameters apart from the formulation aspect, to improve the attributes of gluten-free products, while at the same time not increasing processing costs.

Using the ideal particle size and flour type has proven to be an important determinant of the characteristics of final bread. De la Hera et al. (2013a) used three flours (from yellow maize, milled from yellow semolina, milled from white maize) of five different particle sizes (<80 μm, 80–106 μm, 106–150 μm, 150–180 μm and >180 μm) in combination with the hydrocolloid hydroxypropyl methylcellulose (HPMC) to study their effect on the dough rheological properties and the end-baked bread qualities. The authors discussed how coarser maize flour produced breads with the most desirable loaf volume and lowest crumb hardness. Dough containing flour of a lower particle size reduced dough development times and resulted in smaller loaf volumes. It was hypothesised by the authors that the lower particle size flour caused the creation of a weaker structure, which cannot retain the same quantity of CO_2, resulting in smaller volume (de la Hera, Talegón, Caballero & Gómez, 2013).

Research was also carried out on the effect of particle size on rice based cakes.

They investigated the effects of two types of rice flour (short grain (particle sizes of <80, 80-106, 106-180 and >180 µm) and long grain (particle sizes of <80, 80-106, >106 µm)) on the batter and final cake quality. The data displayed interesting results, coarser flour produced batter with a high specific volume however the baked cake had a low specific volume. It was proposed that this was as a consequence of large bubbles created during mixing which cause coalescence and then bubble loss during baking producing a cake of a lower volume. Overall, finer rice flour fractions were demonstrated to produce cakes with a higher cake specific volume, cake symmetry index and lower crumb firmness (de la Hera, Martinez, Oliete & Gómez, 2013b).

Gomez et al. (2013) investigated the effects of mixing speed, time, mixing attachment and proofing time on a gluten-free dough and batter (that contained 80 % and 110 % water). The authors found that higher water additions led to batter-like consistencies, and required mixing regimes similar to that of cakes i.e. lower mixing speed but longer mixing time, and using a whip wire mixing attachment to incorporate more air and bubbles into the batter. It was also observed that longer mixing times had a positive effect on the amount of CO_2 produced. Two reasons for this were proposed; firstly, longer mixing times permits greater oxygenation which allows yeast to reproduce under its preferred aerobic conditions. Secondly, greater mixing times allow amylase to produce maltose, which is the reserved food source for yeast after sucrose has been consumed during proofing. A shorter mixing time would not allow for these two occurrences, therefore fermentation would cease in the first 15 minutes of proofing leading to a reduction in CO_2 production and a reduced final loaf

volume. Longer proofing time (90 minutes) was required for the sample containing 110 % water, compared to the sample which contained 80 % moisture (50 minutes). The authors suggest that a fluid batter can retain more air and expand more easily during proofing. Beyond the optimal proofing time, the structure developed becomes too weak to support itself and collapses (Gómez, Talegón & de la Hera, 2013).

Demirkesen et al. (2011) compared infrared-microwave baking to conventional baking as a possible cost-saving method. The authors proposed that using microwave ovens offered advantages such as energy efficiency, faster heating, space saving and food which retained better nutritional quality. To optimise the process, a response surface design was used. Findings showed how microwave power and infrared power were two of the prominent factors effecting bake loss, firmness and specific volume. When these two factors were at the maximum levels, a higher level of bake loss was attained, resulting in a drier crumb with a firmer texture. Infrared power particularly affected loaf specific volume. It increased the temperature of the crust more quickly than the crumb, thus reducing the ability of the crumb to develop, resulting in a reduced volume. The optimised baking conditions were calculated to be 40 % infrared, 30 % microwave power and a baking time of 9 minutes. The authors described how the wheat bread was baked for 25 minutes, whereas the gluten-free bread was baked for 9 minutes, thus demonstrating how this method reduced time and energy (Demirkesen, Sumnu, Sahin & Uysal, 2011).

Due to the higher level of starch present in gluten-free products, the staling rate tends to be more rapid, which can create high economic losses. Sciarini et al.

(2012) reported how partially baked gluten-free breads are a potential solution to this problem, as the product can be stored at low temperatures and a final baking step is carried out by the consumer. However, the authors found that the quality of partially baked breads is significantly lessened (decreased loaf volume / firmer crumb) once the final baking step is completed. Therefore, for partially baked products to retain its quality, a hydrocolloid such as CMC / xanthan gum is required (Figure 1-3). It was also noted that the effects of staling which occur during storage of the partial baked breads (i.e. starch retrogradation), can be reversed in the second phase of baking via melting of the amylopectin (Sciarini, Pérez, Lamballerie, León & Ribotta, 2012).

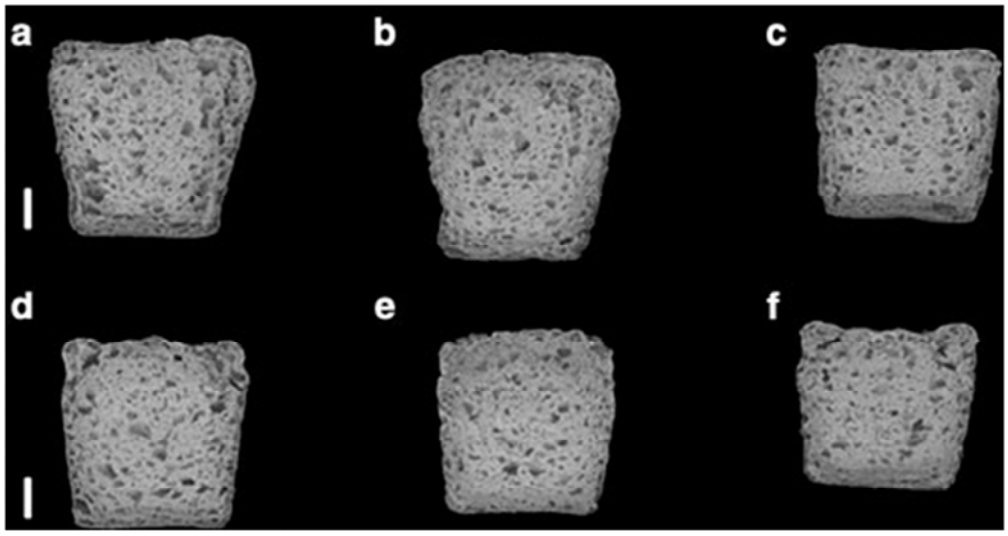


Figure 1-3: a) CMC full-baked bread, b) xanthan full-baked bread, c) no additive full-baked bread, d) CMC final part-baked bread, e) xanthan final part-baked bread & f) no additive full part-baked bread (Sciarini et al., 2012).

Gutierrez et al. (2011) also addressed this issue by investigating the application of antimicrobial active packaging in combination with modified atmospheric packaging (MAP) and conventional sealed packaging. Active packaging uses a

self-adhesive label which has an organic solvent base that is coated with nitrocellulose which contains an essential oil (cinnamon) at levels of 0.0215 g and 0.0374 g. The authors documented how MAP performed the most favourably in preventing contamination and mould growth. This was followed by the active packaging containing the highest level (0.0374 g) of active compounds. Sensory analysis showed positive results for the active packaging regime; the breads were deemed to be softer compared to MAP breads. When MAP was used on its own, the cinnamon flavour of the active packaging was increased when compared to conventionally sealed packaging. The authors also noted how conventional sealing is more cost effective than MAP as no gases, no special packaging machines or high-barrier materials are required (Gutiérrez, Batlle, Andújar, Sánchez & Nerín, 2011).

1.2.6 Conclusion:

The prevalence of coeliac disease has been recorded to vary between 1 in 100 people to 1 in 300 people worldwide in adults (Bai et al., 2013). Hence, a requirement to produce high quality and wide variety of gluten-free products is as important as ever.

This review presents the most recently published research on flours which are highly nutritious and decrease the need for supplements. They can also be potentially cheaper and a more functional alternative to its generic counterparts (rice, corn and potato starch) e.g. chestnut flour and flours developed from fruit by-products e.g. orange pomace and defatted strawberry seeds.

This review also highlights other ingredients which have recently been trialled such as different types of shortenings and doughs enhanced with zein, nutritional additives e.g. calcium salts and inulin.

Novel baking processes for gluten-free products have also been described in this review, as an area where more research would benefit both the producer and the end-user.

1.2.7 References:

Alvarez-Jubete, L., Arendt, E.K., & Gallagher, E. (2010). Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. *Trends in Food Science & Technology*, 21(2), 106-113.

Andersson, H., Öhgren, C., Johansson, D., Kniola, M., & Stading, M. (2011). Extensional flow, viscoelasticity and baking performance of gluten-free zein-starch doughs supplemented with hydrocolloids. *Food Hydrocolloids*, 25(6), 1587-1595.

Anton, A.A., & Artfield, S.D. (2008). Hydrocolloids in gluten-free breads: A review. *International Journal of Food Sciences and Nutrition*, 59(1), 11-23.

Arendt, E.K., Ryan, L.A.M., & Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology*, 24(2), 165-174.

Bai, J.C., Fried, M., Corazza, G.R., Schuppan, D., Farthing, M., Catassi, C., Greco, L., Cohen, H., Ciacci, C., Eliakim, R., Fasano, A., González, A., Krabshuis, J.H., & Le Mair, A. (2013). World gastroenterology organisation global guidelines on celiac disease. *Journal of Clinical Gastroenterology*, 47(2),121-126.

Blanco, C.A., Ronda, F., Pérez, B., & Pando, V. (2011). Improving gluten-free bread quality by enrichment with acidic food additives. *Food Chemistry*, 127(3), 1204–1209.

Capriles, V.D., & Arêas, J.A. (2013). Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance and glycemic response of gluten-free

breads. *Food & Function*, 4(1), 104-110.

De la Hera, E., Talegón, M., Caballero, P., & Gómez, M. (2013a). Influence of maize flour particle size on gluten-free breadmaking. *Journal of the Science of Food and Agriculture*, 93(4), 924-932.

De la Hera, E., Martinez, M., Oliete, B., & Gómez, M. (2013b). Influence of flour particle size on quality of gluten-free rice cakes. *Food and Bioprocess Technology*, 6(9), 2280-2288.

Demirkesen, I., Sumnu, G., Sahin, S., & Uysal, N. (2011). Optimisation of formulations and infrared–microwave combination baking conditions of chestnut–rice breads. *International Journal of Food Science & Technology*, 46(9), 1809-1815.

Demirkesen, I., Sumnu, G., & Sahin, S. (2013). Quality of gluten-free bread formulations baked in different ovens. *Food and Bioprocess Technology*, 6(3), 746-753.

Erickson, D.P., Campanella, O.H., & Hamaker, B.R. (2012). Functionalizing maize zein in viscoelastic dough systems through fibrous, β-sheet-rich protein networks: An alternative, physicochemical approach to gluten-free breadmaking. *Trends in Food Science & Technology*, 24(2), 74-81.

Fevzioglu, M., Hamaker, B.R., & Campanella, O.H. (2012). Gliadin and zein show similar and improved rheological behavior when mixed with high molecular weight glutenin. *Journal of Cereal Science*, 55(3), 265-271.

Gallagher, E., Gormley, T.R., & Arendt, E.K. (2004). Recent advances in the formulation of gluten-free cereal-based products. *Trends in Food Science & Technology,* 15(3-4), 143-152.

Gómez, M., Talegón, M., & de la Hera, E. (2013). Influence of mixing on quality of gluten-free bread. *Journal of Food Quality,* 36(2), 139-145.

Gorgônio, C.M.D.S., Pumar, M., & Mothé, C.G. (2011). Macrocospic and physiochemical characterization of a sugarless and gluten-free cake enriched with fibers made from pumpkin seed (Cucurbita maxima, L.) flour and cornstarch. *Food Science and Technology (Campinas),* 31, 109-118.

Guardeño, L.M., Hernando, I., Llorca, E., Hernández-Carrión, M., & Quiles, A. (2012). Microstructural, physical, and sensory impact of starch, inulin, and soy protein in low-fat gluten and lactose free white sauces. *Journal of Food Science,* 77(8), C859-C865.

Gularte, M.A., de la Hera, E., Gómez, M., & Rosell, C.M. (2012). Effect of different fibers on batter and gluten-free layer cake properties. *LWT - Food Science and Technology*, 48(2), 209-214.

Gutiérrez, L., Batlle, R., Andújar, S., Sánchez, C., & Nerín, C. (2011). Evaluation of antimicrobial active packaging to increase shelf life of gluten-free sliced bread. *Packaging Technology and Science,* 24(8), 485-494.

Hager, A.-S., Ryan, L., Schwab, C., Gänzle, M., O'Doherty, J., & Arendt, E. (2011). Influence of the soluble fibres inulin and oat β-glucan on quality of

dough and bread. *European Food Research and Technology*, 232(3), 405-413.

Han, L., Lu, Z., Hao, X., Cheng, Y., & Li, L. (2012). Impact of calcium hydroxide on the textural properties of buckwheat noodles. *Journal of Texture Studies*, 43(3), 227-234.

Houben, A., Höchstötter, A., & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: an overview. *European Food Research and Technology*, 235(2), 195-208.

Hüttner, E.K., & Arendt, E.K. (2010). Recent advances in gluten-free baking and the current status of oats. *Trends in Food Science & Technology*, 21(6), 303-312.

Juszczak, L., Witczak, T., Ziobro, R., Korus, J., Cieślik, E., & Witczak, M. (2012). Effect of inulin on rheological and thermal properties of gluten-free dough. *Carbohydrate Polymers*, 90(1), 353-360.

Kim, Y., & Yokoyama, W.H. (2010). Physical and sensory properties of all-barley and all-oat breads with additional hydroxypropyl methylcellulose (HPMC) β-Glucan. *Journal of Agricultural and Food Chemistry*, 59(2), 741-746.

Kiskini, A., Kapsokefalou, M., Yanniotis, S., & Mandala, I. (2012). Effect of iron fortification on physical and sensory quality of gluten-free bread. *Food and Bioprocess Technology*, 5(1), 385-390.

Korus, J., Juszczak, L., Ziobro, R., Witczak, M., Grzelak, K., & SÓJka, M. (2012). Defatted strawberry and blackcurrant seeds as functional ingredients of gluten-free bread. *Journal of Texture Studies*, 43(1), 29-39.

Krupa-Kozak, U., Troszyńska, A., Bączek, N., & Soral-Śmietana, M. (2011). Effect of organic calcium supplements on the technological characteristic and sensory properties of gluten-free bread. *European Food Research and Technology,* 232(3), 497-508.

Krupa-Kozak, U., Altamirano-Fortoul, R., Wronkowska, M., & Rosell, C. (2012). Breadmaking performance and technological characteristic of gluten-free bread with inulin supplemented with calcium salts. *European Food Research and Technology,* 235(3), 545-554.

Levent, H., & Bilgiçli, N. (2011). Enrichment of gluten-free cakes with lupin (Lupinus albus L.) or buckwheat (Fagopyrum esculentum M.) flours. *International Journal of Food Sciences and Nutrition,* 62(7), 725-728.

Miñarro, B., Albanell, E., Aguilar, N., Guamis, B., & Capellas, M. (2012). Effect of legume flours on baking characteristics of gluten-free bread. *Journal of Cereal Science,* 56(2), 476-481.

Moreira, R., Chenlo, F., & Torres, M.D. (2011a). Rheological properties of commercial chestnut flour doughs with different gums. *International Journal of Food Science & Technology,* 46(10), 2085-2095.

Moreira, R., Chenlo, F., & Torres, M.D. (2011b). Rheology of commercial chestnut flour doughs incorporated with gelling agents. *Food Hydrocolloids,* 25(5), 1361-1371.

Moreira, R., Chenlo, F., & Torres, M.D. (2012). Effect of shortenings on the rheology of gluten-free doughs: study of chestnut flour with chia flour, olive and

sunflower oils. *Journal of Texture Studies*, 43(5), 375-383.

Moreira, R., Chenlo, F., & Torres, M.D. (2013). Effect of chia (Sativa hispanica L.) and hydrocolloids on the rheology of gluten-free doughs based on chestnut flour. *LWT - Food Science and Technology,* 50(1), 160-166.

O'Shea, N., Arendt, E.K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science & Emerging Technologies,* 16(0), 1-10.

O'Shea, N., Arendt, E., & Gallagher, E. (2013). Enhancing an extruded puffed snack by optimising die head temperature, screw speed and apple pomace inclusion. *Food and Bioprocess Technology*, 1-16.

O'Shea, N., Rossle, C., Arendt, E., & Gallagher, E. (2014). New application of a fruit by-product: gluten-free bread approach. *Journal of Ceral Science,* UNDER REVIEW.

Padalino, L., Mastromatteo, M., Sepielli, G., & Del Nobile, M.A. (2011). Optimization of gluten-free functional spaghetti based on maize flour and oat bran enriched in beta-glucans. *Materials,* 4, 2119-2135.

Padalino, L., Mastromatteo, M., Lecce, L., Cozzolino, F., & Del Nobile, M.A. (2013). Manufacture and characterization of gluten-free spaghetti enriched with vegetable flour. *Journal of Cereal Science*, 57, 333-342.

Sacchetti, G., Pinnavaia, G.G., Guidolin, E., & Rosa, M.D. (2004). Effects of extrusion temperature and feed composition on the functional, physical

and sensory properties of chestnut and rice flour-based snack-like products. *Food Research International,* 37(5), 527-534.

Sciarini, L., Pérez, G., Lamballerie, M., León, A., & Ribotta, P. (2012). Partial-Baking process on gluten-free bread: impact of hydrocolloid addition. *Food and Bioprocess Technology,* 5(5), 1724-1732.

Sedej, I., Sakač, M., Mandić, A., Mišan, A., Pestorić, M., Šimurina, O., & Čanadanović-Brunet, J. (2011). Quality assessment of gluten-free crackers based on buckwheat flour. *LWT - Food Science and Technology,* 44(3), 694-699.

Smith, B.M., Bean, S.R., Herald, T.J., & Aramouni, F.M. (2012). Effect of HPMC on the quality of wheat-free bread made from carob germ flour-starch mixtures. *Journal of Food Science,* 77(6), C684-C689.

Sullivan, P., O'Flaherty, J., Brunton, N., Arendt, E., & Gallagher, E. (2011). The utilisation of barley middlings to add value and health benefits to white breads. *Journal of Food Engineering,* 105(3), 493-502.

Susanna S, & Prabhasankar P. (2013). A study on development of Gluten free pasta and its biochemical and immunological validation. *Lebensm-Wiss Technol,* 50(2), 613-621.

Thompson, T. (2000). Folate, iron, and dietary fiber contents of the gluten-free diet. *Journal of the American Dietetic Association,* 100(11), 1389-1396.

Tsatsaragkou, K., Yiannopoulos, S., Kontogiorgi, A., Poulli, E., Krokida, M., & Mandala, I. (2012). Mathematical approach of structural and textural properties

of gluten free bread enriched with carob flour. *Journal of Cereal Science,* 56(3), 603-609.

Tsatsaragkou, K., Yiannopoulos, S., Kontogiorgi, A., Poulli, E., Krokida, M., & Mandala, I. (2013). Effect of carob flour addition on the rheological properties of gluten-free breads. *Food and Bioprocess Technology,* 7 (3), 868-876.

Van Riemsdijk, L.E., Pelgrom, P.J.M., van der Goot, A.J., Boom, R.M., & Hamer, R.J. (2011a). A novel method to prepare gluten-free dough using a meso-structured whey protein particle system. *Journal of Cereal Science,* 53(1), 133-138.

Van Riemsdijk, L.E., van der Goot, A.J., Hamer, R.J., & Boom, R.M. (2011b). Preparation of gluten-free bread using a meso-structured whey protein particle system. *Journal of Cereal Science,* 53(3), 355-361.

Zandonadi, R. P., Botelho, R.B.A., Gandolfi, L., Ginani, J.S., Montenegro, F.M., & Pratesi, R. (2012). Green banana pasta: an alternative for gluten-free diets. *Journal of the Academy of Nutrition and Dietetics,* 112(7), 1068-1072.

Zannini, E., Jones, J.M., Renzetti, S., & Arendt, E.K. (2012). Functional replacements for gluten. *Annual Review of Food Science and Technology,* 3(1), 227-245.

Ziobro, R., Korus, J., Juszczak, L., & Witczak, T. (2013). Influence of inulin on physical characteristics and staling rate of gluten-free bread. Journal of Food Engineering,116(1),21-27.

Thesis objectives:

It is clear from the literature review that there is potential for fruit by-products to be used as food ingredients. In particular, the review illustrates the successful incorporation of fruit and vegetable by-products into a wide range of food products such as biscuits, cakes, sauces and meats. It also demonstrates their nutritional potential, specifically their dietary fibre contents and bioactivity. The objectives of this thesis are fourfold:

- To undertake a complete characterisation of apple (*Malus domestica* Cv. "Karmijn de Sonnaville") and orange (*Citrus sinensis* L. Cv. "Valencia") pomaces including **(chapter 2)**:
    - Physicochemical properties - particle size, water holding ability, water binding capacity, swelling capacity and oil holding capacity
    - Chemical compositional analysis - ash, dietary fibre, fat, protein, moisture, starch and minerals (calcium, magnesium, potassium, iron and zinc)
    - Pectin characterisation
    - Rheological properties - heated viscous and rheological characteristics
    - Microstructure
- To investigate the incorporation of orange pomace flour into a gluten-free bread formulation using a response surface design, studying in particular the following properties **(chapter 3)**:
    - Bread qualities - loaf volume, crumb texture, crumb moisture, crumb structure

- Dietary fibre

- To determine the role of orange pomace in the optimised gluten-free batter formulation, in particular focussing on **(chapter 4)**:
  - Batter properties - fundamental rheological characteristics
  - Cooked pasting properties - cooked starch properties
  - Microstructure
  - Sensory analysis
- To illustrate the potential novel application of apple pomace flour in a gluten-free extruded snack, with a particular focus on its effects on the following product qualities **(chapter 5)**:
  - Extrudate product characteristics - specific volume, bulk density, radical expansion ratio, moisture and porosity
  - Texture and acoustic properties
  - Cooked starch properties

Chapter 2

Nutritional and physicochemical examination of apple pomace (Malus domestica Cv. “Karmijn de Sonnaville”) and orange pomace (Citrus sinensis L. Cv. “Valencia”).

Under review in Industrial Crops and Products.

2 Chapter 2: Nutritional and physicochemical examination of apple pomace (*Malus domestica* Cv. "Karmijn de Sonnaville") and orange pomace (*Citrus sinensis* L. Cv. "Valencia").

2.1 Abstract:

Fruit and vegetable by-products remaining from processing constitute 25-40 % of the fruit. Due to their high fibre and mineral content, much of the literature has focused on their nutritional viability. Reports on food processing by-products have also highlighted their technical properties; in particular by-products have been described to contain nutritional and functional properties, water holding, binding and gelling ability.

The present study undertakes a characterisation of apple pomace from the *Malus domestica* Cv. "Karmijn de Sonnaville" cultivar and orange pomace from the *Citrus sinensis* L. Cv. "Valencia". "Valencia" is a cultivar commonly used in orange juice production and the cultivar "Karmijn de Sonnaville" is used in the production of apple juice. Analysis comprised of the composition, physicochemical properties, pectin quantity, gelling properties and microstructure of the two raw materials.

Orange pomace was found to contain a favourable nutritional composition which included high dietary fibre (40.47 %), low fat (2.14 %) and a high mineral content. Due to its pectin and starch content, apple pomace flour had the ability to form viscous pastes and visco-elastic structures.

2.2 Introduction:

Due to the manner in which fruit and vegetables are processed, a significant amount is traditionally discarded. This discarded material is usually referred to as a "by-product" or "waste". The by-product or "waste" consists of the core, peel, pips and kernel of the fruit/vegetable being processed. At present, processors may either donate these by-products to farmers for animal food or dispose of them in land fill or by incineration, at a cost to themselves. Apple and orange pomaces are two examples of by-products remaining after processing (O'Shea, Arendt & Gallagher, 2012).

Post apple juice pressing, the remaining waste can represent up to 25 % of the apple fresh weight (Rha et al., 2010). Juices processed from citrus fruits create citrus waste which can constitute up to 45-60 % of the fruit (Fernandez-Lopez et al., 2009). Although considered as waste products, these materials still contain an abundance of unexploited nutrients and bio-actives (Wijngaard, Rößle & Brunton, 2009).

The by-products usually consist of the outer layers of fruit and vegetables. These can be described as cell wall materials, which consist of fibrous substances (cellulose, hemicelluloses and water soluble pectin). These fibrous substances have high functional properties e.g. water holding, binding and gelling abilities (Vetter, Kunzek & Senge, 2001). Therefore, this would suggest that as well as having good nutritional properties they may also have great potential in food formulations.

Other than their functionality and nutrition, by-products from fruit and citrus processing also have the advantage of being gluten and lactose free, making them potentially ideal ingredients for a range of products e.g. bakery products, jams,

drinks and confectionary. Today, it is a common trend for consumers to purchase products which contain natural ingredients.

Previous authors have reported on the quantity and phytochemical properties of fibre found in orange and apple pomace (Grigelmo-Miguel & Martín-Belloso 1999; Gorinstein et al., 2001; Figuerola, Hurtado, Estévez, Chiffelle & Asenjo, 2005; Martí et al., 2010). Much research has been carried out to study the bioactive (e.g. phytochemical content) and composition (mostly fibre) of apple and orange pomace (Chau & Huang 2003; Wolfe & Liu 2003; Topuz, Topakci, Canakci, Akinci & Ozdemir, 2005). The functionality and nutritional properties of these fruit flours can vary depending on their cultivar.

This study builds on the existing knowledge of the nutritional properties of fruit pomace. It highlights the need to comprehend both the nutritional and functional characteristics of a fruit by-product prior to inclusion into a food product which previously has been understated in other studies.

In the present study, apple (*Malus domestica* Cv. "Karmijn de Sonnaville") and orange (*Citrus sinensis* L. Cv. "Valencia") pomaces were obtained from the Irish beverage industry. The main concept of the study was to illustrate the predominant compositional and functional properties of these two pomaces as potential food ingredients.

The over-arching objectives of the study were:

- To determine the compositional and functional properties of the pomaces
- To investigate their rheological and gelling characteristics, and also study their microstructure

- To establish the optimal conditions for pectin extraction, that would produce a high yield without compromising on pectin functionality

2.3 Materials and methods:

2.3.1 Orange and apple pomace preparation:

Orange pomace (Wild Orchard Drinks, Co. Limerick, Ireland) consisted of the peel and pulp remaining after juicing. Apple pomace (The Apple Farm, Co. Tipperary, Ireland) consisted of the peel, pulp and seeds, which remained after juicing. Both pomaces were received in a wet state shortly after juicing. To eliminate fermentation, the pomaces were freeze-dried immediately, milled to a flour of particle size >355 µm and stored at -20 ^{o}C until required for analysis.

2.3.2 Chemical composition:

Moisture content of the flours was analysed using a Brabender moisture oven as described by Ktenioudaki et al. (2012a).

Fat was assessed via acid hydrolysis as described by Alvarez-Jubete et al. (2009). Briefly, 2 g of sample were mixed with 2 ml of ethanol and 10 ml of hydrochloric acid. The samples were placed in a water bath overnight at 80 ^{o}C. The fat in the samples were extracted three times with the addition of three equal amounts of petroleum ether and ethyl ether. Solvents were evaporated over a water bath under a fume hood. Fat was determined gravimetrically.

Protein was determined based on the method described by Alvarez-Jubete et al. (2009). The combustion method based on the Dumas principle using a nitrogen analyser was utilised (FP-328 Leco Instrument; Leco Corporation, St Joseph, Michigan, USA). Blank and the standard compound ethylenediamine tetraacetic acid (9.57 % N) were run prior to the samples. Combustion of the samples took place in a sealed furnace at 1,150 ^{o}C. The nitrogen to protein conversion factor

used was 5.70 for the fruit flours.

Ash was measured in accordance with the AOAC method 923.03 (AOAC 2000).

The content of carbohydrate was calculated based on difference (100 - moisture - fat - protein - ash) (Hager, Wolter, Jacob, Zannini & Arendt, 2012).

Mineral content was measured by atomic absorption spectrometry on previously ashed samples. The method described by Alvarez-Jubete et al. (2009) was utilized and calcium, potassium, magnesium, zinc and iron results were obtained.

Total starch content was analysed using the Megazyme Assay procedure (K-TSTA; Megazyme, Bray, Ireland) based on the AACC 76.13 and AOAC 996.11 methods.

Total fibre analysis was determined according to the AOAC methods 985.29 (AOAC 1990).

Total sugars were measured by using a diagnostic kit by Rhône diagnostics technologies Ltd, (R-Biopharm AG, An der neuen Bergstraße 17, 64297 Darmstadt, Germany).

All experiments were performed in triplicate.

2.3.3 Particle size and hydration properties:

Particle size was determined by placing 50 g of flour on a sieve set which included the following mesh sizes: 500 µm, 355 µm and 250 µm. The sieve set was vigorously shaken and each sieve was weighed to determine the weight of the flour in each individual sieve. The weight of the contents of each sieve was calculated as a percentage of the original weight (Ktenioudaki, O'Shea & Gallagher, 2012b).

The water hydration characteristics (including water holding capacity, water binding capacity and swelling capacity) and oil absorption capacity were based on the methods illustrated by Ktenioudaki et al. (2012b). These tests were carried out in triplicate.

2.3.4 Pectin:

2.3.4.1 Pectin extraction:

The pomace flours were extracted with 0.1 M hydrochloric acid at a ratio of 1:15 w/v. Extraction occurred in an incubator shaker (KS 4000 i control, IKA®-Werke GmbH & Co. KG, Germany) at 150 rpm. To produce the largest yield while still retaining pectin functionality, extractions were carried out at two time points (three hours and seven hours). Following extraction, the extracts were filtered through a muslin bag to remove insoluble plant wall material. The supernatant was neutralised with 10 M sodium hydroxide and then centrifuged (Sigma 6K10, Sigma Laborzentrifugen GmbH, Germany) at room temperature for 10 minutes at 9000 g. It was then blast frozen, freeze-dried and stored at -20 ^{o}C until purification.

Purification of the pectin (derived from the pomace flours) was carried out by ethanol precipitation. Briefly, pectin was dissolved in deionized water in a 60 ^{o}C water bath. The pectin solution was precipitated with ethanol at a ratio of 1:5 and centrifuged (10 minutes by 9000 g), the supernatant was subsequently discarded. To remove ethanol residues the pectin pellet was re-suspended into deionized water, freeze dried and stored at -20 ^{o}C. Pectin content was determined gravimetrically (% DM).

2.3.4.2 Pectin yield and characterisation:

Pectin was further purified to remove simple sugars for nuclear magnetic resonance (NMR) analysis. This was carried out by dissolving the pectin derived from the pomace flours in deionized water in a 60 ^{o}C water bath. The solution was precipitated with a 1:5 extract to acetone ratio and centrifuged (10 minutes by 9000 g). The supernatant was discarded and the remaining pellet was re-suspended in deionized water and freeze-dried (Cuddon FD 80 model Leanne).

Degree of methoxylation: 25 mg of purified pectin was dissolved in 1 ml of deuterated water (D_2O). To aid solubilisation of the pectin, it was placed in a heating block at 60 ^{o}C for 5 minute intervals. The solution was then centrifuged at 12,000 g for 1 minute. The supernatant was placed into 5 mm diameter NMR tubes for measurement. ^{1}H NMR experiments were carried out on a Bruker Avance III 500 MHz spectrometer (Bruker UK Ltd, Coventry, UK) and a 5 mm BBO probe with a transmitter frequency of 500.13 Hz for protons. The pulse programme used was noesypr1d with pre-saturation during relaxation delay and mixing; acquisition mode was DQD. Eight scans and 4 dummy scans were used with a relaxation delay of 10 s and a mixing time of 0.01 s. Spectra were referenced to the residual water signal. All spectra were obtained at 298 K. The NMR processing and acquisition software used was TOPSPIN 2.1 (version 2.1.4, Bruker Biospin, Germany). The integrals of the peaks between 4.51 and 4.62 ppm and 4.90 and 5.27 ppm were used to determine the degree of methylation following the method outlined in Rosenbohm et al. (2003). NMR experiments were executed in triplicate.

Methoxyl content: The methoxyl content of the pectin derived from apple and orange flour was measured as described by Ranganna (1986). Briefly, 5 ml of ethanol and 1 g of sodium chloride (for sharpening the endpoint) were added to 500 mg of pectin extract. 100 ml of deionized water and 6 drops of phenol red indicator were also added and the solution was swirled vigorously at room temperature to dissolve the pectin substance. The pectin solution was then titrated with 0.1 mol/L sodium hydroxide to the endpoint (colour change of pink which persisted for 60 seconds / pH 7.5). 25 ml of 0.25 mol/L of sodium hydroxide was added to the titrated solution. The flask was stoppered and shaken thoroughly for 20 seconds and allowed to stand for 30 minutes at room temperature. 25 ml of 0.25 mol/L hydrochloric acid was included to neutralize the solution. The neutralized solution was titrated with a 0.1 mol/L NaOH till the end point was reached. The methoxyl content was calculated as follows:

$$\frac{\text{ml of alkali} \times \text{molarity of alkali} \times 3.1}{\text{Wt of sample}}$$

Equation 2-1: Methoxyl content (%).

The whole experiment was carried out in triplicate.

2.3.5 Viscous properties:

The viscous properties of the pomace flours were determined using a Rapid-Visco-Analyser (RVA-4D, Newport Scientific, Sydney, NSW, Australia). The recommended adjusted water levels were used. Initially the test had a stirring speed of 960 rpm for 10 seconds to ensure the slurry was well mixed; the

remainder of the test had a stirring speed of 160 rpm. The test profile lasted for 13 minutes. It had a starting temperature of 50 ^{o}C which was held for 1 minute, raised to 95 ^{o}C over 3.7 minutes, held for 2.5 minutes, cooled to 50 ^{o}C over 3.8 minutes and held for 2 minutes. The following results were obtained from the pasting curve: Peak viscosity (highest viscosity during heating (cP)), trough viscosity (lowest viscosity after cooling (cP)) and final viscosity (maximum viscosity after the temperature had returned to 50 ^{o}C (cP)) (Sullivan, O'Flaherty, Brunton, Arendt & Gallagher, 2011). This test was performed in triplicate.

2.3.6 Fundamental rheology:

2.3.6.1 Oscillation:

Rheological measurements were performed on the two fruit samples (apple pomace and orange pomace) on a controlled stress rheometer (Anton Paar GmbH, Graz, Austria) fitted with parallel plates consisting of a 50 mm serrated probe and 50 mm serrated base plate.

Pastes (with adjusted water) were developed using the Rapid-Visco-Analyser (RVA-4D, Newport Scientific, Sydney, NSW, Australia), stirred for ten seconds at 960 rpm (for initial mixing) and then at 160 rpm for five minutes and 50 seconds at 25 ^{o}C.

Approximately 2 g of sample were placed onto the base plate, and the upper plate was brought to a gap of 1.025 mm where excess sample was carefully trimmed away. The outer edge of the sample was lightly covered with petroleum jelly to prevent the sample from drying out. The plate was then lowered to a test gap of

1 mm and testing began. The whole system was covered using a peltier hood, with a temperature setting of 25 ^{o}C (Sullivan et al., 2011).

2.3.6.2 Frequency sweep:

Following a rest time of five minutes, (to allow the sample to relax), the frequency was increased from 0.1 to 10 Hz under a strain (γ), which had been previously identified from an amplitude sweep and was found to be within the linear viscoelastic range (LVR) (0.1 % γ). The frequency sweep was performed in triplicate at a temperature of 25 ^{o}C. Eleven points were measured and from the mechanical spectrum the viscoelastic properties were measured (Ktenioudaki et al., 2012b).

2.3.7 Microstructure:

2.3.7.1 Light microscopy of iodine stained samples:

Approximately 0.5 g of pomace were sprinkled onto a microscope slide and one drop (50 µl) of 1 % w/v iodine in 2 % potassium iodine solution (aq) added with a coverslip placed on top. Starch appears as blue/black particles. To stain polysaccharide/protein, samples were also stained with toluidine blue which is a metachromatic dye staining polysaccharides pink/purple and protein blue (OlgaáFlint 1990). The slides were examined using an Olympus BX51 light microscope (Mason Technology, Dublin) fitted with x 10 or x 20 objectives using either bright field or polarised light with slightly uncrossed polars to highlight birefringent material. Digital colour images (8 bit, TIFF format) were

acquired.

2.3.7.2 Scanning electron microscopy:

Pomace samples were sprinkled onto a carbon adhesive coated stub and sputter coated with chromium. Samples were examined in a Zeiss Supra 40 VP field emission electron microscope (Carl Zeiss, Cambridge, UK) operating at 2 kV. Digital 8-bit TIF images were acquired at a range of magnifications from x 250 to x 5,000.

2.4 Results and discussion:

2.4.1 Chemical composition:

Table 2-1: Chemical composition of apple pomace flour and orange pomace flour.

Composition	Apple Pomace	Orange Pomace
Moisture (%) DM*	9.00 +/- 0.33	10.55+/- 0.15
Fat (%) DM	2.53 +/- 0.26	2.14 +/- 0.33
Protein (%) DM**	2.65 +/- 0.14	6.81 +/- 0.29
Ash (%) DM	1.79 +/- 0.02	4.18 +/- 0.08
Carbohydrate (%)	84.76 +/- 0.56	77.78 +/- 0.67
Starch (%)	5.6 +/- 0.0	3.4 +/- 0.0
TDF (%) DM	30.15 +/- 3.08	40.47 +/- 1.20
Calcium (mg/L)	1265 +/- 142	4112 +/- 414
Potassium (mg/L)	2531 +/- 284	4112 +/- 414
Magnesium (mg/L)	126 +/- 14.22	570 +/- 102
Zinc (mg/L)	1.66 +/- 0.62	2.28 +/- 0.41
Iron (mg/L)	8.39 +/- 1.22	9.76 +/- 0.88
Total sugars	54.2 +/- 0.0	25.0 +/- 0.0

* Dry matter
****** Nitrogen to protein conversion factor was 5.7.

Apple pomace: Table 2-1 illustrates the chemical composition and mineral content of apple pomace (AP) flour from the cultivar “Karmijn de Sonnaville”. As expected, this fruit flour was low in fat and protein. It was also found to be fibrous, containing 30.15 % total dietary fibre, dry matter (DM).

The carbohydrate content of the AP flour was 84.76 %; such a result is to be expected considering the majority of the pomace constitutes the cell wall

material of the apple peel. This is similar to the carbohydrate content (82.2 % DM) of apple pomace as described by Grigelmo-Miguel & Martin-Belloso (1999). Figuerola et al. (2005) reported the apple pomace residues from the "liberty" and "granny smith" cultivars to contain 89.8 % and 60.7 % total dietary fibre (TDF) respectively.

The apple pomace also contained a high level of minerals, in particular the macro-mineral potassium (2531 mg/L) and the minor mineral iron (8.39 mg/L). In comparison, Gorinstein et al. (2001) observed the whole fruit of the "Lobo" cultivar to contain a lower level of potassium (81.9 mg/L) and iron (0.094 mg/L).

Orange pomace: The composition and mineral content of orange pomace (OP) flour "Valencia" is seen in Table 2-1. It had a higher than expected level of protein (6.81 %). The OP flour also contained a high level of total dietary fibre, 40.63 % DM. Chau & Huang (2003) found the TDF of "Liucheng" to be 57.00 % DM.

OP flour had an ash content of 4.18 % DM, demonstrating its potential to contain minerals. From Table 2-1 it can be seen that OP flour contains an abundant quantity of macro minerals e.g. calcium (4112 mg/L), magnesium (570 mg/L) and potassium (4112 mg/L). In comparison, the orange cultivar "Alanya" contained 94 mg/L calcium, 102 mg/L magnesium and 1364 mg/L potassium (Topuz, Topakci, Canakci, Akinci, & Ozdemir, 2005). Figuerola et al. (2005) studied the compositional contents of the cultivar "Valencia". These authors reported higher results for total dietary fibre (64.3 % DM), but similar results for protein (6.70 %) and lower results for lipid (0.89 % DM) and ash (2.71 % DM) contents. The compositional differences are most likely attributed to different

growing conditions and maturity of the fruit (Sturm, Koron & Stampar, 2003).

2.4.2 Physicochemical properties:

Apple pomace: The particle size of the apple pomace (AP) flour is shown in Table 2-2. The AP mainly consisted of particles less than 250 µm in size, with only a slight proportion (8.27 %) greater than 250 µm.

The hydration properties of AP flour are reported in Table 2-2; AP flour had an oil binding capacity of 0.84 g/g DM; this is a similar finding to that of Figuerola et al. (2005) who reported the cultivar "Royal Gala" to contain an oil adsorption capacity of 0.95 g/g. The AP flour investigated in this study also displayed a large water hydration capacity of 3.47 ml/g which is comparable to a commercial standard for apple fibre ingredient (3.85 g/g) (Rosell, Santos & Collar, 2009).

Table 2-2: Physiochemical characteristics of apple pomace flour and orange pomace flour.

Functional Property	Apple Pomace	Orange Pomace
Particle Size		
>355 µm (%)	0.17	0.33
>250 µm (%)	8.27	2.80
<250 µm (%)	91.57	96.87
Oil Binding Capacity (g/g)	0.84 +/- 0.01	1.06 +/- 0.02
Swelling Capacity (ml/g)	12.80 +/- 0.69	8.13 +/- 0.23
Water Holding Capacity (ml/g)	12.25 +/- 1.01	8.54 +/- 0.44
Water Hydration Capacity (ml/g)	3.47 +/- 0.33	4.40 +/- 0.23

The nature of the fibrous properties of the pomace flours governs their application and functionality in a food system. Soluble fibre i.e. gums and

pectin, have shown to possess a high hydration capacity, water holding ability, form viscous solutions and both absorb and maintain other substances e.g. non polar molecules i.e. fat, sugars and minerals. Insoluble fibres (i.e. cellulose and hemi-cellulose) demonstrate similar functional properties within their matrix however unlike soluble fibre, this type of fibre is not able to form viscous solutions (Lecumberri et al., 2007).

Orange pomace: The majority of the orange pomace (OP) flour was found to have a particle size less than 250 µm (96.87 %), with only a slight proportion greater than 250 µm (Table 2-2).

Similar results to Figuerola et al. (2005) were observed for the cultivar "Valencia", where by OP flour had a slightly higher swelling capacity of 8.13 ml/g and a slightly lower oil binding capacity of 1.06 g/g.

OP flour had a high retention capacity of 4.40 ml/g. This is usually related to the functionality and type of fibre present. As described in the compositional analysis section, OP flour contains a high quantity of total dietary fibre (40.63 % DM); therefore this can be related to its high retention capacity (Lecumberri et al., 2007).

2.4.3 Pectin yield and characterisation:

Within plants, pectin plays two roles: it cements the cellulosic network and acts as a hydrating agent (Thakur, Singh, Handa & Rao, 1997). Pectin has been shown to have practical technical advantages i.e. emulsion formulation, gelling and thickening properties (Bhushan, Kalia, Sharma, Singh & Ahuja, 2008). Presently in the food industry, pectin has a wide range of applications, including jams, bakery products, confectionery and beverages (Thakur et al., 1997).

In the present study, extraction of pectin was carried out at two time points. The first time point (3 hours) had the aim of extracting a high yield of pectin without compromising on the functionality of the extracted pectin. A second extraction (7 hours) was prepared to quantify the amount of pectin present in the fruit pomace flours.

2.4.3.1 Pectin yield:

Apple pomace: Table 2-3 shows the quantity of pectin present in apple pomace flour at 3 hours and 7 hours (8.04 % DM and 8.54 % DM).

The pectin content of apple pomace can vary depending on extraction method and cultivar. Results for yield of pectin obtained in this study are within the values (3.50-14.32 %) for apple pectin reported by Bhushan et al. (2008). Garna et al. (2007) investigated the pectin content of apple pomace which contained a number of apple varieties. These authors used similar extraction conditions to those investigated in this study and reported the maximum pectin yield to be 8.9 % DM.

Orange pomace: Orange pomace flour contained a pectin content of 18.69 % DM at 3 hours and 20.56 % DM at 7 hours. Kar & Arslan (1999) obtained a

pectin yield of 29.58 % for "Washington Navel" orange cultivar. It has been illustrated previously that the variety and stage of orange maturity dominate the quality and quantity of pectin extracted from the fruit (Kar & Arslan 1999).

Table 2-3: Pectin characterisation of apple pomace flour and orange pomace flour.

Pectin Analysis	Apple pomace	Orange Pomace
3 hours extraction yield (%) DW**	8.04 +/- 0.08	18.69 +/- 0.65
7 hours extraction yield (%) DW	8.54 +/- 0.25	20.56 +/- 0.49
Moisture content (%)	9.07 +/- 0.51	10.73 +/- 0.45
Degree of methylation 3 hrs (%)	56.38 +/- 3.05	53.77 +/- 0.59
Degree of methylation 7 hrs (%)	39.64 +/- 1.23	38.90 +/- 1.44
Methoxyl content 3 hrs (%)	4.90 +/- 0.19	4.41 +/- 0.20
Methoxyl content 7 hrs (%)	4.11 +/- 0.35	3.65 +/- 0.29

[*]Pectin standards – apple pectin and citrus pectin where found to have the following methoxyl content (7.84%, 7.36%) and degree of methylation (78%, 81%) respectively.

[**] Dry matter (DM).

2.4.3.2 Pectin characterisation: Degree of methylation & methoxyl content

The gelling properties of pectin are largely dependent on pH, molecular weight and concentration of solutes, but in particular the degree of methylation. This is described as the ratio of methylated D-galacturonic acid groups to the total D-galacturonic acid groups (Sriamornsak 2003). Selection and application of pectin in commercial food products is chosen based on this classification i.e. highly-methoxyl (HM) pectin (60-75 %) and low-methoxyl (LM) pectin (20-40 %) (Sriamornsak 2003). HM pectin form gels in an acidified environment in the presence of sugar (e.g. sucrose / glucose) whereas LM pectin form gels in the

presence of calcium ions. Degree of methylation can vary based on the maturity of the fruit and the pectin extraction method (Sriamornsak 2003).

The methoxyl content is another parameter which describes the functionality of the extracted pectin. In the parent protopectin (located in the plant prior to extraction), its methoxyl content has been described as 16.3 % and in commercial pectin this value has been discussed as 8 % (a HM pectin) (Kertesz 1951). This parameter describes the percentage of methoxyl group in relation to the whole structure.

The characterisation of pectin was carried out using the method described by Rosenbohm et al. (2003). It proved to be a rapid and reproducible method that provided results within the expected range with minimally sample preparation required. The pectin samples examined gave similar spectra to those described by Rosenbohm et al. (2003). The method investigated the protons located adjacent to the methylated carboxylic acid in comparison to the protons found adjacent to the free carboxylic acid. This can be seen in Figure 2-1 a & b where the signals for the proton next to the methylated carboxylic acid, that is proton H-5, (4.90-5.27 ppm) and protons positioned next to non-methylated carboxylic acid (4.51-4.62 ppm) were studied. The anomeric proton, H-1 also overlaps with the signal for the H-5 adjacent to methylated carboxylates, however the method by Rosenbohm et al. (2003) allows the integration of the signals to be used to accurately determine the degree of methylation.

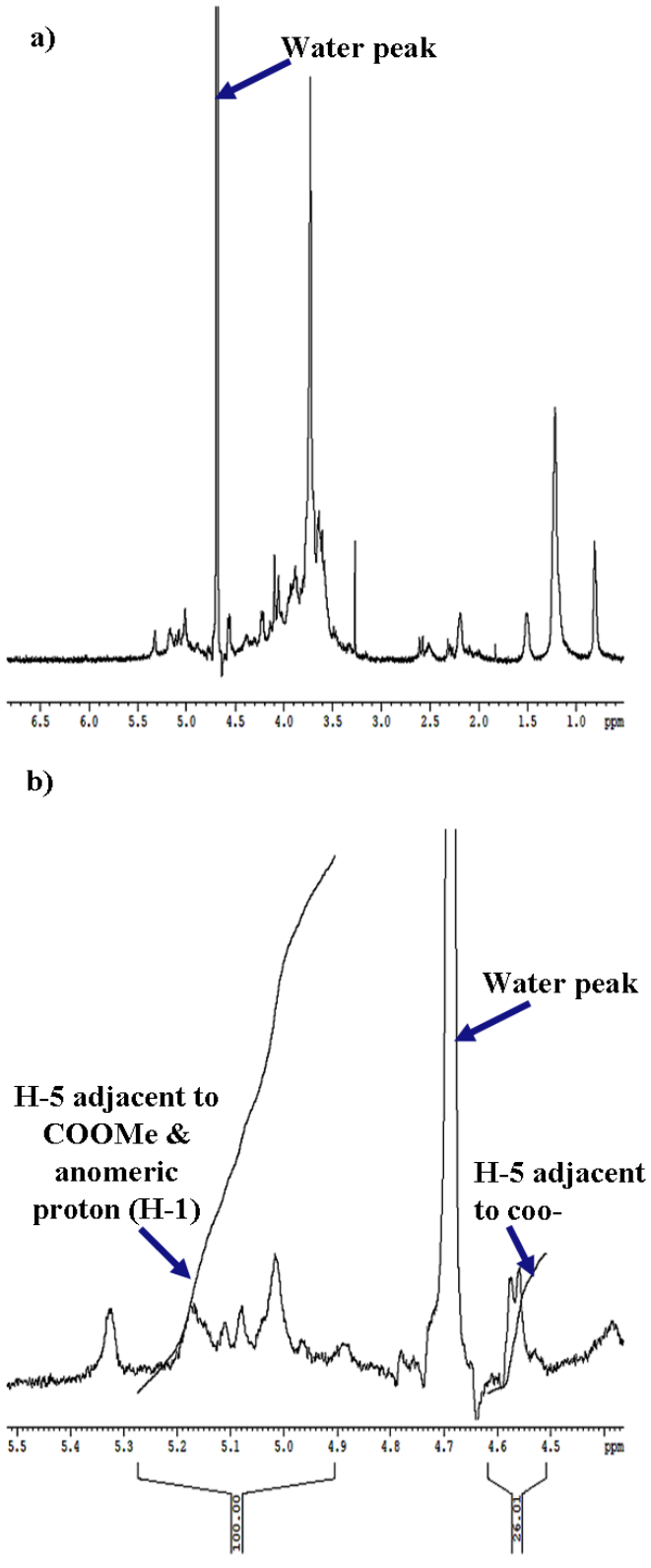


Figure 2-1: Degree of methylation from the ^{1}H NMR spectrum of apple pomace pectin at 3 hours (a) and with integrals (b).

Pectin from apple pomace: Table 2-3 illustrates the degree of methylation, which was found to decrease with increasing extraction time. Apple pomace pectin at three hours contained a degree of methylation of 56 %. Commercial pectin derived from acidic extractions has been described as containing a degree of methylation equivalent to 60 % (Endress, Christensen, Phillips & Williams, 2009). Hence the extraction treatment chosen developed a high yield while maintaining an acceptable level of functionality in comparison to commercial standards. As expected, the methoxyl content of apple pomace was in agreement with the results received for the degree of methylation, where it was found to have a methoxyl content of 4.9 %.

This illustrates high methoxyl pectin which would be suitable in the production of slow set gels such as desert jellies.

Pectin from orange pomace: OP pectin from a three hour extraction (Table 2-3) resulted in a degree of methylation of 39.6 % (Figure 2-2 a & b) which classifies it as low methoxyl pectin.

This reduced level of methylation is most likely as a result of the extraction method in combination with the source of pectin and maturity of the fruit.

The methoxyl content of orange pomace pectin was also investigated and it was found to contain 4.1 % methoxyl hence supporting the findings received for the degree of methylation. As previously discussed, LM-pectin gel in the presence of calcium ions and they have also been described as forming thermo-reversible gels. The applications of LM-pectin gels include improving the mouth-feel of products and in the development of low sugar products.

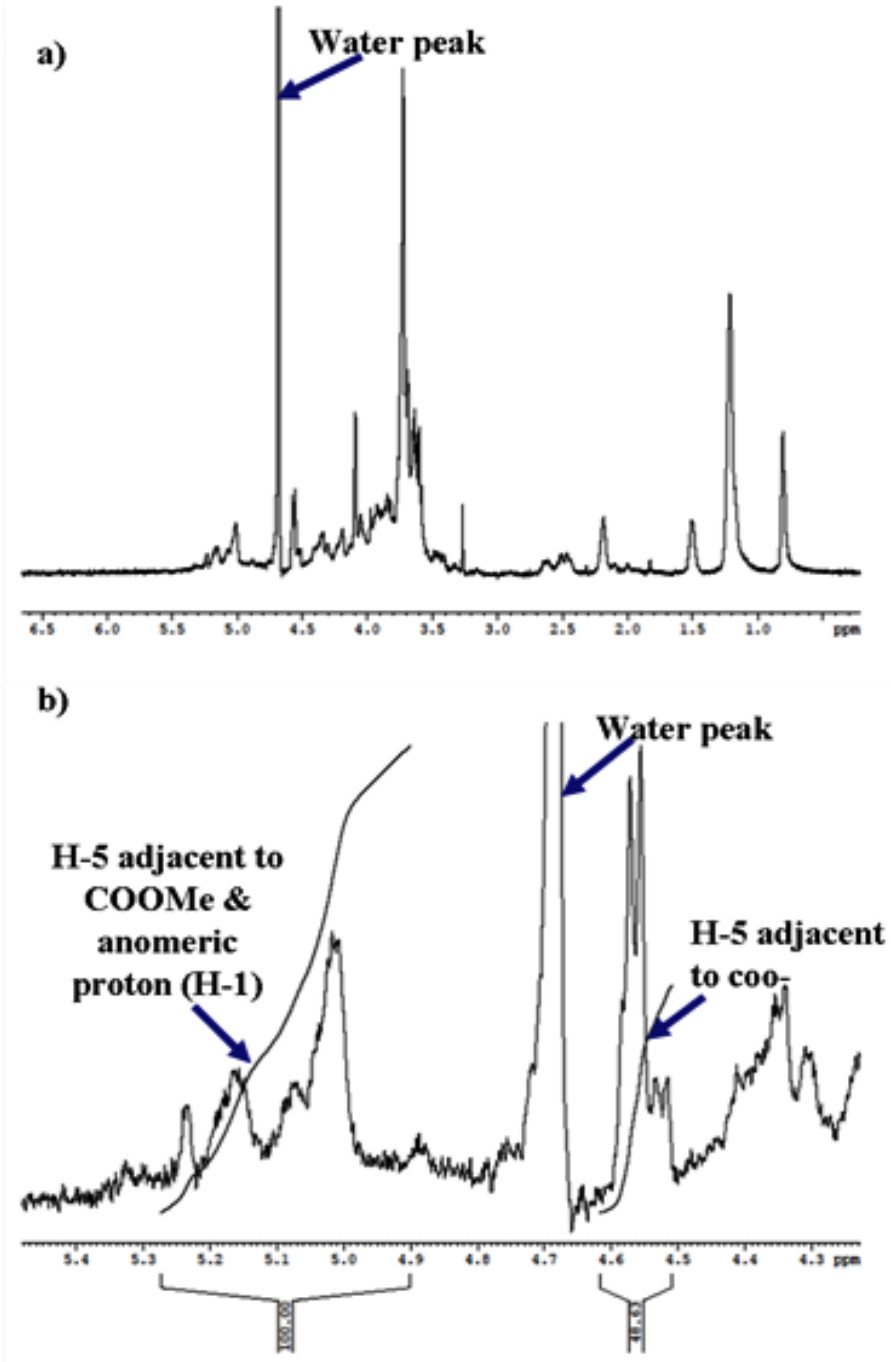


Figure 2-2: Degree of methylation from the ^{1}H NMR spectrum of orange pomace pectin at 3 hours (a) and with integrals (b).

2.4.4 Viscous properties:

Apple pomace: Results from Table 2-4 illustrate the heated viscous characteristics of apple pomace (AP) flour. It had a high peak viscosity (413 cP), trough viscosity (392 cP) and in particular final viscosity (688 cP).

From the compositional analysis, AP flour had a starch content of 5.6 %. The authors reported in the previous section that apple pomace contained a pectin content of 8.54 % DM, and also a high degree of methylation. Pectin with a high degree of methylation is known to increase the gelling ability of a solution (Endress et al., 2009).

It is likely that a synergetic effect occurred between the low of starch and pectin in the apple pomace to create high peak viscosity. The trough viscosity refers to the viscosity created upon cooling. Generally, a lower trough viscosity signifies a reduced level of starch present.

Table 2-4: Rapid-Visco-Analysis of apple pomace flour and orange pomace flour.

RVA characteristics	Apple Pomace	Orange Pomace
Peak Viscosity (cP)	413 +/- 0.58	176 +/- 7.00
Trough Viscosity (cP)	392 +/- 1.53	165 +/- 5.20
Breakdown (cP)	20.67 +/- 2.08	11.00 +/- 2.00
Final Viscosity (cP)	688 +/- 3.79	284 +/- 9.24
Setback (cP)	295 +/- 4.51	119 +/- 4.04

It also illustrates a weak viscosity (usually associated with the original nature of the starch) that cannot withstand high stirring or heat resulting in breakdown in

viscosity (O'Shea, Doran, Auty, Arendt & Gallagher, 2013). This significant reduction in trough viscosity reported in Table 2-4 is to be expected considering the low concentration of starch granules present in the slurry. Finally, the high final viscosity parameter is described as the ability of starch to re-associate itself post heating and upon cooling. In the case of AP flour, it does not contain a high level of starch but it does contain pectin and sugar (54.2 %); sugar is required for pectin to form gel. Therefore, it is most likely a combination of pectin (with a high degree of methylation released during the heating step (Kertesz 1951) combined with starch in the presence of sugars to form a gel/paste during the cooling step.

Orange pomace: The cooked viscosity results for the orange pomace (OP) flour where low. Peak viscosity, trough viscosity and final viscosity of the OP flour slurries were observed to be 176 cP, 165 cP and 284 cP respectively, illustrating a weak, almost liquid-like viscosity. It was reported in Tables 1 & 2 that OP flour contained a minimal starch content of 3.4 %, a total fibre content of 40.47 % DM, a pectin content of 20.56 % DM and sugar content of 25.0 %. The cooked viscosity results shown in Table 2-4 were most likely generated as a result of the high fibre and low sugar and starch contents of the OP flour. Although OP contained a high level of pectin, it possessed a lower degree of methylation compared to other pectin sources (e.g. apple pectin).

Ingredients which are highly fibrous in nature are known to reduce the pasting properties of starchy ingredients due to their high water holding and binding ability. The presence of fibre also reduces the ability of the starch to swell and eventually burst, which would then develop a liquid like paste on cooling.

Ktenioudaki et al. (2012b) described the viscous characteristics of brewer's spent grain (a fibrous by-product from the brewing industry). Their study observed a high reduction in heated viscosity parameters. They attributed these results to the low starch content found in brewer's spent grain in combination with the high fibre content, developing poor heated viscous characteristics.

2.4.5 Oscillatory rheology:

Apple pomace: The rheological spectra, depicted apple pomace (AP) flour slurry to have an increasing storage modulus (G') and complex modulus (G*), which was frequency dependant (it increased with increasing frequency). The G' was observed to be higher the G" (Data not shown). G' is the elastic component, which specifies how much energy the sample retains; G* is a measure of how stiff the sample is (Gunasekaran & Ak 2000). Hence, AP flour slurry was observed to have a visco-elastic like nature.

Table 5 illustrates AP flour slurry had a high complex viscosity (η*) (402 Pa) at 0.1 Hz, however as the frequency increased to 10 Hz, η* rapidly decreased (8.80 Pa). The complex viscosity is described as the viscoelastic flow resistance of the sample (Mezger 2006). As can be seen from Table 5, when the G' (elastic modulus) and as consequence the G* became inflexible and rigid, the ability of the slurry to flow decreased confirming the visco-solid behaviour of the sample. The increased storage of energy (G') and the reduction in the ability of AP flour slurry to flow (η*) may be attributed to the characteristics of the fibre present in the pomace. As discussed previously, fibres which contain soluble fibre have the

ability to develop viscous solutions. AP flour has also been shown in this study to contain a high level of sugar and pectin with a high functionality, which potentially increased the elasticity of the slurry as a function of increasing frequency. Similar results were also shown in the heated viscosity properties that illustrated an increase in peak viscosity and final viscosity. Finally, the degree of methylation of pectin in AP flour was almost 60 %, demonstrating its increased ability to form gels in the presence of sugars.

Orange pomace: It is seen from Table 5, that applying frequency to the non-heated OP flour slurry developed a visco-solid like behaviour. This was observed from the storage modulus (G') being higher than then loss modulus (G'') (data not shown). Consequently, due to the high storage modulus, a high complex modulus was also generated. The effects of frequency was not as obvious with the OP flour slurry, as only slight increases in G' and G* can be noted with increasing frequency. This illustrates the rigidity and firmness of the material.

The elastic properties of OP flour may be attributed to the fibrous properties (in particular potentially the insoluble fraction) of the pomace flour. The heated viscosity properties confirm these findings as it did not produce large peak and final viscosities.

Table 2-5: Rheological parameters of apple pomace flour and orange pomace flour.

Rheological Parameter	Frequency (Hz)	0.1	1	10
Complex Modulus (G*) (Pa)	**Apple Pomace**	252 +/- 66.15	372 +/-128	553 +/- 266
	Orange Pomace	62.90 +/- 7.98	98.93 +/- 12.60	173 +/- 22.59
Storage Modulus (G') (Pa)	**Apple Pomace**	241 +/- 57.27	358 +/- 112	515 +/- 219
	Orange Pomace	59.47 +/- 7.38	94.30 +/- 11.25	159 +/- 20.50
Complex Viscosity (η*) (Pa)	**Apple Pomace**	402 +/- 105	59.20 +/- 20.45	8.80 +/- 4.24
	Orange Pomace	85.43 +/- 22.55	44.33 +/- 50.83	42.47 +/- 56.34

2.4.6 Microstructure of the pomaces:

Apple pomace: Figure 2-3 illustrates the light micrographs and scanning electron microscopy (SEM) images of apple pomace flour. Figure 2-3 a (500 µm) and in particular Figure 2-3 b (200 µm) under closer magnification suggest the presence of starch granules. These can be recognised as the dark particles. This is further confirmed in the SEM images in Figure 2-3 c (10 µm) and 3 d (2 µm). Starch granules can be clearly seen as indicated by white arrows in Figure 2-3 d. A wide variation in particle size was observed, from < 5 µm to 500 µm. These particles were perceived to be of irregular shape with small starch granules of approximately 5-10 µm in diameter.

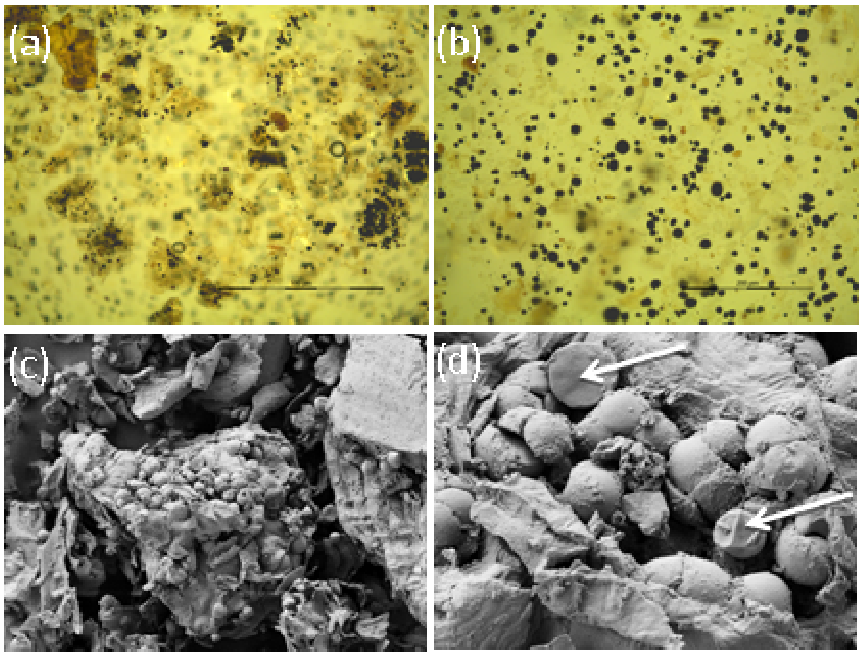


Figure 2-3: Light micrographs and SEM images (10 µm, 2 µm) of apple pomace flour.

As previously discussed, apple pomace was found to contain 5.6 % starch (Table 2-1) and the presence of starch in apple pomace was demonstrated to play an important role in creating a viscous paste and increased elasticity properties. Therefore the verification of the presence of starch in apple pomace from the microscopy images, confirms the results found in both the compositional analysis and viscosity properties.

Orange pomace: The light micrographs of orange pomace flour illustrated in Figure 2-4 demonstrate that the main element found in orange pomace is fibre. This can be clearly observed from Figure 2-4 b, whereby fibrous birefringent particles were observed. It is also clear from Figure 2-4 a & b the absence of any other components in particular protein and starch demonstrating how fibrous in nature orange pomace flour is.

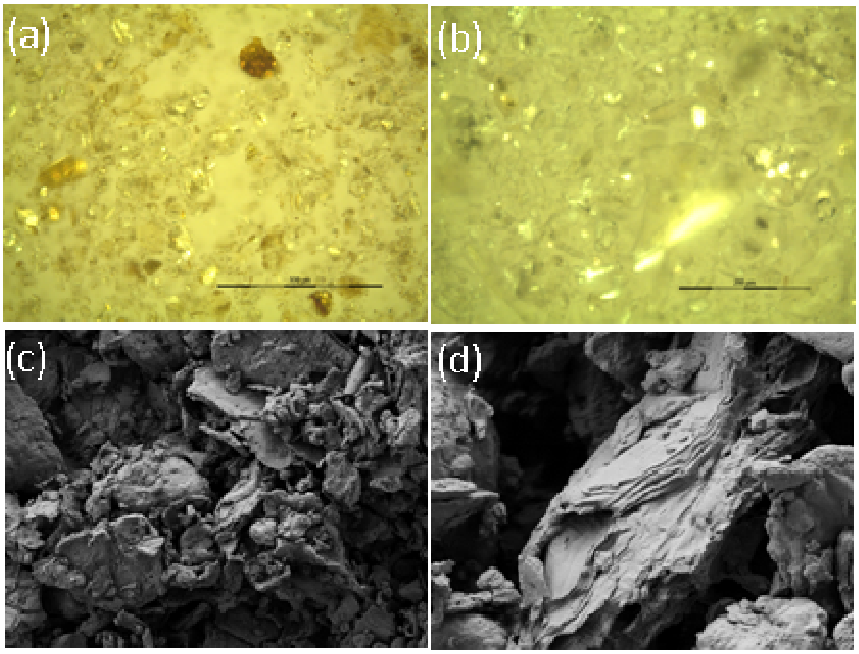


Figure 2-4: Light micrographs and SEM images (10 µm, 2 µm) of orange pomace flour.

These observations are further confirmed from SEM images in Figure 2-4 c & d, where the only visual constituent of orange pomace flour is fibre. The particles highlighted in Figure 2-4 c & d can be depicted as laminar in structure. A laminar structure is consistent with an insoluble fibre which has been described as being linear with no irregularities or branches in its structure (Thebaudin, Lefebvre, Harrington & Bourgeois, 1997).

As fibre was highlighted as the only visible constituent of orange pomace flour, it supports the previous results relating to the reduced viscous and elastic properties of the orange pomace.

2.5 Conclusion:

This study examined the potential of fruit by-products as possible novel food ingredients. The investigation set out to determine the main nutritional, functional, visco-elastic and microstructural properties of two fruit by-products (apple pomace and orange pomace) from known cultivars.

One of the more significant findings to emerge from this study is the functional dominance of fibre present in the fruit pomace. Apple pomace was found to contain a significant amount of functional pectin which aided in the development of a viscous paste and a more visco-elastic structure. Such a result would suggest that it would be more appropriate for inclusion in products such as jellies and baker's jellies.

The application of orange pomace would be more beneficial in products that required an improved water holding and binding capacity.

This work has contributed to existing knowledge of apple and orange pomaces by providing more detailed information about the functionality and composition of their cultivar.

2.6 References:

AOAC. 1990. Official methods of analysis. 15th ed. Arlington, VA: Association of Official Analytical Chemists.

AOAC (2000). AOAC method No 923.03. Official methods of analysis. 15th ed. Arlington, VA: Association of Official Analytical Chemists.

Alvarez-Jubete, L., Arendt, E., & Gallagher, E. (2009). Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. *International Journal of Food Sciences and Nutrition*, 60(s4), 240-257.

Bhushan, S., Kalia, K., Sharma, M., Singh, B., & Ahuja, P.S. (2008). Processing of apple pomace for bioactive molecules. *Critical Reviews in Biotechnology*, 28(4), 285-296.

Chau, C.F., & Huang, Y.L. (2003). Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of Citrus sinensis L. cv. Liucheng. *Journal of Agricultural and Food Chemistry*, 51(9), 2615-2618.

Endress, H., Christensen, S., Phillips, G., & Williams, P. (2009). Pectins. *Handbook of hydrocolloids*, 274-297.

Fernandez-Lopez, J., Sendra-Nadal, E., Navarro, C., Sayas, E., Viuda-Martos, M., & Alvarez, J. A. P. (2009). Storage stability of a high dietary fibre powder from orange by-products. *International Journal of Food Science and Technology*, 44(4), 748-756.

Figuerola, F., Hurtado, M.L., Estévez, A.M., Chiffelle, I., & Asenjo, F. (2005). Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food Chemistry*, 91(3), 395-401.

Garna, H., Mabon, N., Robert, C., Cornet, C., Nott, K., Legros, H., Wathelet, B.,

& Paquot, M. (2007). Effect of extraction conditions on the yield and purity of apple pomace pectin precipitated but not washed by alcohol. *Journal of Food Science,* 72(1), C001-C009.

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M., Weisz, M., Trakhtenberg, S., & Martin-Belloso, O. (2001). Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. *Journal of Agricultural and Food Chemistry,* 49(2), 952-957.

Grigelmo-Miguel, N., & Martín-Belloso, O. (1999). Comparison of dietary fibre from by-products of processing fruits and greens and from cereals. *Lebensmittel-Wissenschaft und-Technologie,* 32(8), 503-508.

Gunasekaran, S., & Ak, M.M. (2000). Dynamic oscillatory shear testing of foods—selected applications. *Trends in Food Science & Technology,* 11(3), 115-127.

Hager, A.-S., Wolter, A., Jacob, F., Zannini, E., & Arendt, E.K. (2012). Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. *Journal of Cereal Science,* 56, 239-247.

Kar, F., & Arslan, N. (1999). Effect of temperature and concentration on viscosity of orange peel pectin solutions and intrinsic viscosity–molecular weight relationship. *Carbohydrate Polymers,* 40(4), 277-284.

Kertesz, Z.I. (1951). *The pectic substances* (Vol. 1): Interscience Publishers New York.

Ktenioudaki, A., Chaurin, V., Reis, S.F., & Gallagher, E. (2012a). Brewer's spent grain as a functional ingredient for breadsticks. *International Journal of Food Science & Technology,* 47(8), 1765-1771.

Ktenioudaki, A., O'Shea, N., & Gallagher, E. (2012b). Rheological properties of wheat dough supplemented with functional by-products of foodprocessing: Brewer's spent grain and apple pomace. *Journal of Food Engineering*, 116, 362-368.

Lecumberri, E., Mateos, R., Izquierdo-Pulido, M., Rupérez, P., Goya, L., & Bravo, L. (2007). Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (Theobroma cacao L.). *Food Chemistry*, 104(3), 948-954.

Martí, N., Saura, D., Fuentes, E., Lizama, V., García, E., Mico-Ballester, M.J., & Lorente, J. (2010). Fiber from tangerine juice industry. *Industrial Crops and Products*, 33(1), 94-98.

Mezger, T.G. (2006). Oscillatory tests. In: Mezger, T.G. (Eds.), *The rheology handbook: for users of rotational and oscillatory rheometers.* Vincentz Network GmbH & Co KG: Hannover, Germany, pp. 53-77.

OlgaáFlint, F. (1990). Micro-technique for the identification of food hydrocolloids. *Analyst*, 115(1), 61-63.

O'Shea, N., Arendt, E.K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science & Emerging Technologies*, 16(0), 1-10.

O'Shea, N., Doran, L., Auty, M., Arendt, E., & Gallagher, E. (2013). The rheology, microstructure and sensory characteristics of a gluten-free bread formulation enhanced with orange pomace. *Food & Function*, 4, 1856-1863.

Ranganna, S. (1986). Pectin. In: Ranganna, S. (Eds.), *Handbook of analysis and quality control for fruit and vegetable products*. Tata McGraw-Hill publishing

company Ltd: New Delhi, India, pp. 31-65.

Rha, H.J., Bae, I.Y., Lee, S., Yoo, S.H., Chang, P.S., & Lee, H.G. (2010). Enhancement of anti-radical activity of pectin from apple pomace by hydroxamation. *Food Hydrocolloids,* 25(3), 545-548.

Rosenbohm, C., Lundt, I., Christensen, T.I., & Young, N.G. (2003). Chemically methylated and reduced pectins: preparation, characterisation by ^{1}H NMR spectroscopy, enzymatic degradation, and gelling properties. *Carbohydrate research*, 338(7), 637-649.

Rosell, C.M., Santos, E., & Collar, C. (2009). Physico-chemical properties of commercial fibres from different sources: A comparative approach. *Food Research International*, 42(1), 176-184.

Sriamornsak, P. (2003). Chemistry of pectin and its pharmaceutical uses: A review. *Silpakorn University International Journal,* 3(1-2), 206-228.

Sturm, K., Koron, D., & Stampar, F. (2003). The composition of fruit of different strawberry varieties depending on maturity stage. *Food Chemistry*, 83(3), 417-422.

Sullivan, P., O'Flaherty, J., Brunton, N., Arendt, E., & Gallagher, E. (2011). The utilisation of barley middlings to add value and health benefits to white breads. *Journal of Food Engineering,* 105(3), 493-502.

Thakur, B.R., Singh, R.K., Handa, A.K., & Rao, M. (1997). Chemistry and uses of pectin—a review. *Critical Reviews in Food Science & Nutrition*, 37(1), 47-73.

Thebaudin, J. Y., Lefebvre, A. C., Harrington, M., & Bourgeois, C. M. (1997). Dietary fibres: Nutritional and technological interest. *Trends in Food Science & Technology,* 8(2), 41-48.

Topuz, A., Topakci, M., Canakci, M., Akinci, I., & Ozdemir, F. (2005). Physical

and nutritional properties of four orange varieties. *Journal of Food Engineering,* 66(4), 519-523.

Vetter, S., Kunzek, H., & Senge, B. (2001). The influence of the pre-treatment of apple cell wall samples on their functional properties. *European Food Research and Technology,* 212(6), 630-635.

Wijngaard, H.H., Rößle, C., & Brunton, N. (2009). A survey of Irish fruit and vegetable waste and by-products as a source of polyphenolic antioxidants. *Food Chemistry,* 116(1), 202-207.

Wolfe, K.L., & Liu, R.H. (2003). Apple peels as a value-added food ingredient. *Journal of Agricultural and Food Chemistry,* 51(6), 1676-1683.

Chapter 3

New application of a fruit by-product: gluten-free bread approach.

Under review in Cereal Foods World.

3 Chapter 3: New application of a fruit by-product: gluten-free bread approach.

3.1 Abstract:

A by-product of fruit processing (orange pomace) was incorporated into a gluten-free bread formulation based on rice and potato flours; its effects on baking properties were studied. It was the aim of this study to demonstrate that such a by-product could be used as a low cost ingredient to benefit characteristics such as the structure, texture and dietary fibre of the resulting gluten-free breads. Three variables were identified as being important from preliminary tests: water (85-100 %) flour weight), orange pomace (0-8 % flour weight) and proofing time (35-50 minutes). Based on these variables, a response surface design (D-optimal) was created. Texture and moisture were analysed two hours after baking and a full analysis (texture, moisture, image analysis, volume and fibre) was carried out 24 hours after baking. Orange pomace demonstrated the greatest effect on crumb hardness ($p<0.0001$) and specific volume ($p<0.0001$). The optimised formulation was calculated to contain 5.5 % orange pomace, 94.6 % water and a proofing time of 49 minutes. A novel and nutritionally viable product of good volume and acceptable texture was developed and successfully validated.

3.2 Introduction:

This experimental study aims to show how a fibrous fruit by-product (orange pomace (OP)) can potentially be used to improve the nutritional content while not compromising on the loaf volume and crumb structure of gluten-free bread.

In some individuals, the ingestion of wheat-containing food and beverage products can trigger such disorders as coeliac disease, gluten intolerance and wheat allergies. Coeliac disease presents itself as an autoimmune response to the protein gluten, where the villi in the small intestine become damaged. As a result of this damage, absorption of essential vitamins and minerals is radically reduced, which potentially puts the individual at risk of deficiencies, some forms of cancers and other autoimmune diseases (Copelton & Valle 2009). As a result of these disorders, suffers have been found to be deficient in vitamins, minerals and dietary fibre (Kupper 2005). At present, the only cure available for these conditions is lifelong avoidance of products containing wheat, rye, spelt, barley, semolina, durum, some forms of oats and the hybrids of these grains such as triticale and kamut.

The majority of gluten-free products (in particular bread) which are available on the market lack flavour, mouth-feel, nutritional content and can be up to twice the cost, when compared to their wheat counterparts (Singh & Whelan 2011). Dietary fibre is one of the main macro-nutrients present in low amounts, or absent altogether in gluten-free breads (Thompson 2000). Previously, authors have investigated the effects of fibre-rich ingredients in gluten-free bread formulations; wheat fibre, oat fibre, barley fibre, maize fibre (Sabanis, Lebesi & Tzia, 2009a, 2009b), oat beta-glucan isolate (Lazaridou, Duta, Papageorgiou,

Belc & Biliaderis, 2007), resistant starch from corn and tapioca sources (Korus, Witczak, Ziobro & Juszczak, 2009), psyllium fibre and pseudocereals (Alvarez-Jubete, Arendt & Gallagher, 2009; Mariotti, Lucisano, Pagani & Ng, 2009).

By-products of fruit and vegetables have been shown to contain high levels of dietary fibre (O'Shea, Arendt & Gallagher, 2012). From a health point of view, dietary fibre has been shown to reduce cardiovascular disease, constipation, some forms of cancer and help in weight management (Champ, Langkilde, Brouns, Kettlitz & Collet, 2003). As an ingredient, fibre contains such positive attributes as high water binding, holding capacity and can act as a good gelling and thickening agent (Rosell, Santos & Collar, 2009).

Previous research with regards to the use of by-products (e.g. apple pomace and mango pomace) has been confined to wheat-based products (Masoodi, Sharma & Chauhan, 2002; Rupasinghe, Wang, Huber & Pitts, 2008; Ajila, Aalami, Leelavathi & Rao, 2009; Bchir, Rabetafika, Paquot & Blecker, 2013). The orange juice industry generates this by-product, commonly called orange pomace, and it can constitute 45 % to 60 % of the fruit (Fernandez-Lopez et al., 2009).

The use of citrus fibres has been well documented in wheat-containing products such as biscuits (Kohajdova, Karovicova, Jurasova & Kukurova, 2011), bread and frozen dough (Bchir, et al., 2013; Ocen & Xu 2013) . As far as the authors are aware, the application of orange pomace as an ingredient has not been researched in the gluten-free field (O'Shea, et al., 2012).

Before the present study commenced, it was envisaged that the addition of

orange pomace to a bread formulation could create two potential problems;

(i) alteration of the water absorption behaviour of the batter (high fibre ingredients require more water to sufficiently hydrate a batter system) (Lazaridou et al., 2007) and

(ii) alteration of the proofing properties, to allow for a more complete development of the batter. A response surface design (RSD) was established to construct a well-rounded study which would encompass the comprehensive investigation of these parameters.

The utilisation of a RSD to show the effect of all three factors (OP addition, proofing time and water addition) on the bread responses has not been previously shown. Studying the combined contribution of the three factors is essential, as ignoring or keeping one factor consistent could have a detrimental consequence on the resulting bread responses.

The main objectives of this study were:

- To assess the effects of orange pomace in a gluten-free formulation
- To investigate its influence on baking properties such as crumb moisture, loaf volume, crumb structure and texture
- To study and optimise the use of orange pomace as a novel, low cost and high fibre ingredient in a gluten-free bread formulation

3.3 Materials and methods:

3.3.1 Materials:

3.3.1.1 Orange pomace preparation:

The orange pomace consisted of the peel, pulp and seeds remaining after juicing. Orange pomace was received in its wet state shortly after juicing from an industrial source. To eliminate fermentation, the pomace was freeze-dried immediately, milled to a particle size of >355 µm and stored at -20 ^{o}C until required. Dietary fibre analysis illustrated the by-product contain 41.1 %. Its addition was based on preliminary trials to establish its limits of usage and following this, values were dictated by the response surface design (Table 3-1).

3.3.1.2 Batter ingredients:

The gluten-free formulation (Table 3-2) consisted of rice flour (S&B Herba, Orpington, Kent, UK), potato starch (Healy Chemicals Ltd, Dublin, Ireland), sunflower oil (Flora, Liverpool, UK), methylcellulose (MC) (All In All Ingredients, Dublin, Ireland), salt (Imeos Enterprises, Runcorn, Cheshire, UK), castor sugar (Tate & Lyle, London, UK) and dried yeast (Doves Farm Foods Ltd, Salisbury Road, Hungerford, Berkshire, UK).

Table 3-1: Means and standard deviations of responses.

Design Point	Orange By-product (%)		Water (%)		Proofing (minutes)		Specific Volume (mL/g)	Number of Cells	Cell volume	Hardness (N) 2hrs	Hardness (N) 24hrs	Moisture (%) 2 hrs	Moisture (%) 24hrs
	actual	coded	actual	Coded	actual	coded	Mean St Dev. (+/-)	Mean St Dev. (+/-)	Mean St Dev. (+/-)	Mean St Dev. (+/-)	Mean / St Dev. (+/-)	Mean St Dev. (+/-)	Mean / St Dev. (+/-)
1	0	-1	100	1	50	1	2.15 +/- 0.00	3191.75 +/- 62.75	4.31 +/- 0.18	1.72 +/- 0.04	4.33 +/- 0.22	54.51 +/- 0.25	53.48 +/- 0.21
2	8	1	92.5	0	42.5	0	1.58 +/- 0.00	2852.00 +/- 83.11	4.35 +/- 0.27	6.71 +/- 0.52	16.05 +/- 1.16	52.81 +/- 0.19	51.34 +/- 0.51
3	2	-0.5	88.75	-0.5	42.5	0	2.32 +/- 0.00	3443.00 +/- 152.66	4.90 +/- 0.29	1.98 +/- 0.05	3.97 +/- 0.18	49.26 +/- 0.13	48.96 +/- 0.71
4	8	1	85	-1	35	-1	1.55 +/- 0.09	3332.25 +/- 63.44	2.98 +/- 0.20	8.43 +/- 0.42	20.49 +/- 2.11	49.15 +/- 0.75	47.88 +/- 0.18
5	4	0	85	-1	35	-1	1.84 +/- 0.00	3421.75 +/- 108.96	3.86 +/- 0.24	3.97 +/- 0.19	9.28 +/- 0.47	49.07 +/- 0.03	49.09 +/- 0.08
6 **(Ctrl)**	0	-1	85	-1	35	-1	1.93 +/- 0.03	3606.00 +/- 125.26	3.69 +/- 0.23	2.11 +/- 0.14	6.16 +/- 0.23	50.16 +/- 0.92	49.01 +/- 0.16
7	0	-1	92.5	0	35	-1	1.90 +/- 0.00	3456.00 +/- 101.79	3.44 +/- 0.18	1.77 +/- 0.11	6.52 +/- 0.72	50.66 +/- 0.01	50.82 +/- 0.10
8	8	1	85	-1	50	1	1.67 +/- 0.03	3301.75 +/- 124.05	4.14 +/- 0.25	5.71 +/- 0.62	17.20 +/- 4.31	48.31 +/- 0.14	47.35 +/- 0.12
9	0	-1	100	1	35	-1	1.98 +/- 0.01	3544.50 +/- 57.02	3.26 +/- 0.07	1.64 +/- 0.04	5.70 +/- 0.29	53.53 +/- 1.02	52.83 +/- 0.74
10	8	1	100	1	50	1	1.85 +/- 0.06	2731.25 +/- 56.91	6.36 +/- 0.35	3.52 +/- 0.08	8.70 +/- 1.44	51.61 +/- 0.08	51.35 +/- 0.17
11	0	-1	85	-1	50	1	2.56 +/- 0.00	3631.00 +/- 64.41	4.87 +/- 0.16	1.34 +/- 0.06	3.98 +/- 0.27	48.83 +/- 0.05	48.48 +/- 0.52
12	8	1	92.5	0	35	-1	1.58 +/- 0.01	3426.00 +/- 69.68	3.27 +/- 0.23	7.09 +/- 0.36	18.87 +/- 0.46	49.97 +/- 0.07	49.25 +/- 0.11
13	8	1	100	1	50	1	1.69 +/- 0.02	2697.00 +/- 55.47	5.03 +/- 0.27	5.51 +/- 0.29	14.25 +/- 0.80	52.84 +/- 0.43	52.41 +/- 0.55
14	8	1	100	1	35	-1	1.49 +/- 0.00	3247.75 +/- 101.92	3.22 +/- 0.22	7.34 +/- 0.20	21.89 +/- 1.72	53.05 +/- 0.72	51.94 +/- 0.24
15	4	0	100	1	42.5	0	2.03 +/- 0.00	3097.75 +/- 23.26	4.37 +/- 0.07	2.20 +/- 0.14	6.03 +/- 0.21	51.47 +/- 0.27	52.29 +/- 0.22
16	0	-1	85	-1	50	1	2.21 +/- 0.01	3778.75 +/- 74.11	3.55 +/- 0.11	2.22 +/- 0.08	6.61 +/- 0.38	50.07 +/- 0.03	50.12 +/- 2.03
17	0	-1	100	1	50	1	2.02 +/- 0.02	3465.25 +/- 21.42	3.50 +/- 0.15	1.68 +/- 0.10	6.01 +/- 0.19	52.14 +/- 0.06	52.26 +/- 0.22
18	4	0	92.5	0	50	1	2.38 +/- 0.01	3094.50 +/- 58.11	5.87 +/- 0.32	1.66 +/- 0.06	4.45 +/- 0.10	50.21 +/- 0.60	49.38 +/- 0.40
19	4	0	92.5	0	50	1	2.47 +/- 0.01	2891.50 +/- 168.97	6.80 +/- 0.21	1.53 +/- 0.07	3.88 +/- 0.07	49.91 +/- 0.27	49.53 +/- 0.09
20	8	1	85	-1	50	1	1.54 +/- 0.02	3642.50 +/- 178.43	3.00 +/- 0.16	8.60 +/- 0.70	19.33 +/- 0.51	48.25 +/- 0.21	47.83 +/- 0.19

Table 3-2: Gluten-free bread formulation.

Material	Composition (%)[a]
Rice flour	50
Potato Starch	50
Sunflower Oil	6
Methylcellulose	0.6
Salt	2
Castor Sugar	5
Yeast	2
Water	85-100[b]
Orange Pomace	0-8[b]

[a] Values (%) are based on total flour weight basis.

[b] Quantities added depend on values created from RSD.

3.3.2 Methods:

3.3.2.1 Breadmaking:

All dry ingredients and yeast were added to the mixer bowl and mixed (Model A120, Hobart, UK) for one minute at speed one to equally disperse the ingredients. Cool tap water and sunflower oil were gradually added to the mixture and mixed for one minute at speed one. The bowl was scraped down and the mixture was mixed for a further three minutes at speed three until a highly viscous batter was formed. Batch sizes of 1500 g were created per each experimental combination. The batter (320 g) was scaled into one lb tins and placed into a proofer (35 ^{o}C, relative humidity 85 %). The proofing times were

dependant on the values created from the RSD. After proofing, the loaves were baked in a deck oven (Tom Chandley Ovens, Manchester, UK) for 25 minutes at 230 ^{o}C. The baked loaves were cooled for two hours. Four loaves were baked for each experimental combination, two of which were analysed after two hours and the remaining two loaves were stored in polyethylene bags until analysis at 24 hours. The bread analysis was carried out at room temperature.

3.3.2.2 Bread analysis:

Analysis at two hours post-baking consisted of crumb moisture and crumb texture profile analysis. Moisture was calculated based on the AACC method 62-05, (1991), and AACC method 44-15A, (1991), using a Brabender oven (Brabender, Duisberg, Germany) (AACC 1991a, 1991b).

Texture profile analysis (TPA) was evaluated on the bread crumb (20 mm slices) using a texture analyser (TA-XT2i, Stable Micro Systems, Surrey, UK) equipped with 30 KG load cell and a 20 mm LAP perspex probe. The test speed was one mm/second under a compression of 40 % strain; each sample took five seconds to run (Alvarez-Jubete et al., 2010). All results were expressed in Newtons (N) and were carried out on four (20 mm thick) slices cut from the centre of two loaves. The bread was cut by hand using a specific bread cutter device that divided each loaf accurately into 20 mm thick slices.

Bread analysis at 24 hours post-baking consisted of loaf volume, digital image analysis, texture profile analysis and crumb moisture. The specific volume of each loaf was calculated using a volume meter (Tex vol instruments BVM-L 370

Viken, Sweden) and software VolCalc 3.2.3.10 on two loaves per bake (mL/g). The properties of the crumb were analysed using the C-Cell imaging system (Calibre Control International LTD, Warrington, UK). High resolution images of the bread slices were captured and analysed using the C-Cell software (C-Cell, Version 2 Software, Calibre Control International LTD, Warrington, UK). Crumb properties such as number of cells and cell volume were determined from the software data. Texture profile analysis and crumb moisture were carried out as previously described.

3.3.2.3 Fibre analysis:

Total fibre analysis was determined on orange pomace flour, optimised and control baked products according to the AOAC methods 985.29 (AOAC 1990).

3.4 Experimental design and statistical analysis:

The experimental design was carried out and analysed using Design Expert 7.1.6. (Stat-Ease Inc., Minneapolis, MN, USA).

The effects of three independent variables (orange pomace (X1), water addition (X2), and proofing time (X3)) on the baking properties of a gluten-free formulation were investigated using a D-optimal design. Upper and lower limits were selected based on preliminary tests; 0-8 % orange pomace addition flour weight basis (FW), 85-100 % water addition FW and 35-50 minutes proofing time.

The dependant variables that best described the parameters of bread (as responses) consisted of cell volume (Y1), number of cells (Y2), moisture two hours (Y3), moisture 24 hours (Y4), specific volume (Y5), hardness two hours (Y6) hardness 24 hours (Y7).

A quadratic model was selected, where 20 combinations were generated via the Design Expert software. Experimental combination six (0 % orange pomace addition, 85 % water, proof time 35 minutes) reflects the original control formulation. Within these twenty combinations five combinations were repeated twice to assess error within the model.

A polynomial quadratic regression equation (as seen below Eq. 3-1) was used to describe the effects of the three factors on the nine responses (Hossain et al., 2012).

$$Y=\beta_o+\sum_{i=1}^{3}\beta_i x_i+\sum_{i=1}^{3}\beta_{ii}x_i^2+\sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}x_i x_j$$

Equation 3-1: Polynomial quadratic regression equation.

The model that best fitted the response was selected during analysis of measurements. Analysis of variance (ANOVA) was carried out on each response model to identify the coefficient (R^2), lack of fit and significant difference ($p<0.05$, $p<0.01$, $p<0.001$, $p<0.0001$).

3.5 Optimisation and validation of model:

The Design Expert optimisation tool was used as a means to calculate optimal levels for orange pomace, water addition and proofing times. Using the predictive tool, the maximum level of orange pomace (factor), maximum specific volume (response) and minimum crumb hardness (response) where chosen. All remaining factors were left in range and all remaining responses were left out of the optimisation. As a result, the most appropriate formulation would then be recommended by the software.

The validation of the model was carried out by assessing the model performance indices (the accuracy factor (Eq. 3-2) and bias factor (Eq. 3-3)) described by Hossain et al. (2012).

$$10^{\frac{\sum^{\log} | V_p / V_e |}{ne}}$$

Equation 3-2: Accuracy factor.

$$10^{\frac{\sum^{\log} (V_p / V_e)}{ne}}$$

Equation 3-3: Bias factor.

These performance indices are essential in evaluating the predicted performances of the developed models in illustrating the effects of the independent variables (X1-X3) on the response (dependant) variables (Y1-Y7) thus resulting in a validated final optimised product.

The average mean deviation (Eq. 3-4) was used to evaluate the competence of the data fit on the model.

$$\sum(\%) = \frac{1}{n_e}\sum_{i=1}^{n}\left\|\frac{V_E - V_P}{V_E}\right\| \times 100$$

Equation 3-4: Average mean deviation.

Σ, can be seen to be the average mean deviation, ne is the number of experimental data, Ve is the experimental value and Vp is the calculated value.

3.6 Results and discussion:

3.6.1 Experimental design:

The experimental design generated twenty experimental runs (Table 3-1 (combinations)). The means and standard deviation results of all twenty combinations were calculated (Table 3-1). For comparative purposes, design point six (0 % orange pomace, 85 % water, proof time 35 minutes) can be considered as the original control formulation. The error value for each model was based on five combinations repeated twice, combination (C) 1=C17, C8=C20, C10=C13, C11=C16 and C18=C19. Each model created from the responses was shown to be significant with p values ranging from $p<0.05$ to $p<0.0001$. The lack of fit for each model was insignificant, with values ranging from $p>0.3395$ to $p>0.9526$. Finally, satisfactory coefficients (R^2) were received for each parameter (Table 3-3).

3.6.2 Specific volume:

Loaf volume is an important quality feature of bread. Many factors can affect the volume of bread, such as viscosity, amylose and amylopectin content, protein aggregation once heated and surface active components (Alvarez-Jubete, Auty, Arendt & Gallagher, 2010). In the present study, results of the specific volume response propose that addition of the orange pomace had the most significant effect on specific volume ($p<0.0001$). As anticipated, the proofing variable had a significant effect on the specific volume ($P<0.001$) (Figure 3-1).

Table 3-3: Regression models for the response variables.

	Specific Volume (mL/g)	Number of Cells	Cell Volume	Hardness 2hrs (N)	Hardness 24 hrs (N)	Moisture 2 hrs (%)	Moisture 24 hrs (%)
Orange Pomace (X_1)	-0.071****	177.777***	-1.207	1.859****	3.838****	-0.608	-0.720**
Water (X_2)	0.362	-366.452***	2.002	-1.482*	-3.154	-0.053****	-0.985****
Proofing (X_3)	-0.008***	109.547*	-0.721***	0.529**	0.516***	0.612	0.864
Orange Pomace/Water (X_1/X_2)	0.002	-2.089	0.015*	-0.016	-0.032	0.001	0.004
Orange Pomace/Proofing (X_1/X_3)	-0.001	-1.780	0.007	-0.014	-0.039*	-0.004	-0.002
Water/Proofing (X_2/X_3)	-0.001	-2.182**	0.004	-0.002	-0.017	0.003	0.002
Orange Pomace^2	-0.019**	5.980	-0.065*	0.103**	0.283***	0.072	0.036
Water^2	-0.002	2.410	-0.012	0.008	0.021	0.001	0.006
Proofing^2	0.001	1.019	0.005	-0.004	0.011	-0.010	-0.012
R^2	0.901	0.860	0.805	0.941	0.952	0.833	0.907
p (Lack of fit)	>0.421	>0.483	>0.808	>0.975	>0.664	>0.340	>0.430
p (Model)	<0.0006	<0.0029	<0.0130	< 0.0001	< 0.0001	<0.0067	<0.0004

Values significantly different at: p<0.05*, p<0.01**, p<0.001***, p<0.0001****.

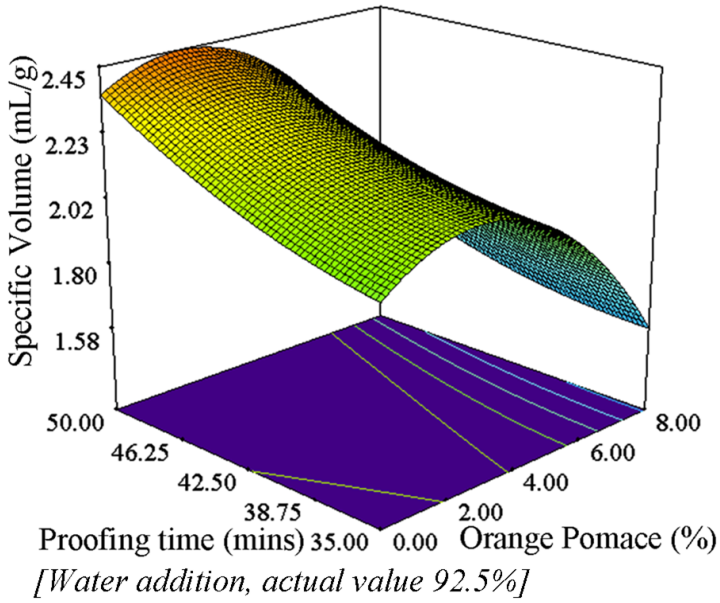


Figure 3-1: Specific volume (mL/g) as a function of proofing (minutes) and orange pomace (%).

The positive relationship between orange pomace addition and proofing can clearly been seen from the repeated experimental combinations 18 and 19 whereby orange pomace was added at 4 % and the batter was proofed for 50 minutes. This produced a loaf specific volume of 2.38 mL/g and 2.47 mL/g respectively. This demonstrates a synergetic relationship between the both factors when compared to the control (1.93 mL/g). A high specific volume (2.3 mL/g) was also achieved with lower inclusions of orange pomace (2 %) and high levels of water (88.8 %). This improvement in specific volume may be attributed to the fibre found in the orange pomace supporting the methylcellulose starch matrix, however only to a certain extent. After a particular level, as the orange pomace and water increase, the specific volume begins to decrease. This incident may be explained by a weakening in the methylcellulose starch matrix,

resulting in a loss of carbon dioxide (CO_2) gas retention. The hydrocolloid, methylcellulose, which aids in the structure-building properties of the batter may also become, diluted as a result of higher levels of orange pomace addition, therefore weakening the structure formed. Sabanis et al. (2009a) documented similar results (i.e. a lowering of volume) with the addition of maize fibre to a gluten-free formulation. These authors suggest another potential reason for the lower loaf volumes may be due to the extra water available in the gluten-formulation. This additional water creates a low dough consistency; consequently creating unstable air vacuoles within the crumb structure which can become weak and uneven, developing large holes in the crumb or it may even cause the structure to collapse.

3.6.3 Crumb structure:

Bread crumb can be described as a complex, viscoelastic foam structure (Arendt, Ryan & Dal Bello, 2007). An ideal crumb structure would consist of a high number of cells in a slice; each cell would have thin walls. It has been found that the quantity of cells present in a crumb structure is a good measure of the amount of carbon dioxide (CO_2) retained during proofing.

In the present study, the number of cells in the bread slices were found to be predominately effected by the presence of orange pomace ($p<0.001$) and the addition of water ($p<0.001$). The number decreased with the inclusion of orange pomace levels and increasing water additions. This is evident from experimental combinations two and 10 where a high level of orange pomace and a high level

of water addition are included in the formulations (Figure 3-2).

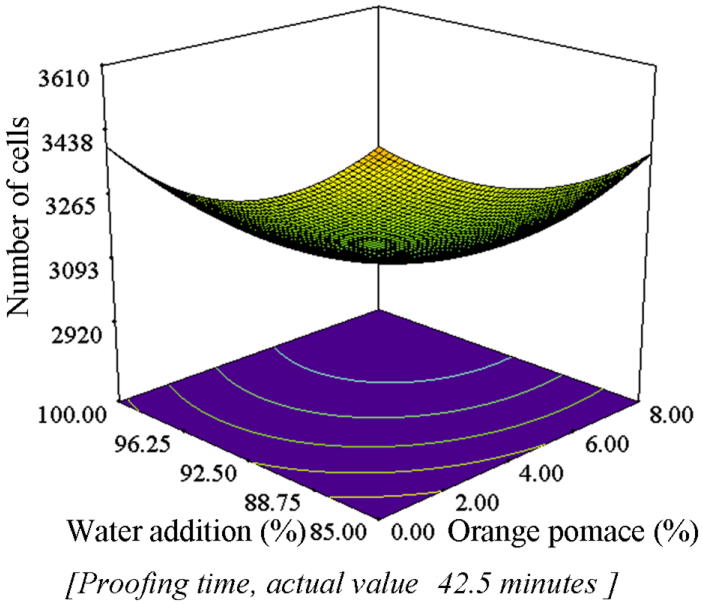


Figure 3-2: Number of cells as a function of water (%) & orange pomace (%).

A more porous and rigid like cell structure has been associated with the inclusion of hydrocolloids such as that used in this study (methylcellulose) thus potentially explaining the greater number of cells in the control slice (Anton & Artfield 2008). Orange pomace in an excess of 4.0 % with a reduced water addition and proofing time, was seen in some cases to cause a detrimental effect on crumb structure of the bread; resulting in a dense and compact loaf. A reduction in the ability of dough to retain gas can lead to a disrupted crumb structure possibly due to dilution of the methylcellulose and starch network, in this case most likely due to orange pomace addition (Sivam, Sun-Waterhouse, Quek & Perera, 2010). The control formulation (0 % orange pomace and 85 % water) presented a high number of cells (3606 cells). A negative correlation between cell volume and the number of cells present was seen (R^2 = -0.67). The correlation highlights

that as the cell volume decreases, the number of cells present increase, creating a more descriptive explanation of crumb cellular effects.

The cell volume provides information on the general volume of the crumb cells, which is related to the overall volume of the loaf. This parameter was found to be strongly influenced by the length of proofing ($p<0.001$). Proofing time even when compared to orange pomace was found to have a more dominant effect on this response (data not shown).

3.6.4 Texture analysis:

It has been well documented that gluten-free breads can exhibit greater crumb hardness, and increased rate of hardening in comparison to wheat breads. One such reason for this is the absence of gluten, which has been found to delay staling by its presence in the starch matrix and the interactions it has with starch (Ahlborn, Pike, Hendrix, Hess & Huber, 2005). As the majority of gluten-free breads contain a high percentage of starch and a low percentage of protein, staling is likely to occur more rapidly (Ahlborn et al., 2005). In the present study hardness values were taken two hours after baking and 24 hours after baking.

Crumb hardness values two hours after baking ranged from 1.34 N to 8.60 N; 24 hours after baking results had increased significantly, varying from 3.88 N to 21.89 N. Orange pomace had the most significant ($p<0.0001$, Figure 3-3 & 3-4) effect on hardness values at both two hours and 24 hours after baking.

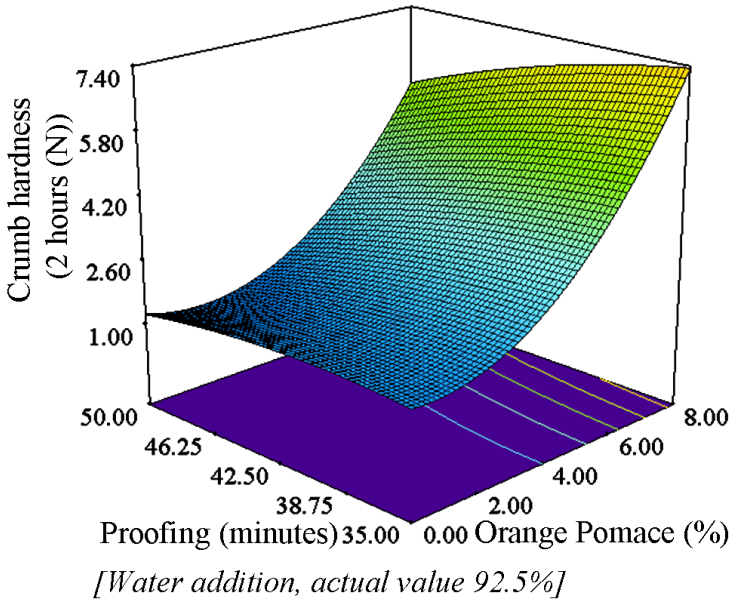


Figure 3-3: Crumb hardness (N) at two hours as a function of proofing (minutes) and orange pomace (%).

Similar to the results found in the specific volume section, proofing time was also found to significantly affect hardness at both two hours and 24 hours post-baking (Figure 3-3 & 3-4). This is evident from the 3D contour plots (Figure 3-3) two hours after baking, which demonstrated that orange pomace had decreased the hardness of the crumb texture to a certain point, but increasing levels further lead to significant increase in hardness values. Experimental combinations 18 and 19 confirm the positive effects of combining orange pomace and proofing on hardness at both two hours and 24 hours, with reduced crumb hardness results when compared to the control (Table 3-1). Orange pomace at 2 %, 88.75 % water and 42.5 minutes resulted in breads of a comparable texture to the control (experimental combination six (C6)) (and it also yielded enhanced loaf volumes). At 24 hours, the hardness of the breads containing 2 % orange pomace was significantly reduced compared to the control.

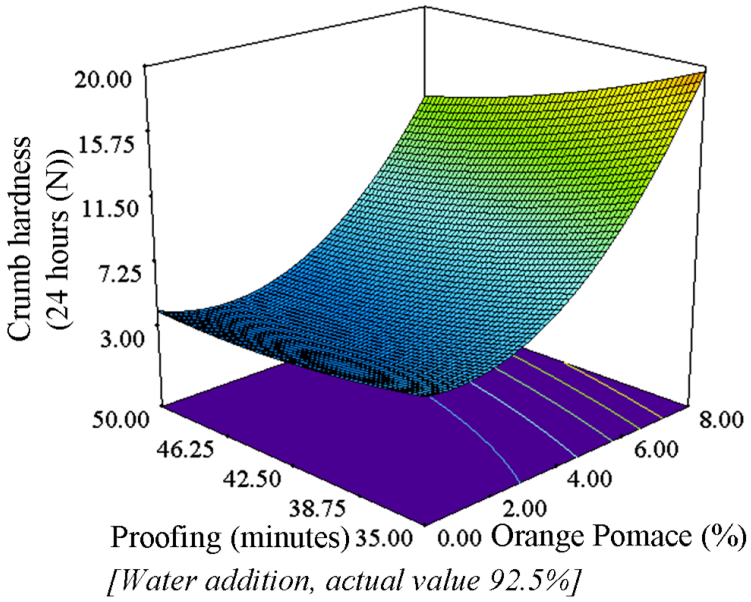


Figure 3-4: Crumb hardness (N) at 24 hours as a function of proofing (minutes) and orange pomace (%).

Orange pomace at higher levels (e.g. experimental combinations four (C4)) yielded breads of a harder texture (8.43 N two hours after baking and 20.49 N 24 hours after baking). The softer texture obtained at the lower levels of addition may have occurred due to the water binding capacity of the fibre present in the orange pomace. High fibre ingredients have the ability to bind available water, which assists in reducing moisture loss during storage, as a result of the hydrogen bonding between the fibre and starch matrix, thus delaying starch retrogradation (Sabanis et al., 2009a). These authors illustrated that the level of fibre added had a significant effect on crumb hardness. Specific volume results were directly correlated with crumb hardness. Generally, a smaller loaf will have a hard crumb texture with a dense and tightly packed crumb structure. Such an observation can be directly related to the results which were achieved in this study (breads containing orange pomace at 8 % had a small volume, coupled

with a hard crumb). It is suggested by Sabanis et al. (2009b) that a firm crumb and small loaf volume occurs due to the cells present in the crumb structure developing a thickening of the cell wall.

Water addition was only seen to have a significant ($p<0.05$) effect on hardness at two hours; however at 24 hours no major effect could be reported. At low levels of orange pomace and high levels of water addition, the hardness values were decreased, however, with high levels of orange pomace addition, hardness could not be reduced by water addition alone. McCarthy et al. (2005) how a soft crumb texture can be achieved through increased water addition. This extra moisture can accelerate staling at 24 hours, as there is no gluten present to slow down the migration of water from the crumb to the crust staling can occur rapidly. Therefore, as discussed by McCarthy et al. (2005) without the presence of gluten or a replacement ingredient to help retain the moisture staling can occur faster, thus explaining why no improvement was seen at the 24 hour time point.

To conclude this section, it may be deduced that the addition of orange pomace at an appropriate level in combination with longer proofing times developed breads with positive texture results when compared to the control bread.

3.6.5 Moisture:

The rate at which staling of gluten-free bread occurs can be more rapid than that of wheat bread. The most likely reason for this is due to the high percentage of starch present and its subsequent retrogradation. Retrogradation occurs via the formation of three dimensional gel networks by amylose containing starches

which form ‘entanglements’. When cooling occurs, the water is trapped in the gel network from the loss of translational motion of these entangled molecules. Rigidity then begins to set in due to crystallites forming at the junction zones in the swollen discontinuous phase (Kent & Evers 1994a). Starch retrogradation leads to crumb hardening, loss of flavour and a migration of moisture from crumb to crust (Kent & Evers 1994b).

The moisture results from analysis at two hours demonstrate that values ranged from 48.3 % to 54.5 %. At 24 hours, these results decreased, with results ranging from 47.3 % to 53.5 %. As expected the model illustrates that the water addition variable had a significant effect ($p<0.0001$) on moisture content of the bread crumb at both two hours and 24 hours. Increasing the levels of water added also increased the moisture content of the resulting crumb.

Proofing time had no effect on crumb moisture over both time points. Orange pomace addition had a significant effect ($p<0.01$) (Figure 3-5) on the formulation at the 24 hour time point only.

At the 24 hour time point the experimental combinations 10 and 13, which contained the highest amount of orange pomace (8.0 %) and the highest amount of water addition (100 %), retained the most moisture over the both time points, in particular when compared to the control combination (C6), which was to seen to lose moisture.

A longer staling period such as a 7 day trial would be required to confirm the potential of orange pomace to reduce the rate of water loss.

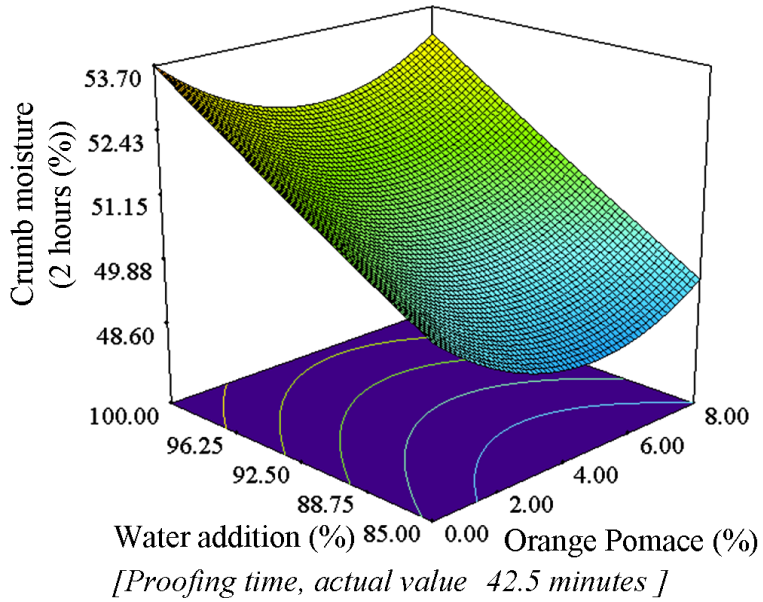


Figure 3-5: Crumb moisture at two hours as a function of water (%) and orange pomace (%).

Gallagher et al. (2003) found that additional water can have positive effects on bread such as softer crumb and a larger volume. Majzoobi et al. (2011) investigated the inclusion of tomato pomace in flat bread. The authors established that moisture content could be retained within the crumb following tomato pomace addition.

3.7 Proofing time and formulation optimisation:

Based on the above results, the optimisation tool was used to calculate optimal levels for orange pomace addition, water addition and proofing times. It was clear from the results presented that all three variables play an important role in some or all of the responses measured. The most appropriate formulation was chosen to produce a high quality bread product, which contained a level of orange pomace to significantly increase fibre content, while not negating important quality properties such as hardness and specific volume. The software suggested the following: orange pomace addition (5.5 %), water addition (94.6 %) and proofing time (49 minutes).

3.8 Validation of the optimised formulation:

To confirm that this model can be used to predict and suggest appropriate results, validation of the model must be carried out to ensure quality and dependability of its results. Validation results can be seen in the form of the model's predicted values versus the actual experimental values received in Table 3-4. The bias and accuracy factors act as a guide to show the overall performance of the model. The bias factor can be used to describe the reliability of the model, whereas the accuracy factor can be described as a measure of how close the experimental values are to the predicted values of the model.

Table 3-4: Predicted, experimental values, accuracy & bias factors and average mean.

Response	Predicted	Experimental	Bias Factor	Accuracy Factor	Average mean diavation (Σ)
Specific Volume (mL/g)	2.02	2.19	1.08	0.93	8.03
Cell volume	5.17	6.06	0.85	1.17	14.68
Number of Cells	3096	2772	1.12	0.90	11.69
Hardness 2 hrs (N)	2.47	2.84	0.87	1.15	13.10
Hardness 24 hrs (N)	5.52	6.95	0.79	1.26	20.66
Moisture 2 hrs (%)	51.00	50.43	0.99	1.01	1.11
Moisture 24 hrs (%)	50.38	50.24	1.00	1.00	0.27

If the model has a perfect accuracy factor, it is indicated by receiving a value of 1; anything above or below 1 illustrates how less precise the values of the model are (Miles, Ross, Olley & McMeekin, 1997).

However, 0.10 - 0.15 have been added on for each variable in the model, therefore an ideal model that will predict the effects of orange pomace, water addition and proofing would need to have an accuracy factor within the range 1.2 - 1.3 or a percentage error range of 20 % - 30 % (Tiwari, Muthukumarappan, O' Donnell & Cullen, 2008).

Finally, the average mean deviation is a measure of the error of fitting the data to the model (Tiwari et al., 2008). From Table 3-4 it can be seen that the accuracy values fall either close to 1 or well within the error limits, thus implying that the model had a good fit, or the experimental values achieved were adequately close to the models predicted values. It can also be seen from Table 3-4 that the bias factor followed a similar trend to the accuracy factor with values falling close to 1. Therefore the bias factors for all responses would suggest a good association between the predicted and experimental factors. Finally, from Table 3-4 it is evidenced that a sufficiently low standard error was received for all responses. To support these findings the chosen validated formulation gave an impressive desirability level of 0.73.

3.9 Dietary fibre content of the optimised formulation:

As the orange pomace by-product flour was found to be high in dietary fibre, analysis of the control and optimised breads, for its fibre content was carried out. For a product to be labelled "high in fibre" it must contain 6.0 % fibre, or for a product to be labelled a "source" of fibre, it must contain 3.0 % fibre (Eur-lex 2006). The control gluten-free bread contained 2.0 %. The optimised gluten-free bread, which contained 5.5 % orange pomace, had an enhanced fibre level of 4.0 %.

3.10 Conclusion:

This study has shown that orange pomace (retrieved from the orange juice industry) may be used as a healthy, novel, economical ingredient in gluten-free bread formulations to produce a bread of acceptable baking properties.

Gluten-free bread was successfully formulated based on the application of a D-optimal, response surface design (RSD). The use of RSD to highlighted the potential utilization and limitations of orange pomace as an ingredient in gluten-free bread. It also highlighted the effects water addition and proofing can have on a gluten-free formulation; such parameters can sometimes be overlooked. Results show that low levels of orange pomace may be present with the other ingredients to develop structurally appealing bread. Upon optimisation (5.5 % orange pomace, 94.6 % water and 49 minutes proofing) a novel and nutritionally viable product of good loaf volume and acceptable texture was developed and successfully validated. Further trials relating to the sensory aspects of the bread, its microstructure and rheology are ongoing.

3.11 References:

AACC. (1991a). Method 44-15A, Moisture: air oven methods, approved methods of the American Association of Cereal Chemists International, St. Paul, Minnesota, USA.

AACC. (1991b). Method 62-05 Preparation of sample: bread Brabender moisture oven, manufacturer's handbook, approved methods of the American Association of Cereal Chemists International, St. Paul, Minnesota, USA.

Ahlborn, G.J., Pike, O.A., Hendrix, S.B., Hess, W.M., & Huber, C.S. (2005). Sensory, mechanical, and microscopic evaluation of staling in low-protein and gluten-free breads. *Cereal Chemistry*, 82(3), 328-335.

Ajila, C.M., Aalami, M., Leelavathi, K., & Rao, U. (2009). Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. *Innovative Food Science & Emerging Technologies*, 11(1), 219-224.

Alvarez-Jubete, L., Arendt, E., & Gallagher, E. (2009). Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. *International Journal of Food Sciences and Nutrition*, 60(s4), 240-257.

Alvarez-Jubete, L., Auty, M., Arendt, E.K., & Gallagher, E. (2010). Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. *European Food Research and Technology*, 230(3), 437-445.

Anton, A.A., & Artfield, S.D. (2008). Hydrocolloids in gluten-free breads: A review. *International Journal of Food Sciences and Nutrition*, 59(1), 11-23.

AOAC. (1990). AOAC Method No 985.29. Official methods of analysis. 15th

ed. Arlington, VA: Association of Official Analytical Chemists.

Arendt, E.K., Ryan, L.A.M., & Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology*, 24(2), 165-174.

Bchir, B., Rabetafika, H.N., Paquot, M., & Blecker, C. (2013). Effect of pear, apple and date fibres from cooked fruit by-products on dough performance and bread quality. *Food and Bioprocess Technology*, 1-14.

Champ, M., Langkilde, A.M., Brouns, F., Kettlitz, B. & Collet, Y.L.B. (2003). Advances in dietary fibre characterisation. 1. Definition of dietary fibre, physiological relevance, health benefits and analytical aspects. *Nutrition Research Reviews*, 16(1), 71-82.

Copelton, D.A., & Valle, G. (2009). "You don't need a prescription to go gluten-free": The scientific self-diagnosis of celiac disease. *Social Science & Medicine*, 69(4), 623-631.

Eur-lex. (2006). "Regulation (EC) No 1924/2006 of the European parliament and of the council of 20 December." Viewed on the 9th of January 2012. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:404:0009:01:EN:HTML

Fernandez-Lopez, J., Sendra-Nadal, E., Navarro, C., Sayas, E., Viuda-Martos, M., & Alvarez, J.A.P. (2009). Storage stability of a high dietary fibre powder from orange by-products. *International Journal of Food Science and Technology*, 44(4), 748-756.

Gallagher, E., Gormley, T.R. & Arendt, E.K. (2003). Crust and crumb

characteristics of gluten-free breads. *Journal of Food Engineering*, 56, 153-161.

Hossain, M.B., Brunton, N.P., Patras, A., Tiwari, B., O'Donnell, C.P., Martin-Diana, A.B., & Barry-Ryan, C. (2012). Optimization of ultrasound assisted extraction of antioxidant compounds from marjoram (Origanum majorana L.) using response surface methodology. *Ultrasonics Sonochemistry*, 19(3), 582-590.

Kent, N.L., & Evers, A.D. (1994a). Chemical components. In: Kent, N.L., Evers, A.D. (Eds.), *Technology of cereals.* BPC Wheatons Ltd., Great Britain, pp. 53-77.

Kent, N.L., & Evers, A.D. (1994b). Bread-baking Technology. In: Kent, N.L., Evers, A.D. (Eds.), *Technology of cereals.* BPC Wheatons Ltd., Great Britain, pp. 191-217.

Kohajdova, Z., Karovicova, J., Jurasova, M., & Kukurova, K. (2011). Application of citrus dietary fibre preparations in biscuit production. *Journal of Food and Nutrition Research*, 50(3), 182-190.

Korus, J., Witczak, M., Ziobro, R., & Juszczak, L. (2009). The impact of resistant starch on characteristics of gluten-free dough and bread. *Food Hydrocolloids*, 23(3), 988-995.

Kupper, C. (2005). Dietary guidelines and implementation for celiac disease. *Gastroenterology*, 128(4, Supplement 1), S121-S127.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C.G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in

gluten-free formulations. *Journal of Food Engineering*, 79(3), 1033-1047.

Mariotti, M., Lucisano, M., Pagani, M.A., & Ng, P.K.W. (2009). The role of corn starch, amaranth flour, pea isolate, and psyllium flour on the rheological properties and the ultrastructure of gluten-free doughs. *Food Research International*, 42(8), 963-975.

Majzoobi, M., Ghavi, F.S., Farahnaky, A., Jamalian, J., & Mesbahi, G. (2011). Effect of tomato pomace powder on the physicochemical properties of flat bread (Barbari bread). *Journal of Food Processing and Preservation*, 35, 247-256.

Masoodi, F.A., Sharma, B., & Chauhan, G.S. (2002). Use of apple pomace as a source of dietary fiber in cakes. *Plant Foods for Human Nutrition*, 57(2), 121-128.

McCarthy, D.F., Gallagher, E., Gormley, T.R., Schober, T.J., & Arendt, E.K. (2005). Application of response surface methodology in the development of gluten-free bread. *Cereal Chemistry*, 82, 609-615.

Miles, D.W., Ross, T., Olley, J., & McMeekin, T.A. (1997). Development and evaluation of a predictive model for the effect of temperature and water activity on the growth rate of Vibrio parahaemolyticus. *International Journal of Food Microbiology*, 38(2–3), 133-142.

O'Shea, N., Arendt, E.K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science & Emerging Technologies*, *16*, 1-10.

Ocen, D., & Xu, X. (2013). Effect of citrus orange (citrus sinensis) by-product dietary fiber preparations on the quality characteristics of frozen dough bread. *American Journal of Food Technology*, 8, 43-53.

Rosell, C.M., Santos, E., & Collar, C. (2009). Physico-chemical properties of commercial fibres from different sources: A comparative approach. *Food Research International*, 42(1), 176-184.

Rupasinghe, H.P.V., Wang, L.X., Huber, G.M., & Pitts, N.L. (2008). Effect of baking on dietary fibre and phenolics of muffins incorporated with apple skin powder. *Food Chemistry*, 107(3), 1217-1224.

Sabanis, D., Lebesi, D., & Tzia, C. (2009a). Development of fibre-enriched gluten-free bread: a response surface methodology study. *International Journal of Food Sciences & Nutrition*, 60, 174-190.

Sabanis, D., Lebesi, D., & Tzia, C. (2009b). Effect of dietary fibre enrichment on selected properties of gluten-free bread. *Lwt-Food Science and Technology*, 42(8), 1380-1389.

Singh, J., & Whelan, K. (2011). Limited availability and higher cost of gluten-free foods. *Journal of Human Nutrition and Dietetics*, 24(5), 479-486.

Sivam, A.S., Sun-Waterhouse, D., Quek, S., & Perera, C.O. (2010). Properties of bread dough with added fiber polysaccharides and phenolic antioxidants: A review. *Journal of Food Science*, 75(8), R163-R174.

Thompson, T. (2000). Folate, iron, and dietary fiber contents of the gluten-free

diet. *Journal of the American Dietetic Association*, 100(11), 1389-1396.

Tiwari, B.K., Muthukumarappan, K., O' Donnell, C.P., & Cullen, P.J. (2008). Modelling colour degradation of orange juice by ozone treatment using response surface methodology. *Journal of Food Engineering*, 88(4), 553-560.

Chapter 4

The rheology, microstructure and sensory characteristics of a gluten-free bread formulation enhanced with orange pomace.

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4 Chapter 4: The rheology, microstructure and sensory characteristics of a gluten-free bread formulation enhanced with orange pomace.

4.1 Abstract:

The present manuscript studied a previously optimised gluten-free bread formulation containing 5.5 % orange pomace in relation to the batter characteristics (i.e. pre-baking), microstructure (of the flours, batter and bread) and sensory characteristics of the bread. Rheology, RVA and mixolab results illustrated that orange pomace improved the robustness of the gluten-free batter and decreased the occurrence of starch gelatinisation. This was confirmed from the confocal laser scanning microscopy (CLSM) images, which showed potato starch granules to be more expanded in the control batter when compared to the sample containing orange pomace. Starch granules were also observed to be more enlarged and swollen in the CLSM bread images, suggesting a higher level of gelatinisation occurred in the control sample. Sensory analysis was carried out on the optimised and control bread; panellists scored the flavour, crumb appearance and overall acceptability of the Orange pomace containing breads comparable to the control.

4.2 Introduction:

The presence and prevalence of coeliac disease is increasing every year. It is described as a life-long intolerance to gluten, in particular the gliadin fraction of wheat and prolamin fraction of rye and barley (Gallagher, Gormley & Arendt, 2004). Gluten is responsible for the three-dimensional protein network which helps retain carbon dioxide (CO_2) in dough during proofing and is the basis for developing the crumb structure and volume of the bread loaf. The rheology of the dough is drastically changed when gluten is absent, hence generating production, processing and final product quality problems. Due to the physical properties of gluten-free dough (less cohesive and elastic, highly smooth, sticky and difficult to handle), batter is a more acceptable term to describe gluten-free dough (Houben, Höchstötter & Becker, 2012).

Descriptive analysis of gluten-free batter properties is lacking due to the inability of many pieces of equipment to work with batter-like consistencies. Equipment such as the rheometer, Rapid-Visco-Analyser (RVA) and Mixolab has been employed in this study to analyse the rheological properties of the batters (Dobraszczyk & Morgenstern 2003).

Orange pomace is a by-product created from the fruit and vegetable processing industry (O'Shea, Arendt & Gallagher, 2012). The pomace of oranges generally consists of peel (albedo) and seeds (Schieber, Stintzing, & Carle, 2001). This by-product can constitute 45 % to 60 % of the fruit (Fernandez-Lopez et al., 2009). From a functional point of view, orange pomace has been found to contain a high level of dietary fibre. Dietary fibre has also proven to have many

processing applications such as water binding, gelling and structure building and can potentially be used as a fat replacer (Rosell, Santos & Collar, 2009).

A response surface design (RSD) was previously carried out to optimise a gluten-free bread formulation containing orange pomace. From preliminary trials, orange pomace, water and proofing time were identified as three factors that would effect the baking and batter characteristics of the gluten-free formulation. The response surface design chosen was a D-optimal design, which consisted of 20 experimential combinations, error was assessed on five combinations repeated twice. The optimised formulation was developed using the optimisation tool. The optimised formulation was validated using performance indices. In comparison to the control the optimised bread had a greater loaf volume and comparable textural and crumb characteristics. In the previous study, orange pomace was established as a viable ingredient to improve the structure and nutrition of gluten-free bread.

The present study follows on from this work, where a particular focus was on the following objectives:

- To study the rheology and microstructure of an optimised level of orange pomace in a gluten-free batter in comparison to a standard control batter
- To investigate the effects of orange pomace on the starch pasting properties of the gluten-free batter
- To assess the sensory characteristics of orange pomace in gluten-free bread

4.3 Materials and methods:

All experiments were performed in compliance with the relevant laws and Teagasc guidelines, under the approval of Teagasc. Informed consent was also obtained from human subjects prior to the commencement of sensory anaylsis.

4.3.1 Batter ingredients:

The ingredients utilized were similar as described in Alvarez-Jubete et al. (2010) except methylcellulose was used instead of xanthan gum and the formulation can be seen in Table 4-1.

Table 4-1: Gluten-free formulation.

Material	Composition (%)[a]
Rice flour	50
Potato Starch	50
Sunflower Oil	6
Methylcellulose	0.6
Salt	2
Castor Sugar	5
Yeast	2
Water	85-100[b]
Orange Pomace	0-8[b]

[a] Values (%) are based on total flour weight basis.

[b] Quantities added depend on values created from RSD.

4.3.2 Batter and bread preparation:

The batter and bread preparation was carried out as illustrated in Alvarez-Jubete et al. (2010).

4.3.3 Flour pasting properties (RVA):

The pasting properties of a slurry containing rice flour, potato starch and methylcellulose (control) and a slurry containing rice flour, potato starch, methylcellulose and 5.5 % orange pomace (dry ingredients from the formulation described above for the control, and 5.5 % OP) was determined using a Rapid-Visco-Analyser (RVA-4D, Newport Scientific, Sydney, NSW, Australia). Using the RVA-4D sample and moisture correction equations, the moisture and sample weights were adjusted to reflect the samples moisture contents. This is an important step to carry out, to ensure maximum accuracy of the test results. The method was carried out as described by (Sullivan, O'Flaherty, Brunton, Arendt & Gallagher, 2011). This test was performed in triplicate.

Batter properties were investigated using a Mixolab (Chopin Technologies, Villeneuve-la-Garenne, France). The batter was prepared as previously described without the addition of yeast. The Chopin + protocol was used. This had a mixing speed of 80 rpm and the test duration was 45 minutes. The protocol is split into three sections: Section 1 had a starting temperature of 30 ^{o}C for eight minutes; section 2 consisted of a temperature increase to 90 ^{o}C at 4 ^{o}C per minute, with the temperature being held at 90 ^{o}C for seven minutes. In section 3, the temperature decreased to 50 ^{o}C at 4 ^{o}C per minute and finally held at 50 ^{o}C for five minutes.

Batter (75 g) was added to the heating block. As the water hydration of the batter was known, the water absorption and moisture content parameter was ignored. The water delivery nozzle was left connected to the tank and testing began. Once all moisture from the delivery nozzle had been redirected back into the tank, the delivery nozzle was placed over the mixing block so as not to lose any moisture from the batter during the mixolab protocol. The Mixolab properties were obtained from the mixolab graph (Koksel, Kahraman, Sanal, Ozay & Dubat, 2009). The test was preformed in triplicate and results were expressed in nanometre (Nm).

4.3.4 Fundamental oscillatory rheology:

Rheological measurements were performed on the two batter samples (control, control with 5.5 % orange pomace) on a controlled stress rheometer (Anton Paar GmbH, Graz, Austria) fitted with parallel plates consisting of a 50 mm serrated probe and 50 mm serrated base plate. The mixing and preparation of the batter samples were as described above but without the addition of yeast.

Approximately 5 g of sample was placed onto the base plate, and the upper plate was brought to a gap of 1.025 mm where excess sample was carefully trimmed away. The outer edge of the sample was lightly covered with petroleum jelly to prevent the sample from drying out. The plate was then lowered to a test gap of 1 mm and testing began. The whole system was covered using a peltier hood, with a temperature setting of 25 ^{o}C (Sullivan et al., 2011).

4.3.4.1 Frequency sweep:

Following a rest time of five minutes, (to allow the sample to relax), the frequency was increased from 0.1 to 10 Hz under a strain (γ), which had been previously identified from an amplitude sweep and was found to be within the linear viscoelastic range (LVR) (0.05 % γ control & 0.1 % γ orange pomace sample). The frequency sweep was performed at a temperature of 25 ^{o}C. Eleven points were measured and from the mechanical spectrum the viscoelastic properties were measured (Ktenioudaki, O'Shea & Gallagher, 2012).

4.3.5 Microstructure:

Control and control with 5.5 % orange pomace batter were prepared as described above (i.e. no yeast included) and stored in sealed tubs in the fridge for no longer than one hour before examination.

4.3.5.1 Cryo-scanning electron microscopy of the batters:

Cryo-SEM was used to illustrate the structural properties of the batters. All imaging was performed on a Zeiss Supra 40 VP field emission SEM (Carl Zeiss AG, Darmstadt, Germany) and fitted with a Gatan Alto 2500 Cryo-preparation system (Gatan UK, Abingdon, Oxon).

The batter was prepared for Cryo-SEM by rapidly immersing into a liquid nitrogen slush (-210 ^{o}C). Following freezing, the frozen specimens were immediately transferred using the vacuum transfer device into the cryo-preparation chamber. With the aid of an externally fitted binocular microscope, the sample was fractured using a chilled scalpel blade at -170 ^{o}C in the chamber, which was maintained under a high vacuum of 10^{-4} Pa. The specimen was then

etched, facilitating the removal of ice from the surface of the fractured sample at -95 ºC for 30 minutes and coated using a cold magnetron sputter coater using 300 V, 10 mA of sputtered gold/ palladium alloy (60/40) for 120 seconds. It was then transferred under vacuum onto the cooled SEM stage and imaged at -125 ^{o}C.

4.3.5.2 Confocal laser scanning microscopy (CLSM) of the batters:

A small piece of batter, approximately 10 mm in diameter was placed onto a slide using a spatula. To simultaneously visualise the protein, starch and plant cell wall (cellulosic) material, a triple fluorescent labelling technique was developed. Nile Blue (an oxazine salt) has been shown to label proteins in bread when excited at 633 nm (Kenny, Wehrle, Auty & Arendt, 2001) and also contains an oxidation product, Nile Red (oxazone), which labels lipids when excited at 488 nm. Fluorescein iso-thiocyanate (FITC) can be used to label starch granules (van de Velde, van Riel & Tromp, 2002) when excited at 515 nm. Fluorescent Brightener 28 is an optical brightener based on a reaction product of 4,4'diamino-22'-stilbenedisulfonic acid with diethanolamine and aniline and is used instead of Calcofluor White MR which binds to structural polysaccharides such as cellulose but is no longer produced (Green 1990). The sample was examined with a Leica SP5 confocal scanning laser microscope (Leica Microsystems, Mannheim, Germany) using blue diode (405 nm), 179 argon (488 nm) and HeNe (633 nm) lasers in three separate channels simultaneously 180 and pseudo-coloured to differentiate channels. The 405, 488 and 633 nm channels were pseudo-coloured purple, green and red, respectively.

4.3.5.3 Cryo-SEM of the gluten-free breads**:**

A small piece was removed from the centre of a pup loaf and fixed to a carbon-coated stub. The procedure and imaging of the bread is the same as for the batter above**.**

4.3.5.4 CLSM of the gluten-free breads:

A small flat slice was cut from inside the pup loaf and placed on a slide. The staining and imaging protocol are as for the batter above.

All chemicals and stains used were standard analytical grade and purchased from Sigma Aldrich (Arklow, Co. Wicklow, Ireland).

4.3.6 Sensory analysis:

Sensory analysis was carried out on the control and the control containing 5.5 % orange pomace bread. Twenty semi-trained panellists were asked to assess the bread based on the following characteristics; crumb appearance, texture while chewing, overall flavour and overall acceptability. This was conducted by the panellists marking a 9 cm line (0 = dislike extremely, 9 = like extremely) in accordance with their opinion. The panellists were also asked to rank the sample they preferred the most.

4.3.7 Statistical analysis:

Descriptive statistics of RVA, Mixolab and rheology results were calculated. The RVA and Mixolab data were analysed by analyses of variance using a randomised block design, with days as blocks to compare treatments (control and control with 5.5 % orange pomace). Oscillatory rheology data was

analysed using a factorial arrangement of treatments (control, control with 5.5 % orange poamce) x frequency (0.1, 1.0, 10.0) with days as blocks. Means were compared using the F-protected least significant difference procedure. Sensory characteristics were analysed using ANOVA in a randomised block design with panellists as blocks to compare control sample and control with 5.5 % orange pomace bread.

Statistical analysis was carried out using GenStat statistical software programme (GenStat 14.1, VSN International Ltd., UK).

Panellists preferences data were analysed by means of the Chi-Square test. (SAS 9.3, SAS Institute Inc., Cary, NC, USA.)

4.4 Results and discussion:

4.4.1 Pasting properties of the flour slurries (RVA):

As gluten is absent in gluten-free formulations, starch takes its place and is the main governing factor in the formulation. Starch plays a role in many of the crucial elements in the pre- and post-development of bread i.e. creating a network to replace gluten. The type of starch present can have a significant impact on the quality of the final product in particular, starch retrogradation, which is a major determinant of staling. Therefore, to help assess the quality of the bread, it is important to understand the starch pasting properties and how the presence of other ingredients such as fibrous and hydrocolloid ingredients influence this (Ji, Zhu, Zhou & Qian, 2010).

The addition of orange pomace to the control formulation had the most significant effect on the trough viscosity ($p<0.001$) and final viscosity ($p<0.001$) parameters of the RVA graph, represented in Figure 4-1.

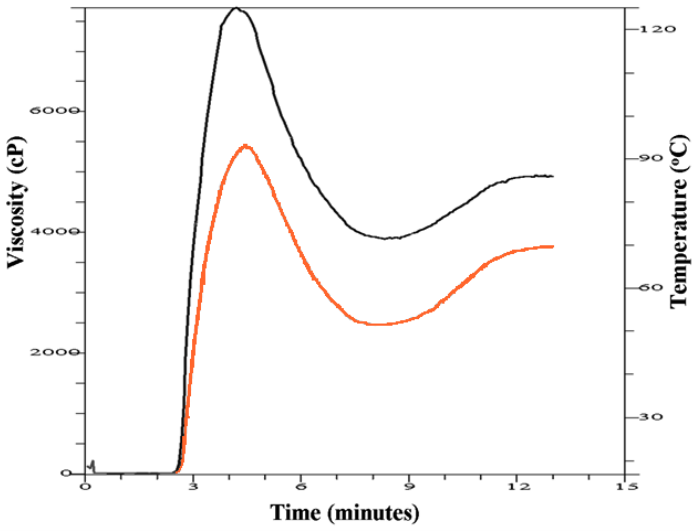


Figure 4-1: RVA graph of the control slurry and control with 5.5 % orange pomace starch slurry. (black = control, orange = orange pomace).

Trough viscosity is described as the minimum viscosity once the cooling cycle has started; it illustrates the ability of the starch to withstand shear stress and heating. Adding orange pomace to the rice and potato flours created a batter with a lower trough viscosity compared to the control; it also had lower final viscosity compared to the control sample as seen in Table 4-2.

Table 4-2: Pasting properties from the RVA.

Starch/flour mix:	Peak Viscosity (cP) [a]	Trough Viscosity (cP) [a]	Final Viscosity (cP) [a]	Setback (cP) [a]
Control	6932 ± 710	3729 ± 151	4906 ± 22	1177 ± 129
Orange Pomace	5483 ± 52	2485 ± 16 [b]	3776 ± 17 [b]	1290 ± 3

[a]Values are means +/- standard deviations.

[b]Indicates a significant difference at ($p<0.001$).

Final viscosity is described as the viscosity created during the second holding period (Lazaridou & Biliaderis 2009).

This parameter relates to quality of the paste or gel formulated after the initial heating period with a holding period, a cooling period and finally a second holding period. It reflects the ability of the starch granules to re-associate after the heating and cooling cycles. The decrease in viscosity seen in the sample containing orange pomace is most likely a consequence of the following reasons. The starch is diluted (via the fibrous nature of the orange pomace) thus a reduced amount of swollen starch granules are present, resulting in a decrease in viscosity. Also the high fibre present in the orange pomace slurry may be

competing for water with the starch; as water has a higher affinity to the fibre, a decrease in the amount of fully hydrated starch granules occur, hence decreasing viscosity of the overall batter. In the previous study where the final baked bread characteristics were investigated, the number of cells found in the control bread slices were higher, possibly as a result of this higher final viscosity parameter where the gas bubbles were retained during proofing. Mais & Brennan (2008), who investigated the addition of sweet potato starch and fibre in a wheat starch slurry and reported similar results with respect to a reduction in final viscosity. When a high fibre containing ingredient is added to starch slurry, the starch becomes diluted and the pasting properties decrease. The physco-chemical properties of starch are changed due to the presence of the fibre.

As mentioned previously, another potential reason for a decrease in viscosity is due to water. Fibre has a high water binding capacity; therefore the starch and fibre compete for the formulations water. As fibre absorbs more of the water then starch, a reduction in starch granule swelling takes place leading to a decrease in viscosity (Angioloni & Collar 2009).

Ktenioudaki et al. (2012) studied the effects of apple pomace and brewer's spent grain in a wheat flour blend. They reported that both by-products decreased the pasting properties and the resulting RVA curves were generally lower than the wheat control. Both by-products are known to be high in dietary fibre, hence emphasising the effects fibre have in a dough system.

4.4.2 Batter pasting properties (mixolab):

The Mixolab produces results which can be seen as a combination of the Farinograph and the rapid visco analyzer (RVA). Hence, it was possible to investigate the effect of orange pomace on the mixing and pasting properties of the entire gluten-free formulation using the Mixolab (Figure 4.2).

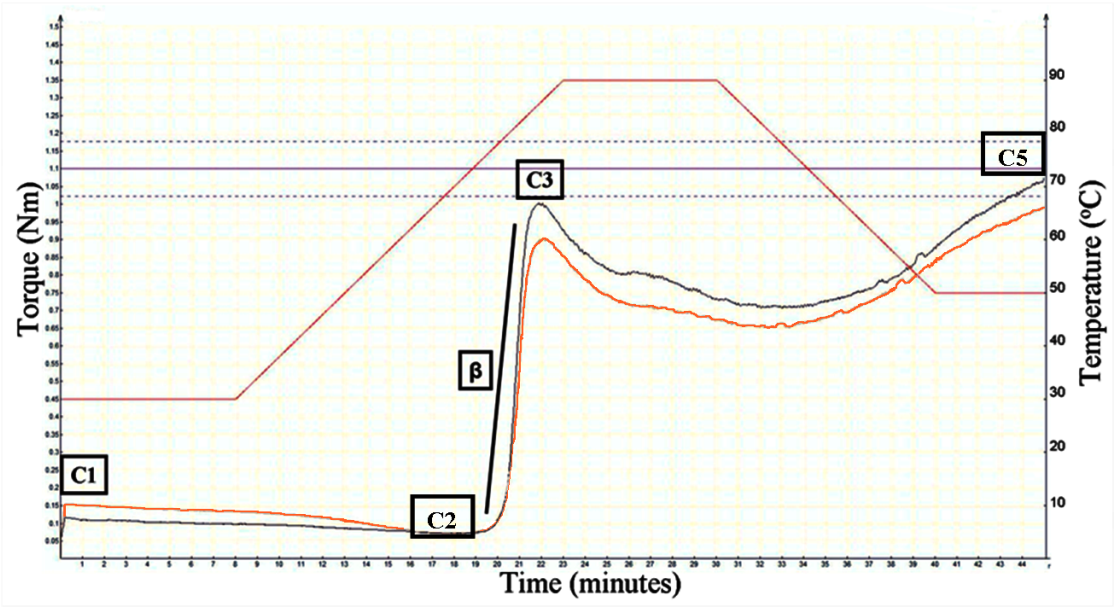


Figure 4-2: Mixolab graph of the control batter and control with 5.5 % orange pomace batter formulation. (black – control, orange–orange).

From Table 4-3, it can be seen that the inclusion of orange pomace created a significantly different C1 value compared to the control ($p<0.05$). The C1 parameter is described as the initial mixing properties of the batter. The Mixolab measures the torque required to mix the batter (in real time) by two kneading arms in a mixing block at a constant temperature (30 ^{o}C) (Collar, Bollain & Rosell, 2007). It initially helps to distinguish a water absorption level, and then provides information relating to the strength of the batter. As the optimal

hydration of the batter had previously been calculated, this mixing property was the main interest for the present study. The batter containing orange pomace at 5.5 % developed a stronger consistency (0.160 Nm) when compared to the control (0.109 Nm, $p<0.05$). As the orange pomace batter presented a higher C1 value this signifies greater firmness than the control batter, indicating a stronger batter formulation. The next section of the Mixolab curve, (C2) describes the protein weakening properties of the material being tested. However, this was not of relevance to the present study, as none of the raw materials used were particularly proteinaceous in nature.

Table 4-3: Mixolab properties.

Gluten-free batter:	**C1 (Nm)[a]**	**β (Nm)[a]**	**C3 (Nm)[a]**
Control	0.109 ± 0.02	0.973 ± 0.003	1.028 ± 0.01
Orange pomace	0.160 ± 0.02 [b]	0.870 ± 0.011[c]	0.935 ± 0.01 [c]

[a]Values are means +/- standard deviations.

[b]Indicates a significant difference at $p<0.05$.

[c]Indicates a significant difference at $p<0.01$.

The section from C3 to C5 of the graph represents the starch pasting properties of the batter. C3 measures the maximum torque required for gelatinisation during the heating stage (Bonet, Blaszczak & Rosell, 2006). The control batter was found to require a higher torque at this stage than the orange pomace batter. Therefore, the control had a higher gelling ability; or in the presence of orange pomace there was a reduction in granule swelling ($p<0.01$). Similar to the low

RVA viscosity results, one such reason for a lower C3 parameter for the batter containing orange pomace could be attributed to starch dilution as a result of the incorporation of orange pomace batter, which also contains a high level of fibre. Torbica et al. (2010) demonstrated comparable results to this study. The authors compared the properties of a blend of rice flour and un-husked buckwheat to rice flour on its own. They found the rice flour/buckwheat blend resulted in lower gelatinisation results when compared to 100 % rice flour. They described how the un-husked buckwheat flour contained higher cellulose compared to the rice flour cellulose content, and this additional cellulose was seen to have contributed to the reduction in the torque required for gelatinisation of the starch. The final Mixolab parameter on the graph (C5) is described as the torque recorded when cooling the batter to 50 ^{o}C. During this time, the consistency of the batter increases with decreasing temperature to re-form a gel (Koksel et al., 2009).

Beta (β) is described as the rate of gelatinisation; it is calculated as C3-C2 (Rosell, Marco, Garcia-Alvarez & Salazar, 2011). Batter containing orange pomace had a lower rate of gelatinisation when compared with the control ($p<0.01$). Thus this lower gelatinisation rate, coupled with a lower torque requirement for gelatinisation highlights the initial potential of orange pomace to reduce the rate of gelatinisation and have positive effects on the final texture of the baked bread.

4.4.3 Batter rheology (rheometer):

4.4.3.1 Frequency sweep:

Oscillatory testing was carried out to examine the viscoelastic properties of the gluten-free batters. Generally, oscillatory tests can be described as submitting a sample to either a stress or a frequency that varies with time (Miri 2011).

Results from the mechanical spectrum illustrate that the batter containing orange pomace had a higher storage modulus (G') when compared to the control batter (Table 4-4). After the oscillation has been removed, the energy stored in the material is described as the G'. It can be seen as a measure of elasciticty (Sullivan, O'Flaherty, Brunon, Arendt & Gallagher, 2010). The orange pomace containing batter had greater elasticity then the control ($p<0.05$). As seen in Table 4-4 it can be observed that as the frequency increased (0.1-10 Hz) so too did the storage modulus of the samples ($p<0.001$).

Table 4-4: Storage Modulus (G') & Complex Modulus (G*).

	Frequency (HZ)	**0.1** [b]	**1** [b]	**10** [b]
Storage Modulus (G')	Control	5.70E+03	1.08E+04	1.83E+04
	Orange Pomace [a]	7.42E+03	1.43E+04	2.43E+04
Complex Modulus (G*)	Control	6.43E+03	1.17E+04	1.96E+04
	Orange Pomace [a]	8.30E+03	1.54E+04	2.61E+04

[a]Indicates a significant difference at ($p<0.05$).

[b]Indicates a significant difference at ($p<0.001$).

Torbica et al. (2010) investigated the effects of husked and un-husked buckwheat flour when included in rice flour dough and subjected to a frequency sweep. The authors found that up to a certain level of inclusion, the buckwheat produced a more elastic batter when compared to the control, however at levels greater than 10 %, G' decreased, yielding a dough which became less elastic and required less force to deform it, when compared to the control. The G' values of buckwheat and rice dough were also seen to increase slightly with frequency these authors propose this increase was a characteristic of the gel formed. They suggested that changes in gel strength were as a result of changes occurring between macromolecular organisations such interactions between protein and polysaccharides.

As stated already, orange pomace batter produced a higher G' compared to the control, which increased with increasing frequency. The increase in G' following orange pomace addition may be attributed to the interactions occurring between the methylcellulose (MC), orange pomace and starch. In the previous baking study, orange pomace was suggested to have a supportive role with MC; whereby a starch/MC/fibre matrix could be created, which was visco-elastic enough to retain carbon dioxide (CO_2), therefore producing baked loaves of a higher specific volume.

The complex modulus (G*) is another important parameter when describing the batter system. It is explained as the overall measure of stiffness or firmness of the sample (Miri 2011).

It can be observed from Table 4-4 & Figure 4-3, that under the test conditions, the orange pomace batter yielded a higher G* when compared to the control ($p<0.05$).

Similar to the G' result, as the frequency increased (0.1-10 Hz) so too did the G* ($p<0.001$). As seen in the previous sections, the addition of orange pomace to the batter created a stiffer or stronger batter when compared to the control. Sullivan et al. (2011) considered the effects of barley middlings (a fibrous by-product of barley milling) in wheat dough. It was observed that the presence of barley middlings created a higher G* compared to the control wheat dough. The increase in G* was proposed to occur due to the high level of fibre. This fibre has a stronger affinity then the gluten present for water thus generating firmer dough.

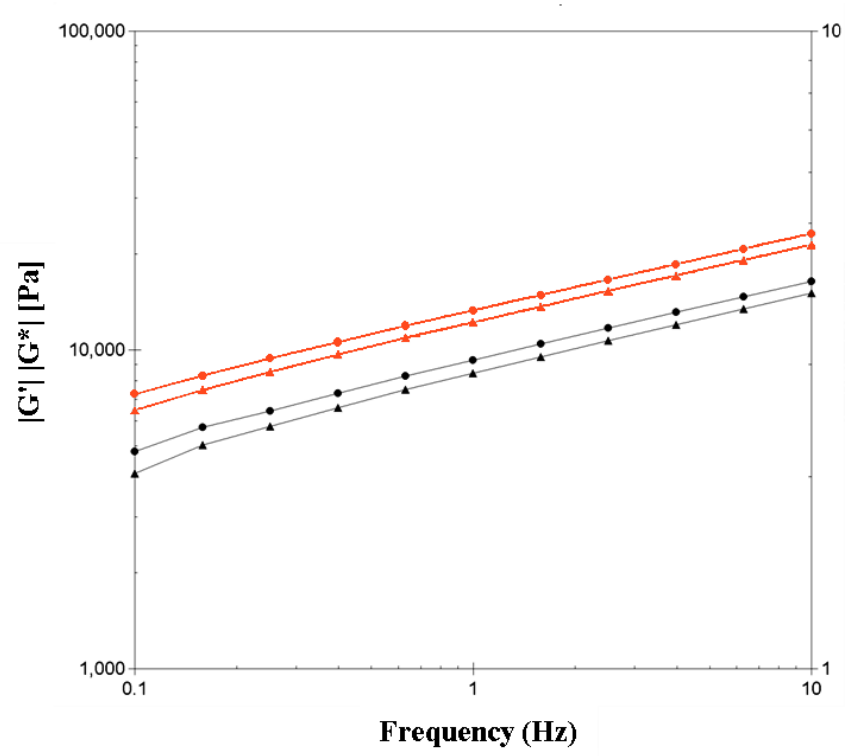


Figure 4-3: Frequency sweep of control batter and control with 5.5 % orange pomace batter (Black-control, orange-orange pomace). G' - ▲, G* - ●.

4.4.4 Batter and bread microstructure:

4.4.4.1 Cryo-scanning electron microscopy:

Cryo-scanning electron microscopy (Cryo-SEM) was used to help identify and make initial observations on the structure and components visible in the gluten-free batters and breads.

Representative images of the batters and breads are shown in Figure 4-4.

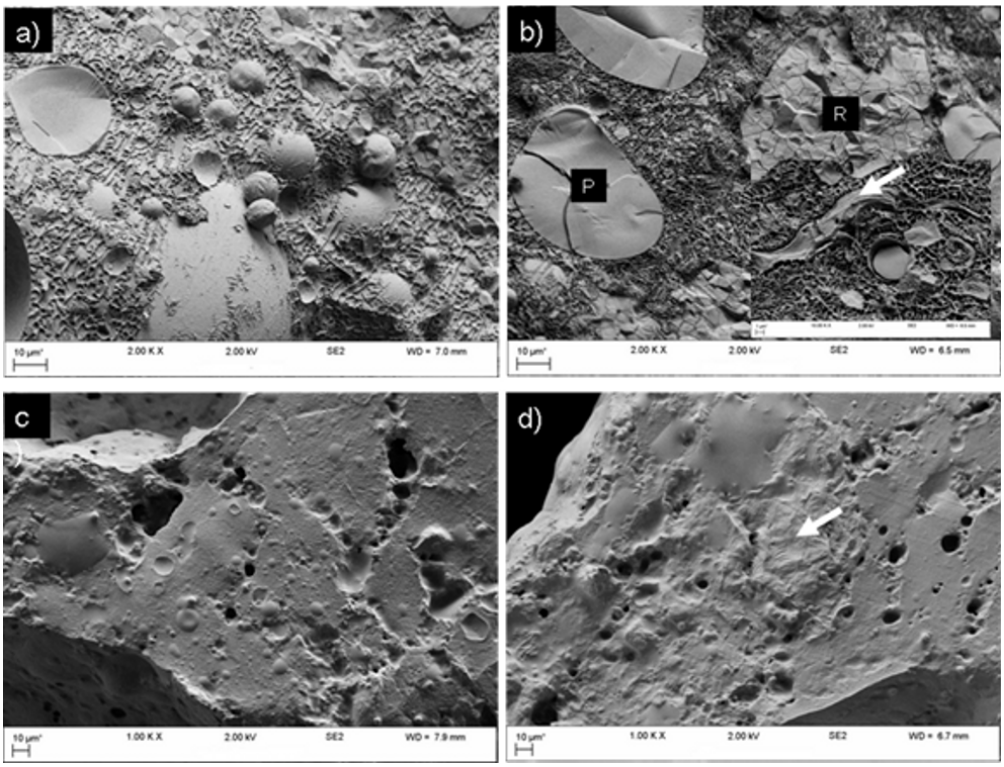


Figure 4-4: Cryo-scanning electron micrographs of batters (a & b) and breads (c & d) [Control samples (a & c); control + 5.5 % orange pomace samples (b & d). Orange pomace is highlighted by an arrow (b & d). Potato starch grains (P) and rice starch complexes (R) can be clearly seen in batter samples. Scale bar = 10 µm.]

Cryo-SEM of the batters showed the presence of rice starch complexes and individual large potato starch granules dispersed in an aqueous matrix in

both control and control + 5.5 % orange pomace samples (Fig 4-4 a & b). The control and 5.5 % orange pomace, in addition, contained fibrous inclusions (Fig 4-4 b, arrow) that are most likely orange pomace fragments of cellulosic tissue. These fibrous particles were not present in the control sample. The images were selected from at least 30 other cryo-SEM images to be representative. The fibrous structures were seen in nearly all the orange pomace samples but not in the control. The size and shape of the fibrous regions correspond to those seen in the confocal microscope and do not appear to be due to ice crystal formation, which is the most common artefact generated by cryo-SEM. The use of cryo-fixation was chosen as this eliminates artefacts that can occur during traditional wet fixation and dehydration EM techniques. Light microscopy also confirmed the fibrous nature of the orange pomace ingredient (results not shown).

A more subtle difference seen between the two samples was the size of ice crystals. The control sample had noticeably larger ice crystals, seen as angular spaces in the aqueous phase, compared to the control and 5.5 % orange pomace sample. During cryo preparation, unbound moisture tends to form ice crystals. The size of these crystals is related to the amount of free water and the presence of solutes. Therefore, the higher the free moisture available for freezing, the larger the ice crystals developed. Results indicate that there is more free moisture in the control compared to the control and 5.5 % orange pomace batter. This observation is consistent with the theory that the orange pomace, being hydrophilic, competes for moisture and reduces water available for starch hydration (see CLSM results below).

Cryo-SEM of the breads showed the presence of fibrous material in the control and 5.5 % orange pomace sample (Fig 4-4 d, arrow). The starch granules are

more easily visible in the control bread, compared to the orange pomace bread where they are not as easily visible. More circular air pockets can be viewed in the control bread compared to the control and 5.5 % orange pomace bread. A reduction in starch viscosity as a result of it being diluted by the orange pomace may create more uneven cell sizes in the crumb structure of bread containing orange pomace.

4.4.4.2 Confocal laser scanning microscopy:

Confocal Laser scanning microscopy (CLSM) uses a bulk specimen to produce optical sections, which can be further analysed with the use of stains to identify and differentiate different structures in a batter or bread system (Arendt, Renzetti & Dal Bello, 2009).

For the first time, we have used multiple labelling to localise all the main ingredients of gluten-free batter and bread containing orange pomace.

The control batter can be seen in Fig 4-5 a Starch is green in colour (FITC labelled); larger green granules represent individual potato starch granules. Rice flour particles comprise complexes of smaller starch granules bounded by a fine layer of protein. Fat could be seen as small (<3 μm) bright yellowish green droplets and protein is seen as discrete red particles. From the control image it can be observed that potato starch granules are well labelled with dye penetrating to the centre of the granule.

The batter containing orange pomace is shown in Fig 4-5 b. The orange pomace itself is pseudocoloured purple and has been effectively labelled with Calcofluor White. The control with 5.5 % orange pomace batter has potato starch granules

that have not been labelled throughout the grain, just the outer region, resulting in a darker centre and do not appear to be as swollen compared to the control. This suggests that the starch is less hydrated in the orange pomace sample and is corroborated by the cryo-SEM results showing less free moisture. It is therefore likely that the orange pomace prevents starch from being fully hydrated; hence less starch is swollen thus confirming results discussed in the RVA section. Instead, the presence of orange pomace creates orange pomace and starch complexes, which potentially cause the batter to become stiffer (as discussed in the frequency sweep section) potentially offering more strength to the batter system.

Mariotti et al. (2009), viewed the presence of psyllium in a gluten-free batter and similar findings were reported. These authors found a reduction in swollen starch granules when psyllium was present. The presence of psyllium also promoted groups of starch psyllium objects which they hypostasised helped offer stability to the batter. The authors offered a second reason for viewing a more uniform structure; some characteristics of psyllium such as gelling and water-absorbing abilities may help limit starch swelling and gelatinisation.

When a gluten-free batter is compared to a wheat dough, wheat dough develops a more homogenous and continuous matrix after mixing; when there is no gluten present, the starch is free to swell creating an uneven and heterogeneous structure. CSLM of the control and control and 5.5 % orange pomace breads is shown in Fig 4-5 c & d.

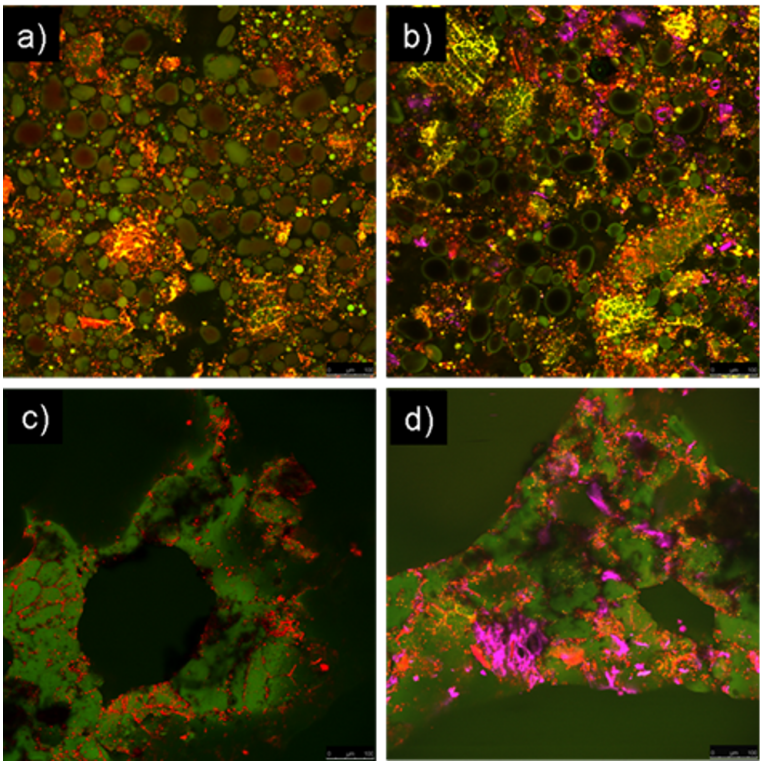


Figure 4-5: Confocal scanning laser micrographs of batters (a & b) and breads (c & d). [Control samples (a & c); control & 5.5 % orange pomace samples (b and d). Quadruple labelled to show starch (pale/dark green); protein (red); liquid fat (yellow) and cellulosic orange pomace (purple). Scale bar = 10 µm.]

Starch granules in the control bread appeared significantly larger and more swollen than those of the control and 5.5 % orange pomace bread suggesting that they were more highly gelatinised. Fig 4-5 d represents the orange pomace bread and clearly shows the presence and distribution of the orange pomace (purple). The reduced apparent gelatinised starch area is most likely a combination of volume exclusion due to the large orange pomace particles in addition to reduced moisture availability, suggested that a reduction in gelatinisation occurred, confirming results discussed in the Mixolab section, where the rate and peak of gelatinisation were reduced when orange pomace was present.

Alvarez-Jubete et al. (2010) documented, similar results when high fibrous pseudocereals replaced part of a rice flour and potato starch base gluten-free formulation. They reported that a greater degree of starch gelatinisation occurred in control samples; which they described as starch granules swelling, fusing together and losing their original shape. From the CLSM images, it could be highlighted that gelatinisation was reduced when pseudocereals were added.

4.4.5 Sensory analysis:

Figure 4-6, depicts the sensory analysis results. It can be seen that there was no significant difference among the sensory bread characteristics, apart from 'texture while chewing' ($p<0.001$). For this parameter, the control sample was found to have a more acceptable texture when compared to the orange pomace sample. Panellists were also asked to rank which sample they preferred (control versus control with 5.5 % orange pomace inclusion). No significant difference was found, indicating that the 5.5 % orange pomace bread received similar results when compared to the control bread.

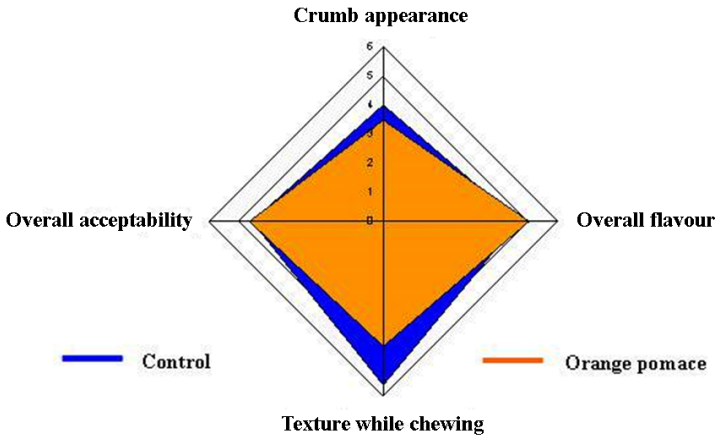


Figure 4-6: Sensory results. [Blue line - control bread & orange line - orange pomace bread.]

4.5 Conclusion:

Orange pomace played a significant role in improving the structure of a gluten-free batter. The RVA and Mixolab results were in agreement as to how orange pomace affected the pasting properties of the batter. In particular, the trough viscosity and final viscosity parameters illustrated the high water binding effects of the fibrous by-product ingredient. CLSM images of the batter that showed a continuous matrix of orange pomace/starch complexes that produced a firm and elastic batter system.

Finally, sensory analysis revealed no significant difference between characteristics such as appearance, flavour and acceptability, although the control was preferred slightly for texture while chewing.

After an extensive batter and baking investigation, the studies have highlighted how orange pomace can be used as a novel, low cost and value-added ingredient in gluten-free bread.

The authors have now extensively studied a gluten-free system which includes a novel ingredient. This has traversed pre-baking characteristics, post-baking properties and sensory acceptability.

4.6 References:

Alvarez-Jubete, L., Auty, M., Arendt, E.K., & Gallagher, E. (2010). Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. *European Food Research and Technology,* 230(3), 437-445.

Angioloni, A., & Collar, C. (2009). Gel, dough and fibre enriched fresh breads: Relationships between quality features and staling kinetics. *Journal of Food Engineering,* 91(4), 526-532.

Arendt, E.K., Renzetti, S., & Dal Bello, F. (2009). Dough Microstructure and textural aspects of gluten-free yeast bread and biscuits. In: Gallagher, E. (Eds.), *Gluten-free food science and technology*. Blackwell Publishing Ltd., Singapore, pp. 106-129.

Bonet, A., Blaszczak, W., & Rosell, C.M. (2006). Formation of homopolymers and heteropolymers between wheat flour and several protein sources by transglutaminase-catalyzed cross-linking. *Cereal Chemistry,* 83, 655-662.

Collar, C., Bollain, C., & Rosell, C.M. (2007). Rheological behaviour of formulated bread doughs during mixing and heating. *Food Science and Technology International,* 13(2), 99-107.

Dobraszczyk, B.J., & Morgenstern, M.P. (2003). Rheology and the breadmaking process. *Journal of Cereal Science,* 38(3), 229-245.

Fernandez-Lopez, J., Sendra-Nadal, E., Navarro, C., Sayas, E., Viuda-Martos, M., & Alvarez, J.A.P. (2009). Storage stability of a high dietary fibre powder from orange by-products. *International Journal of Food Science and*

Technology, 44(4), 748-756.

Gallagher, E., Gormley, T.R., & Arendt, E.K. (2004). Recent advances in the formulation of gluten-free cereal-based products. *Trends in Food Science & Technology*, 15(3-4), 143-152.

Green, F,L. (1990). Monograph on Fluorescent Brightener 28. In: *The SigmaAldrich Handbook of Stains, Dyes and* Indicators. Aldrich Chemical Company, Wisconsin, US, p. 382.

Houben, A., Höchstötter, A., & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: an overview. *European Food Research and Technology*, 1-14.

Ji, Y., Zhu, K., Zhou, H., & Qian, H. (2010). Study of the retrogradation behaviour of rice cake using rapid visco analyser, fourier transform infrared spectroscopy and X-ray analysis. *International Journal of Food Science & Technology*, 45(5), 871-876.

Kenny, S., Wehrle, K., Auty, M., & Arendt, E. (2001). Influence of sodium caseinate and whey protein on baking properties and rheology of frozen dough. *Cereal Chemistry*, 78(4), 458-463.

Koksel, H., Kahraman, K., Sanal, T., Ozay, D.S., & Dubat, A. (2009). Potential utilization of mixolab for quality evaluation of bread wheat genotypes. *Cereal Chemistry*, 86(5), 522-526.

Ktenioudaki, A., O'Shea, N., & Gallagher, E. (2012). Rheological properties of wheat dough supplemented with functional by-products of

foodprocessing: Brewer's spent grain and apple pomace. *Journal of Food Engineering*, 116(2), 362-368.

Lazaridou, A, & Biliaderis, C.G. (2009). Gluten-free Doughs: Rheological properties, testing procedures – methods and potential problems. In: Gallagher, E. (Eds), *Gluten-free food science and technology*. Blackwell Publishing Ltd, Singapore, pp.52-82.

Mais, A., & Brennan, C.S. (2008). Characterisation of flour, starch and fibre obtained from sweet potato (kumara) tubers, and their utilisation in biscuit production. *International Journal of Food Science & Technology*, 43(2), 373-379.

Mariotti, M., Lucisano, M., Pagani, M.A., & Ng, P.K.W. (2009). The role of corn starch, amaranth flour, pea isolate, and Psyllium flour on the rheological properties and the ultrastructure of gluten-free doughs. *Food Research International*, 42(8), 963-975.

Miri, T. (2011). Viscosity and Oscillatory Rheology. In: Norton, I.T., Spyrocpoulose, F., Cox, P. (Eds), *Practical Food Rheology: An Interpretive Approach*. Markono Print Media Pte Ltd, Singapore, pp.7-28.

O'Shea, N., Arendt, E.K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science & Emerging Technologies*, 16, 1-10.

Rosell, C.M., Santos, E., & Collar, C. (2009). Physico-chemical properties of

commercial fibres from different sources: A comparative approach. *Food Research International*, 42(1), 176-184.

Rosell, C.M., Marco, C., Garcia-Alvarez, J., & Salazar, J. (2011). Rheological properties of rice-soybean protein composite flours assessed by mixolab and ultrasound. *Journal of Food Process Engineering*, 34(6), 1838-1859.

Schieber, A., Stintzing, F.C., & Carle, R. (2001). By-products of plant food processing as a source of functional compounds — recent developments. *Trends in Food Science & Technology*, 12(11), 401-413.

Sullivan, P., O'Flaherty, J., Brunton, N., Arendt, E. & Gallagher, E. (2010). Fundamental rheological and textural properies of doughs and breads produced from milled pearled brley flour. *European Food Research and Technology*, 231(3), 441-453.

Sullivan, P., O'Flaherty, J., Brunton, N., Arendt, E., & Gallagher, E. (2011). The utilisation of barley middlings to add value and health benefits to white breads. *Journal of Food Engineering*, 105(3), 493-502.

Torbica, A., Hadnadev, M., & Dapcevic, T. (2010). Rheological, textural and sensory properties of gluten-free bread formulations based on rice and buckwheat flour. *Food Hydrocolloids*, 24(6-7), 626-632.

Van de Velde, F., van Riel, J., & Tromp, R.H. (2002). Visualisation of starch granule morphologies using confocal scanning laser microscopy (CSLM). *Journal of the Science of Food and Agriculture*, 82(13), 1528-1536.

Chapter 5

Enhancing an extruded puffed snack, by optimising die head temperature, screw speed and apple pomace inclusion.

Published in Food and Bioprocess Technology (2013), 1-16.

5 Chapter 5: Enhancing an extruded puffed snack, by optimising die head temperature, screw speed and apple pomace inclusion.

5.1 Abstract:

Extrusion cooking is recognised as a smart technology for food processors. It requires low cost, high temperature, short-time process and few ingredients to create a puffed snack. The only drawback is that it contains multiple parameters that need to be rigorously trialled to develop an optimal process. This study investigated the effects of two extruding parameters (die head temperature and screw speed) and examined the addition of apple pomace into a corn flour-based extruded snack formulation. A response surface design was utilised. A D-optimal design was chosen, which generated 21 combinations; within these combinations, the control formulation existed. Extrudate characteristics, i.e. bulk density and porosity, textural properties, cooked starch properties and moisture, were analysed. Screw speed was found to have the greatest effect on extrudate quality, e.g. bulk density increased as the screw speed increased ($p < 0.001$). Both apple pomace addition and screw speed impacted expansion ratio; as they increased, expansion decreased ($p < 0.0001$). The optimised and validated formulation contained the following parameter levels: 7.7 % apple pomace, 150 °C die head temperature and a screw speed of 69 rpm. As apple pomace and corn flour are naturally gluten free, the extruded product would appeal to people who suffer from intolerances, allergies and coeliac disease.

5.2 Introduction:

Due to the cost of alternative cereals, food processing aids (e.g. enzymes, emulsifiers and anti-staling agents) and processing, manufacturing gluten-free products can be challenging and expensive for the producer. Extrusion cooking presents itself as a low cost, high temperature and short time process (HTST). Such products as breakfast cereals, flat breads, puffed snacks and modified starches can be developed with extrusion cooking.

During extrusion cooking, the raw materials experience chemical and structural transformations e.g. protein denaturation, starch gelatinization and complex creations i.e. amylase and fat complexes. The high temperature environment modifies moistened expansible starchy material via a combination of high pressure and shear forces to produce a puffed end-product (Asare, Sefa-Dedeh, Sakyi-Dawson & Afoakwa, 2004; Singh, Sekhon & Singh, 2007; Yang, Peng, Lui & Lin, 2008). The driving force helping build up this high pressure and shear force is the screw. Extruders generally consist of a single or twin screw; the twin screws usually operate in a co-rotating or counter-rotating manner within the extruder barrel (Brennan, Derbyshire, Tiwari & Brennan, 2013).

Although extrusion cooking is a quick and cost effective technology, it does contain many variables which need to be considered and optimised. Such variables as screw speed, temperature, moisture content and the physical characteristics of the raw materials must be taken into consideration before the process can start. Optimal screw speeds have been described as being one of the main variables responsible for an increased expansion, as they cause the shearing action which results in a decreased melt viscosity, creating a greater expanded

end snack (Moraru & Kokini 2003). Temperature has been discussed as changing the rheological properties of the extruded melt which similar to screw speed can help increase the final expansion. Excess screw speeds and high temperature can develop extreme melt softening and degradation of the starch melt which develop a weak structure and poor extrudate expansion. The moisture content is the accelerating force behind expansion and contributes to the rheological attributes of the melt. Excessive moisture content decreases melt viscosity developing a week structure which can collapse on being extruded; hence these three factors need numerous trials to produce an optimised process (Moraru & Kokini 2003).

Cereals such as maize are ideal for extrusion; they are gluten-free, easily accessible and have a high starch content which can produce excellent expansion characteristics. The main disadvantage of using maize is that it is likely to be relatively low in nutrients such as dietary fibre and minerals (Pastor-Cavada et al., 2011).

Incorporation of by-products unwanted after fruit processing may be a solution to enhancing the nutrition of an extruded puffed snack without compromising the snacks structure. During fruit processing e.g. the pressing of apples, to produce apple juice up to one third of the fruit is discarded. Researchers have shown that by-products such as apple pomace contain a high level of dietary fibre and bio actives e.g. vitamins, minerals and phytochemicals (Gorinstein et al., 2001; Wijngaard, Rößle & Brunton, 2009). When used at an optimal level they have been shown to have a high functionality in products, such as fat replacers, bulking agents, water binding capacity and increased water holding ability of a

product (Rosell, Santos & Collar, 2009; O'Shea, Arendt & Gallagher, 2012). Previous researchers have studied the effects of by-product pomace blends (that included orange peel, grape seed and tomato pomace) in a single screw extruder on the physical and functional properties of a rice grit based extruded snack (Yagci & Gogus 2008; Yagci & Gogus 2009). Extensive work has been carried out using twin screw extruders to investigate the effects of tomato pomace, grape pomace, brewers spent grain and red cabbage trimmings on the macro-structure and functional aspects of extruded puffed snacks (Altan, McCarthy & Maskan, 2008a, 2008b; Altan, McCarthy & Maskan, 2009; Stojceska, Ainsworth, Plunkett & İbanoğlu, 2009). Twin-screw extruders have also been utilised to study the effects of apple pomace and apple powder on the physical and nutritional contents of an extruded snack (Stojceska, Ainsworth, Plunkett & İbanoğlu, 2010; Karkle, Alavi & Dogan, 2012). Where literature is lacking or absent is in investigating the effects of apple pomace using a single screw extruder. To explore apple pomaces effect on the structural and functional aspects of an extruded puffed corn snack. This is of particular interest when apple pomace has been described as having so much potential as an ingredient due to its nutrition and physiochemical attributes.

Response surface design (RSD) is a unique statistical tool, as it evaluates the effects of variables on processes or formulations. It then allows an optimised product or process to be developed based on the factors impact on the responses (McCarthy, Gallagher, Gormley, Schober & Arendt, 2005). Due to the nature of extrusion experiments (containing a variety of extrusion parameters) RSD is an ideal statistical tool. It reduces the amount of experiments while conserving time

without neglecting the integrity of the study. It has been successfully used in extrusion studies previously (Singh et al., 2007; Yang et al., 2008). To examine the full effects of apple pomace in a corn puffed snack, extensive preliminary trials were carried out to produce a control with favourable expansion characteristics and known moisture content. This would allow the authors to make strong comparisons between combinations and see the effects of the two chosen extrusion parameters.

The main objectives of this study were:

- To include apple pomace as a value added ingredient into an extruded formulation using a single screw extruder
- To assess the product quality characteristics of the extruded snack via observing bulk density, specific volume, porosity and expansion ratio
- To assess the effects of apple pomace on an extruded snack by investigating the starch cooking properties and the texture of the snack
- To optimise both the process and the formulation to create a snack of equal or better than quality compared to the control extruded snack

5.3 Materials and methods:

5.3.1 Raw materials:

Medium corn flour (polenta) was obtained from Shakeltons Mills (Ireland). Corn flour was dried to < 8.5 % moisture content at 80 ^{o}C for 3 hours (Tom Chandley Ovens, Manchester, UK) vacuum packed in polyethylene bags and stored at room temperature until required. Gluten-free sodium bicarbonate (Dr Oetker purchased in local supermarket) was added to each formulation at 1 % flour weight basis.

The apple pomace (The Apple Farm, Co. Tipperary, Ireland) consisted of the peel, pulp and seeds remaining after juice separation. Apple pomace was received in its wet state shortly after juice separation. To eliminate fermentation, the pomace was freeze dried immediately, milled to a particle size of <355 microns and stored at -20 ^{o}C until required. Its addition was based on preliminary trials to establish its limits of usage and following this, values were dictated by the response surface design.

5.3.1.1 Sample preparation:

Initial preparation of the corn flour consisted of sieving corn flour and sodium bicarbonate together. The corn flour and sodium bicarbonate were then pre-hydrated with filtered water to 10.5 % moisture content. Corn flour was supplemented and sieved with apple pomace according to the combinations generated from the response surface design and pre-hydrated to 10.5 % moisture as already described. The blends of ingredients were stored in polyethylene bags

and left to equilibrate overnight before extrusion. Prior to extrusion, moisture analysis was again carried out to ensure the blend of ingredients contained 10.5 % moisture.

5.3.2 Extrusion:

Extrusion trials were performed with a KE 19/25 D single-screw, laboratory-scale extruder (Brabender, Germany). The barrel diameter and L/D ratio were 19 mm and 25:1, respectively. The screw compression ratio was 3:1 and the die opening had a diameter of 3 mm. Feed rate was 20 g/min, which remained constant for all experiments. Barrel temperature in the feed zone was set to 75 °C (zone 1), transition zone was 105 °C (zone 2) and the final zone was 135 °C (zone 3). Barrel temperatures and feed rate were established following extensive preliminary trials. Die head temperature for each experiment was set as outlined in Table 5-1 and 5-2. The extruder was powered by a 2.5 kW motor with speeds variable from 0 to 150 rpm. Screw speeds for each experiment are shown in Table 5-1 and 5-2. The extruder was equipped with a torque indicator, which showed the percentage of torque relative to the current drawn by the drive motor. Extrudates were collected when extruder parameters (motor torque, die pressure and die temperature) were stable (after 3-4 minutes). Extruded samples were cooled to room temperature for approximately 30 minutes.

Table 5-1: Response mean and standard deviations for extrudate physical characteristics results.

Design	Apple Pomace (%)		Screw Speed (rpm)		Die Head Temp (^{o}C)		RER	Bulk Density (g/cm^{3})	Porosity	Spec Vol (mL/g)	Moisture (%)
No.	Coded	Actual (%)	Coded	Actual (RPM)	Coded	Actual (^{o}C)	Avg / St. Dev	Avg / St. Dev	Avg / St. Dev	Avg / St. Dev	Avg / St. Dev
1	1	10	-1	60	0	175	3.64 +/- 0.11	0.20 +/- 0.02	0.42 +/- 0.05	5.27 +/- 0.42	7.17 +/- 0.15
2 (Ctrl)	-1	0	0	80	0	175	4.45 +/- 0.15	0.15 +/- 0.02	0.51 +/- 0.05	6.25 +/- 0.40	7.20 +/- 0.10
3	0.5	7.5	0	80	-0.5	162.5	4.01 +/- 0.13	0.14 +/- 0.01	0.58 +/- 0.03	6.68 +/- 0.36	6.50 +/- 0.10
4	1	10	1	100	1	200	2.50 +/- 0.09	0.46 +/- 0.05	0.00 +/- 0.00	2.85 +/- 0.27	6.20 +/- 0.17
5	0	5	-1	60	-1	150	5.01 +/- 0.12	0.13 +/- 0.00	0.51 +/- 0.01	7.05 +/- 0.23	8.43 +/- 0.12
6	-1	0	-1	60	1	200	4.17 +/- 0.15	0.16 +/- 0.01	0.49 +/- 0.03	5.94 +/- 0.73	8.00 +/- 0.10
7	1	10	1	100	-1	150	3.77 +/- 0.13	0.21 +/- 0.01	0.47 +/- 0.04	4.74 +/- 0.64	6.70 +/- 0.00
8	0	5	0	80	1	200	3.85 +/- 0.09	0.17 +/- 0.01	0.50 +/- 0.04	5.51 +/- 0.09	6.73 +/- 0.15
9	1	10	1	100	-1	150	3.71+/- 0.12	0.27 +/- 0.02	0.40 +/- 0.04	3.66 +/- 0.26	6.60 +/- 0.10
10	1	10	-1	60	1	200	3.04 +/- 0.12	0.22 +/- 0.02	0.32 +/- 0.06	5.01 +/- 1.26	6.67 +/- 0.06
11	-1	0	1	100	-1	150	4.17 +/- 0.19	0.24 +/- 0.02	0.38 +/- 0.08	4.15 +/- 0.11	7.33 +/- 0.06
12	0	5	1	100	0	175	3.61 +/- 0.26	0.19 +/- 0.02	0.43 +/- 0.08	5.22 +/- 0.72	6.90 +/- 0.10
13	-1	0	-1	60	1	200	4.50 +/- 0.24	0.15 +/- 0.02	0.48 +/- 0.07	5.94 +/- 0.36	7.53 +/- 0.06
14	1	10	-1	60	-1	150	4.10 +/- 0.09	0.17 +/- 0.00	0.48 +/- 0.02	5.82 +/- 0.78	7.40 +/- 0.00
15	0	5	0	80	0	175	4.26 +/- 0.12	0.13 +/- 0.01	0.52 +/- 0.04	8.21 +/- 1.05	7.00 +/- 0.10
16	-1	0	1	100	1	200	3.04 +/- 0.18	0.34 +/- 0.04	0.14 +/- 0.10	3.13 +/- 0.08	6.87 +/- 0.15
17	-1	0	1	100	1	200	3.19 +/- 0.18	0.27 +/- 0.04	0.32 +/- 0.12	4.06 +/- 0.56	6.80 +/- 0.00
18	-1	0	-1	60	-1	150	5.48 +/- 0.25	0.12 +/- 0.03	0.62 +/- 0.07	7.32 +/- 0.34	8.20 +/- 0.10
19	1	10	1	100	1	200	2.91 +/- 0.16	0.27 +/- 0.03	0.21 +/- 0.08	4.53 +/- 1.09	6.30 +/- 0.10
20	-1	0	1	100	-1	150	4.07 +/- 0.14	0.32 +/- 0.03	0.25 +/- 0.07	3.29 +/- 0.29	7.33 +/- 0.12
21	-1	0	0	80	-1	150	4.54 +/- 0.17	0.14 +/- 0.01	0.59 +/- 0.03	6.50 +/- 0.47	7.47 +/- 0.12

5.3.3 Analysis:

5.3.3.1 Extrudate characteristics:

Radical expansion ratio (RER): The cross sectional diameter of the extrudate was measured using a calliper (Electronic Digital Caliper, Ultra Präzision Messzeuge GmbH, Germany). The expansion ratio was calculated as the cross sectional diameter of the extrudate (d) divided by the diameter of the die opening (d_o), 15 extrudates per experiment was measured (equation 5-1) (Meng, Threinen, Hansen & Driedger, 2010).

$$\mathrm{Exp} = \frac{\mathrm{d}}{\mathrm{d_o}}$$

Equation 5-1: Radical expansion ratio (RER).

Bulk density (ρ_b): The diameter (d) and length (l) of the extrudates was measured using a calliper (Electronic Digital Calliper, Ultra Präzision Messzeuge GmbH, Germany). The length per weight (l_x) of extrudate was determined by weighing a 10 cm length of extrudate and dividing the extrudates length (10 cm) by its associated measured weight. The bulk density was then calculated using the below formula **(Eq. 5-2)**, assuming the extrudate took a cylindrical shape.

$$\rho_b = 4/\pi d^2 l_x$$

Equation 5-2: Bulk density (g/cm^3).

where: ρb is described as bulk density (g/cm^3), d is diameter of the extrudate (cm), l_x is the calculated length per weight (cm/g) of the extrudate, 5 extrudates per experiment were measured.

Note: Results were expressed in g/cm^3 (Yagci & Gogus 2008).

Porosity: Porosity was calculated using the following equation (Equation 5.3):

$$\text{Porosity} = \frac{\text{Bulk volume - Apparent volume}}{\text{Bulk volume}}$$

Equation 5-3: Porosity.

where: bulk volume was calculated (Equation 5-4) by dividing 1 by the bulk density calculated in the bulk density section.

$$\text{Bulk volume} = \frac{\text{Mass}}{\text{Density}}$$

Equation 5-4: Bulk volume (g/cm^3).

To calculate apparent volume, the extrudates were milled using a Cemotec mill (Cemotec 1090 sample mill (Foss, Slangerupgade 69, Denmark)), and the flour produced was sieved through a 500 µm sieve. A 5 ml graduated measuring cylinder was tared and gently filled with extrudate powder. The bottom of the cylinder was repeatedly tapped gently until there was no further reduction of sample volume and it was weighed.

Apparent volume was then calculated by dividing 1 by the apparent density (Equation 5-5) (Yagci & Gogus 2009).

$$\text{Apparent volume} = \frac{\text{Mass}}{\text{Density}}$$

Equation 5-5: Apparent volume (g/cm^3).

Specific volume: The specific volume of each extrudate was calculated using the equation:

$$\text{Specific volume} = \frac{\text{Volume}}{\text{Mass}}$$

Equation 5-6: Specific volume (mL/g).

via the software VolCalc 3.2.3.10 which is connected to the volume meter (Tex Vol Instruments BVM-L 370 Viken, Sweden). Measurements were taken using laser topography, whereby a laser takes measurements of the surface of the 10 cm extrudate as it is being rotated. An adjustable attachment was used to accurately secure the 10 cm long extrudate on the volume meter and five 10 cm (length) extrudates were measured per experiment.

Table 5-2: Response mean and standard deviations for texture & cooked starch properties results.

Design	Apple Pomace (%)		Screw Speed (rpm)		Die Head Temp (°C)		Hardness (N)	Acoustic Energy dB(SPL).sec	Linear Distance dB(SPL).sec	Cold Peak (cP)	Final Viscosity (cP)
No.	Coded	Actual (%)	Coded	Actual (RPM)	Coded	Actual (°C)	Avg / St. dev	Avg / St. Dev	Avg / St. Dev	Avg / St. Dev	Avg / St. Dev
1	1	10	-1	60	0	175	178.5 +/- 23.3	387.1 +/- 41.4	17897.2 +/- 2399.6	235.0 +/- 11.30	590.0 +/- 18.4
2(Ctrl)	-1	0	0	80	0	175	166.8 +/- 12.9	477.5 +/- 56.9	36014.5 +/- 4075.4	527.5 +/- 4.90	586.5 +/- 3.5
3	0.5	7.5	0	80	-0.5	162.5	126.6 +/- 17.5	348.3 +/- 76.6	23047.6 +/- 4076.4	502.5 +/- 16.3	603.0 +/- 4.2
4	1	10	1	100	1	200	173.2 +/- 34.3	98.10 +/- 12.3	7421.50 +/- 2873.5	101.5 +/- 36.1	826.0 +/- 43.8
5	0	5	-1	60	-1	150	205.0 +/- 9.8	504.3 +/- 99.4	22448.7 +/- 4988.1	505.5 +/- 30.4	506.5 +/- 43.1
6	-1	0	-1	60	1	200	237.7 +/- 25.8	396.9 +/- 97.5	28439.5 +/- 6000.5	691.5 +/- 215.7	795.0 +/- 1.40
7	1	10	1	100	-1	150	110.2 +/- 25.1	285.1 +/- 107.1	11432.6 +/- 3952.9	169.0 +/- 7.10	757.5 +/- 31.8
8	0	5	0	80	1	200	136.5 +/- 27.7	396.2 +/- 52.00	16755.8 +/- 1351.7	486.0 +/- 32.5	746.0 +/- 4.20
9	1	10	1	100	-1	150	147.4 +/- 16.8	235.4 +/- 82.30	10460.8 +/- 4737.3	98.50 +/- 0.70	800.0 +/- 1.40
10	1	10	-1	60	1	200	115.8 +/- 22.3	193.3 +/- 36.80	10111.0 +/- 4021.3	288.0 +/- 0.00	600.5 +/- 10.6
11	-1	0	1	100	-1	150	201.9 +/- 25.6	328.5 +/- 81.70	16439.7 +/- 4333.9	222.5 +/- 10.6	931.0 +/- 21.2
12	0	5	1	100	0	175	180.6 +/- 21.7	371.8 +/- 90.0	17555.8 +/- 7149.0	368.0 +/- 36.8	837.5 +/- 3.50
13	-1	0	-1	60	1	200	236.0 +/- 14.2	401.3 +/- 59.40	18107.5 +/- 3216.5	579.5 +/- 50.2	800.0 +/- 4.20
14	1	10	-1	60	-1	150	135.3 +/- 28.8	372.6 +/- 39.90	15529.0 +/- 1915.5	367.0 +/- 53.7	608.5 +/- 43.10
15	0	5	0	80	0	175	138.8 +/- 15.6	381.7 +/- 69.40	21323.9 +/- 2956.0	677.5 +/- 37.5	550.5 +/- 14.80
16	-1	0	1	100	1	200	206.3 +/- 37.6	213.1 +/- 39.40	15877.4 +/- 5752.8	145.5 +/- 4.90	1106 +/- 22.60
17	-1	0	1	100	1	200	162.3 +/- 14.6	298.9 +/- 101.6	15936.6 +/- 5615.1	214.0 +/- 8.50	1042 +/- 2.80
18	-1	0	-1	60	-1	150	147.1 +/- 28.4	412.2 +/- 49.70	17973.0 +/- 2027.2	337.0 +/- 4.20	480.5 +/- 19.10
19	1	10	1	100	1	200	126.5 +/- 28.3	174.0 +/- 45.30	8698.80 +/- 1867.5	286.5 +/- 14.8	722.0 +/- 11.30
20	-1	0	1	100	-1	150	206.6 +/- 49.5	332.8 +/- 71.20	17873.2 +/- 3488.5	148.0 +/- 8.50	951.0 +/- 9.90
21	-1	0	0	80	-1	150	134.9 +/- 20.4	527.7 +/- 150.0	27474.6 +/- 6365.0	393.0 +/- 14.1	486 .0+/- 12.70

Extrudate moisture: Moisture of the end extrudates were calculated based on the AACC method 62-05, (1991), 'Preparation of sample: bread Brabender moisture oven manufacturer's handbook' and AACC method 44-15A, (1991), 'Moisture: air oven methods', using a Brabender oven (Brabender, Duisberg, Germany). The method was altered slightly; extrudates were ground using the Cemotec 1090 sample mill (Foss, Slangerupgade 69, Denmark) and sieved (1,680 µm) to create a homogenous sample. Ten grams of each sample were weighed and dried in a Brabender air oven (130 ^{o}C) for one hour. Brabender oven results were expressed as a percentage (AACC 1991a, 1991b).

5.3.3.2 Textural properties:

Mechanical: The mechanical proprieties of the snacks were evaluated by a downward force on four 5 cm length extrudates sitting in a guillotine deformed by a Warner–Bratzler blade using a texture analyser (TA-XT2i, Stable Micro Systems, Surrey, UK) equipped with 30 g load cell. The pre-test speed was 1 mm/s, test speed was 1.50 mm per second and post-test speed was 2 mm/s under 20 g of force; each sample took 4.5 seconds to run. All results were expressed in N and each experiment was repeated 5 times. The following parameters were measured: hardness and work of shear.

Acoustic: A texture analyser was used in combination with an acoustic envelope detector (A/RAED) to measure acoustic emissions created, simultaneously with the mechanical properties of the compression. Acoustics are recorded by

amalgamating all the frequencies within the band pass range and producing a voltage proportional to the sound pressure level (SPL).

The acoustic attachment consisted of a microphone (Brual & Kjaer 4188-A-021) held in a stand 20 mm away from the sample. The test was performed in a quiet laboratory and at room temperature, a gain amplifier was used at level 1, set to signal and filter out any surrounding noise from the laboratory while the test was being carried out. The sampling rate was set to 500 points per second for acoustic emissions collected by the Texture Exponent software. At the beginning of the study, calibration of the microphone was performed using an Acoustic Calibrator Type 4231 (94 dB and 114 dB SPL-1000 Hz). The following acoustic parameters were measured: Acoustic energy and linear distance (Taniwaki & Kohyama 2012).

5.3.3.3 Cooked starch properties (RVA):

The flour blend prepared from the extrudates were analysed using a Rapid Visco Analyser, the "RVA^{TM} extrusion method" was selected to investigate the starch properties (RVA-4D, Newport Scientific, Sydney, NSW, Australia).

5.3.4 Experimental design and analysis:

The experimental design was created and analysed using Design Expert 7.1.6. (Stat-Ease Inc., Minneapolis, MN, USA). The effects of three independent variables (apple pomace (X1), screw speed (X2), and die head temperature (X3)) on the physical, mechanical, acoustic, cooked starch properties and moisture of an extruded puffed snack were investigated.

A D-optimal design was chosen, upper and lower limits for the design were selected based on preliminary tests; 0-10 % apple pomace addition flour weight basis (fwb), 60-100 rpm screw speed and 150-200 ^{o}C die head temperature.

Dependant variables (or responses) were selected based on descriptors which would best describe a high quality puffed extruded snack. These were of bulk density (Y1), porosity (Y2), radical expansion ratio~~n~~ ((RER) Y3), specific volume (Y4), hardness ((mechanical texture) Y5), acoustic energy ((acoustics of texture) Y6), linear distance ((acoustic of texture) Y7), peak viscosity ((cooked starch properties) Y8), final viscosity ((cooked starch properties) Y9) and moisture (Y10).

A quadratic model with one centre point was selected, resulting in 21 combinations generated via the Design Expert software. Within these 21 combinations, the control was created and five combinations were repeated twice to assess error within the model. A polynomial quadratic regression equation (as seen below Equation 5-7) was used to describe the effects of the three factors on the twelve responses (Hossain et al., 2012).

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j$$

Equation 5-7: Polynomial quadratic regression equation.

The model that best fitted the response was selected during analysis of measurements. Analysis of variance (ANOVA) was carried out on each response model to identify the coefficient (R^2), lack of fit and significant difference ($p<0.05$, $p<0.01$, $p<0.001$, $p<0.0001$).

5.3.5 Validation of model:

The validation of the model was carried out by assessing the model performance indices (the accuracy factor (Equation 5-8) and bias factor (Equation 5-9) described by Hossain et al. (2010).

$$10^{\frac{\sum^{\log} | V_p / V_e |}{ne}}$$

Equation 5-8: Accuracy factor.

$$10^{\frac{\sum^{\log} (V_p / V_e)}{ne}}$$

Equation 5-9: Bias factor.

These performance indices are essential in evaluating the predicted performances of the developed models in illustrating the effects of the independent variables (X1-X3) on the response (dependant) variables (Y1-Y9) thus resulting in a validated final optimised product.

The average mean deviation (AMD, Equation 5-10) was used to evaluate the competence of the data fit on the model.

$$\sum (\%) = \frac{1}{n_e} \sum_{i=1}^{n} \left\| \frac{V_E - V_P}{V_E} \right\| \times 100$$

Equation 5-10: Average mean deviation.

Σ, can be seen to be the average mean deviation, n_e is the number of experimental data, V_e is the experimental value and V_p is the calculated value.

A D-optimal design was chosen from response surface software. This design was based on a quadratic model with one centre point which generated 21 combinations (Table 5-1, 5-2). The physical characteristics, textural characteristics and cooked starch properties of the 21 combinations were investigated, means and standard deviations of each combination was calculated (Table 5-1, 5-2). The calculated regression coefficients for each response are displayed in Table 5-3.

The error of the model was assessed based on five combinations repeated twice within the 21 generations. These repeated combinations (C) can be seen as C4 = C19, C6 = C13, C7 = C9, C11 = C20 and C16 = C17.

The best fit model which significantly evaluated the effect of the variable on the response was chosen. Response results were reported for quadratic models unless otherwise stated as a linear model. The response results were discussed based on models which received satisfactory coefficient (R^2) and adjusted coefficients (R^2), gave a significant p-value and created an insignificant lack of fit indicating the models fitted well.

A Pearson's correlation matrix was designed to investigate the relationships between each response and this was evaluated using an ANOVA (Table 5-4).

Table 5-3: Regression coefficients and statistical information of all responses.

Model	RER Quadratic	Bulk Density (g/cm^3) Quadratic	Porosity Quadratic	Specific Volume (mL/g) Quadratic	Moisture (%) Quadratic	Hardness (N) Linear	Acoustic energy dB(SPL).sec Quadratic	Linear Distance dB(SPL).sec Quadratic	Cold Peak (cP) Quadratic	Final Viscosity (cP) Quadratic
Apple Pomace (A) (%)	-0.13261****	-0.02584	0.048677	-0.06065	-0.03627****	-4.93887**	24.18696***	-545.53040***	39.68890*	121.99194***
Screw Speed (B) (rpm)	-0.01415****	-0.01747***	0.046193***	0.21135****	-0.23560****	-0.21600	16.46387***	2207.71713**	43.85052****	-37.66586****
Die Head Temp (C) (^oC)	-0.00326****	-0.01499	0.016583***	0.30919*	-0.04776****	0.29177	16.95482***	3203.41837	33.77569	-21.18574****
AB	0.00226***	-0.00014	0.0003	0.00429*	0.00078	-	0.05084	5.59571	0.52945	-0.53619***
AC	-0.00008	0.00013	-0.0003	-0.00001	0.00003	-	-0.12182	-8.89732	-0.19991	-0.4582***
BC	0.00002	0.00002	-0.00005	0.00038	0.00011	-	0.00531	0.24319	-0.04430	-0.06364
A^2	-0.01181**	0.00186	-0.0024	-0.03368	-0.01135*	-	-1.90815	76.94056	-5.96381*	-0.99401
B^2	-0.00013	0.00011	-0.0003	-0.00219	0.00119***	-	-0.12852	-14.99547**	-0.27722	0.36353***
C^2	-0.00005	0.00004	-0.00004	-0.00102	0.00008	-	-0.05385	-9.23304**	-0.07999	0.08939
R^2	0.97	0.80	0.83	0.88	0.95	0.38	0.85	0.84	0.86	0.95
adjusted R^2	0.94	0.63	0.70	0.78	0.90	0.27	0.74	0.71	0.74	0.91
p-value	< 0.0001	< 0.0088	< 0.0033	< 0.0007	< 0.0001	< 0.0407	< 0.0017	< 0.0028	< 0.0016	< 0.0001
lack of fit	>0.5504	> 0.9809	> 0.8310	> 0.6721	> 0.2660	> 0.1762	> 0.1302	> 0.3417	> 0.2747	> 0.1951

***** Illustrates a significant difference at: p<0.05*, p<0.01**, p<0.001***, p<0.0001****

Adjusted R^2, demonstrates an adjustment of the R-squared that penalizes the addition of unrelated predictors to the model

Lack of fit, illustrating model adequacy

5.4 Results and discussion:

5.4.1 Physical characteristics of the extrudates:

5.4.1.1 Radical expansion ratio:

Radical expansion ratio is a key parameter in describing the degree of cross-sectional puffing developed from the melt as it exits the extruder (Asare et al., 2004).

The radical expansion ratio (RER) was considerably effected by all three factors ($p<0.0001$, Table 5-1). Results calculated for RER ranged from 2.50 to 5.48 (Table 5-1). From Figure 5-1, it can be seen that as the factors increased, a significant decrease in expansion of the extruded material occurred.

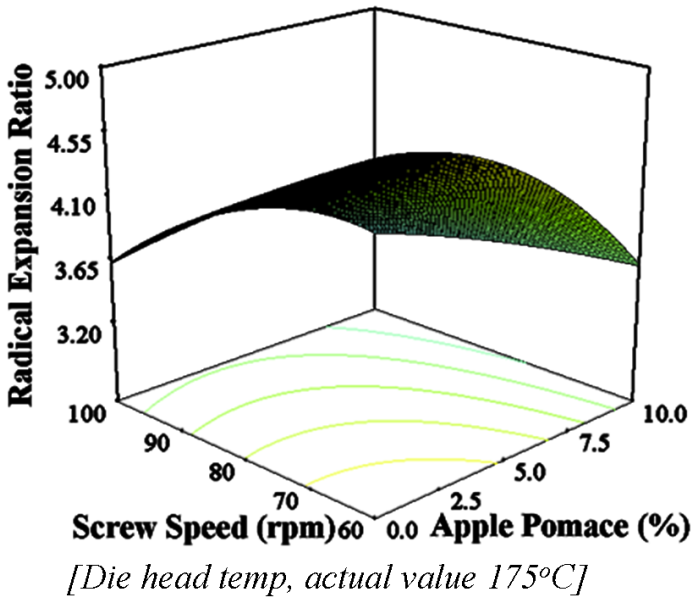


Figure 5-1: Radical expansion ratio as a function of screw speed (rpm) & apple pomace (%)**.**

Table 5-4: Pearson's correlation (R^2) matrix.

	Bulk Density (g/cm^3)	**RER**	**Porosity**	**Specific Volume (mL/g)**	**Final Viscosity (cP)**	**Moisture (%)**
Bulk Density	1					
RER	-0.764***	1				
Porosity	-0.955***	0.818***	1			
Specific	-0.91***	0.731***	0.834***	1		
Final	0.68**	-0.54*	-0.636**	-0.798***	1	
Moisture	-0.551*	0.834***	0.545*	0.524*	-0.334	1
Where:						

***** Illustrates a significant difference at: $p<0.01$*, $p<0.001$**, $p<0.0001$***

Apple pomace has been found to be a highly insoluble fibrous ingredient. Insoluble fibre has been reported to lower expansion via increasing extensional viscosity, which decreases the elastic properties hence lowering the affinity between the starch and insoluble fibre. Insoluble fibres also have higher hydrophilic properties which have been shown to absorb more water, therefore modifying the glass transition temperature of the melt. It can also lower expansion by adhering to the bubble structure and consequently puncturing the cell, reducing cell extensibility (van der Sman & Broeze 2013). Chang et al. (1998) investigated the inclusion of the fibrous jatobá fruit pulp in a puffed snack. The authors reported similar results to those reported in the present study; a decrease in expansion properties of the extruded material as the jatobá fruit inclusions increased. They proposed the following reasons for this result: firstly, increasing levels of the fibrous ingredient diluted the starch content therefore reducing the starches swelling ability and secondly, the presence of jatobá increased the mass viscosity restricting expansion ability and finally the fibre ruptured the cell walls of the bubbles as they formed (Chang, Silva, Gutkoski, Sebio & Da Silva, 1998).

As expected, RER is directly related to porosity, as evidenced by the strong positive correlation (R^2 = 0.82, Table 5-4). Previous authors have also reported similar findings e.g. Pérez et al. (2008) reported the RER of maize decreased, particularly between 160 to 180 ^{o}C. Singh et al. (2007) described how die head temperature decreased the expansion ratio of rice and pea blends once temperatures went beyond 150 ^{o}C, which also support the findings of this study.

Once screw speed increases beyond a certain limit, it began to have a minimizing

effect on porosity of the product. Bubble size development is decreased, producing a large number of smaller bubbles and hence a smaller expansion occurs (de Mesa et al., 2009).

5.4.1.2 Bulk density:

Bulk density is an important quality parameter in the post production of extruded snacks. As both bulk density and texture are affected by the expansion ratio, bulk density can be used as a quality parameter to examine the textural characteristics of a snack (Chessari & Sellahewa 2000). In this study, the method used to analyse the bulk density was carried out according to Yagci & Gogus (2008) the length and weight of five different extrudate samples per experimental combination were measured. The bulk density of each sample was calculated using the described equation (mass per length/volume of a cylinder). The average of five extrudate samples per experimental combination was calculated, to illustrate the effects of the extrusion parameters and apple pomace.

Screw speed was shown to be the major factor observed to have a significant effect on bulk density of the individual extrudate sample groups ($p<0.001$, Table 5-1). This can be seen in Figure 5-2, whereby increasing screw speeds served to increase the bulk density of the extruded material. The values for bulk density varied from 0.12 - 0.46 g/cm^3 (Table 5-1). Die head temperature and apple pomace had no significant effect on the model.

This response has been shown to be indirectly related to the degree of expansion of a melt as it exits the extruder.

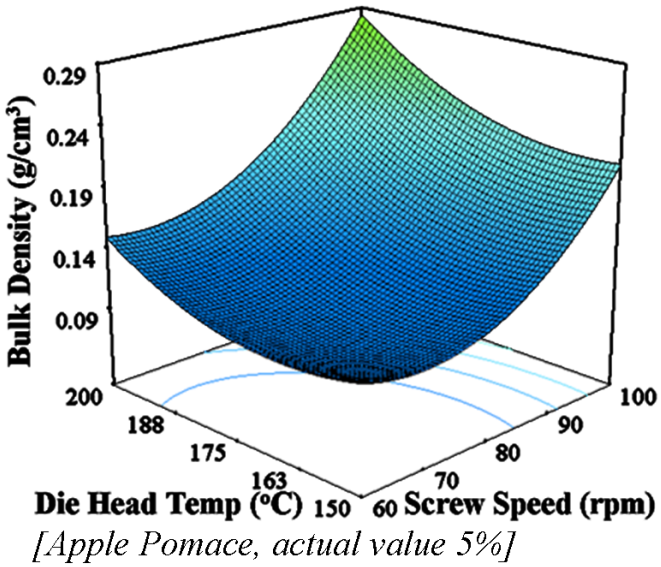


Figure 5-2: Bulk density (g/cm^3) as a function of die head temperature (^{o}C) & screw speed (rpm).

Therefore, a lower bulk density and a higher expansion ratio are desirable characteristics of an extruded snack. Bulk density can also be seen to be a measure of volumetric expansion, which has been shown to have less variability compared to other measurements of expansion (Meng et al., 2010).

The relationship between bulk density and expansion ratio is confirmed from the correlation analysis, where a negative correlation (R^2 = -0.76, Table 5-4) was found, demonstrating a lower expansion rate produces a larger bulk density of the melt.

5.4.1.3 Porosity:

Porosity is one of the main parameters which describe the solid foam-type structure formed in an extruded snack. Once the melt has been extruded and the temperature begins to dramatically decrease, it is governed by the gaseous volume present, and stabilization of the matrix then begins to occur. The matrix proceeds to a glassy state and finally solidifies to form the porous solid extrudate (van der Sman & Broeze 2013).

The porosity response was found to decrease significantly with increasing screw speed ($p<0.001$) and increasing die head temperature ($p<0.01$, Table 5-3). Values for porosity are illustrated in Table 5-1, as demonstrated in Figure 5-3.

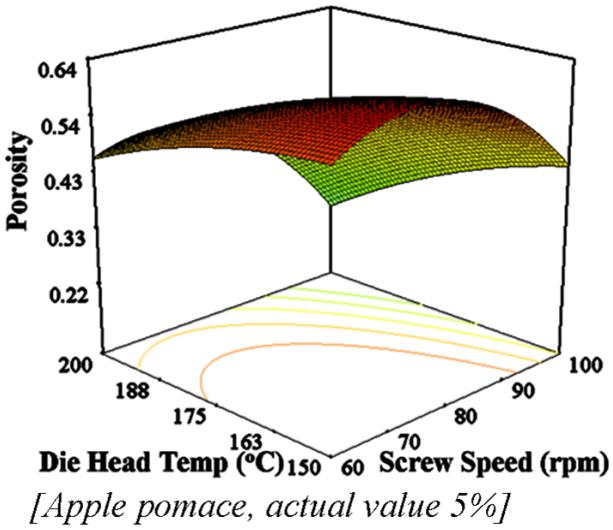


Figure 5-3: Porosity as a function of die head temperature (^{o}C) & screw speed (rpm).

The effect of screw speed on porosity can be attributed to its control of shear stress placed on the melt being extruded (Frame 1994). Too much shear stress

can cause starch to become over degraded reducing its ability to swell and gelatinize. The centre of starch, the hilum is claimed to be one of the primary nucleation sites for the commencement of bubble formation. If the starch becomes damaged it cannot absorb water, reducing its ability to carry the vapour and begin nucleation which creates the porous structure (Moraru & Kokini 2003).

Die head temperature in general, affects the porosity of the extrudate, as it is required for vaporisation of moisture, which is responsible for enlarging a porous structure. Once past a critical temperature, structural degradation of the starch melt occurs. This weakens the structure reducing, its ability to withstand high vapour pressure and ultimately it collapses (Yagci & Gogus 2009). These two parameters (temperature and screw speed) in combination can have detrimental effect on porosity if extreme conditions are set for the extrusion process.

5.4.1.4 Specific volume:

As far as the authors are aware this is the first time that the BVM-L volume meter has been used to calculate specific volume of extruded puffed snacks.

Analysing the overall volume of the final extrudate can be time constraining and expansion equations can be variable and quite specific. For example, expansion ratio only addresses the cross sectional part of the extrudate and porosity observes the porous nature of the extrudate. The utilization of the volume meter was found to be a quick and accurate method to gain information relating to volume of the extrudate.

The model determined that specific volume was significantly affected by both screw speed ($p<0.0001$) and die head temperature ($p<0.05$, Figure 5-4, Table 5-3), no significant effect was noticed from the apple pomace.

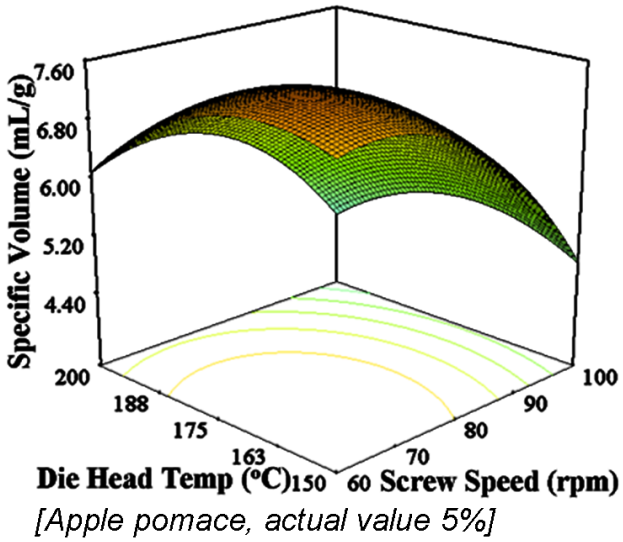


Figure 5-4: Specific Volume (mL/g) as a function of die head temperature (^{o}C) & screw speed (rpm).

A positive correlation between specific volume and the radical expansion ratio was found ($R^2 = 0.73$). Pearson's correlation matrix also displayed a negative relationship ($R^2 = -0.91$, Table 5-4) between specific volume and bulk density, which is to be expected.

5.4.1.5 Moisture:

Unlike other cereal products such as bread, cake and muffins which contain high moisture, extruded snacks tend to contain a lower level of moisture, hence potentially creating a more shelf stable product (Asare et al., 2004).

Values for the moisture response of the end extrudate varied from 6.20 – 8.43 % (Table 5-1). The moisture response was found to be significantly affected by all three factors, screw speed ($p<0.0001$), die head temperature ($p<0.0001$) and apple pomace inclusion ($p<0.0001$, Figure 5-5, Table 5-3).

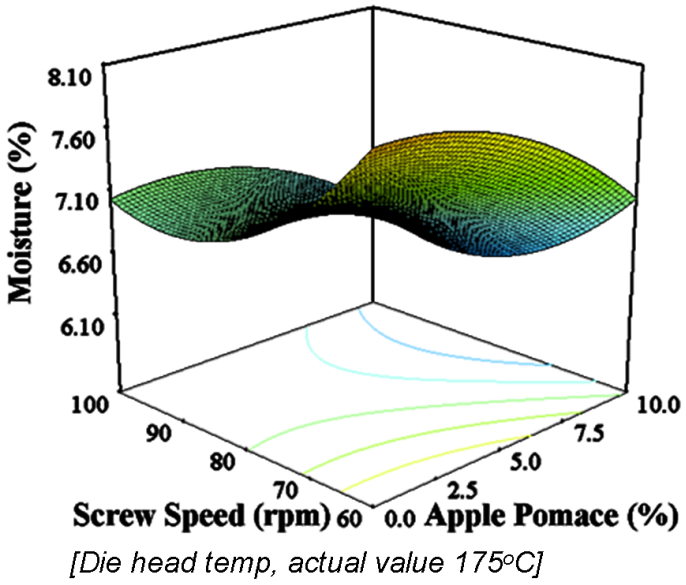


Figure 5-5: Moisture (%) as a function of screw speed (rpm) & apple pomace (%).

Die head temperature and screw speed play a similar role in decreasing the moisture content of extrudates, when both of these parameters are increased at a high intensity the moisture content of the extrudates is decreased due to intense shearing and evaporation as a result of high temperatures. This can be seen in

experimental combinations which contain high die head temperatures and screw speed (Moraru & Kokini 2003).

The gaseous pressure which creates expansion is delivered by water vapour beyond a critical heating limit, the moisture is decreased which creates snacks with smaller expansion and a low extrudate moisture (van der Sman & Broeze 2013). As apple pomace is high in insoluble fibre, beyond a significant level it binds the free water available in the matrix therefore reducing water availability for gas expansion while also reducing the moisture content of the melt and extrudate (Moraru & Kokini 2003).

The moisture content of a snack is the driving force behind nucleation and bubble expansion, for this reason the final expansion of the extrudate is also affected. Consequently, this parameter is closely related to the expansion parameters such as RER where higher moisture gave a larger expansion ratio. A correlation of R^2= 0.83 was found between these two parameters (Table 5-4).

5.4.2 Textural properties:

5.4.2.1 Mechanical properties:

Hardness: This parameter is described as the maximum force required to fracture or break the product on the texture analyser. Previous research has found that hardness correlates well with the initial bite or texture of hardness when a person begins to bite into a snack (Dehghan-Shoar, Hardacre & Brennan, 2010).

Figure 5-6, shows how additions of apple pomace ($p<0.01$, Table 5-3) linearly affect hardness. Increasing levels of apple pomace decreased the hardness of the snack. Although this response did not show a very high regression coefficient ($R^2 = 0.38$), due to large variability in the model from the unknown variables it is satisfactory (Table 5-4).

Yagci & Gogus (2009) investigated the effects of blends which contained durum clear flour, partially defatted hazelnut flour (PDHF) and fruit waste (orange peel, grape seeds and tomato pomace) in different combinations. The authors found that low and high levels of fruit waste coupled with low levels of PDHF produced an extrudate with decreased hardness properties. However at a medium level of fruit waste and an increased PDHF level the hardness of the extrudate was increased considerably due to the combination of the fruit waste and PDHF.

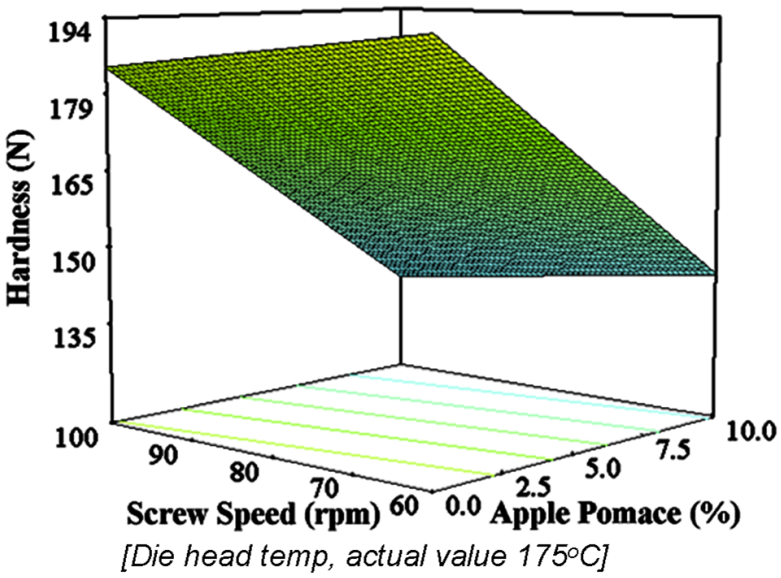


Figure 5-6: Hardness (N) as a function of screw speed (rpm) & apple pomace (%).

Apple pomace is known to contain a high quantity of pectin, which may decrease the hardness of the end product by acting as a lubricant and produce a crispier rather than a harder snack (van der Sman & Broeze 2013). Formation of a crispier snack from soluble fibre has been reported previously by Van der Sman & Broeze (2013). However, as previously described, the presence of soluble fibre can also have a detrimental effect on expansion, as the fibre absorbs surrounding water in the melt, decreasing the availability of water for vaporised steam which drives nucleation and then expansion. Soluble fibre can also enhance the elastic properties through an anisotropic process, via the extruder aligning the soluble fibre in an axial direction (van der Sman & Broeze 2013). This affects sectional expansion; if too much fibre is present it reinforces the longitude structure not allowing the sectional structure to expand developing a low expansion ratio (Moraru & Kokini 2003).

5.4.2.2 Acoustic properties:

Extruded snacks have a cellular nature typically filled with air. The crispy/crunchy quality is usually affected by the size of the air cells and thickness of the cell walls. When this structure is deformed, the noise it generates can be associated with biting into the food and the resulting crispy/crunchy perception of the food. This sound pressure is produced by snapping the back walls that bend before breaking within the structure (Saeleaw, Dürrschmid & Schleining, 2012).

Acoustic Energy: The models developed for this response were found to be significantly affected by all three factors but particularly by screw speed ($p<0.001$) and apple pomace Figure 5-7, 5-8 ($p<0.001$, Table 5-3). This indicates that as the screw speed and apple pomace increased, the resulting area under the curve (i.e. acoustic energy) decreased, signalling a snack with a higher level of crispiness.

The extruded snacks which contained no or low amounts of apple pomace created a crunchier snack compared to snacks containing higher amounts of apple pomace. As previously hypothesised, this could be attributed to pectin (soluble fibre source) being present in the apple pomace. This can have a lubricating effect forming a crispier snack but with a reduced expansion ratio (van der Sman & Broeze 2013).

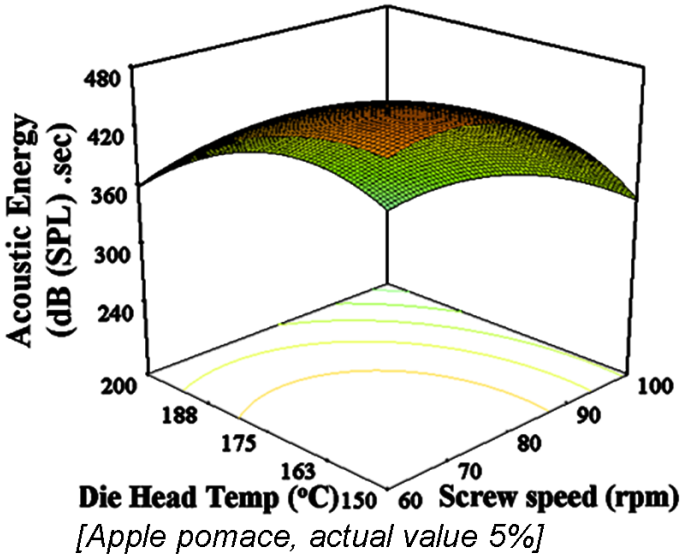


Figure 5-7: Acoustic Energy (dB (SPL) .sec) as a function of die head temperature (^{o}C) and screw speed (rpm).

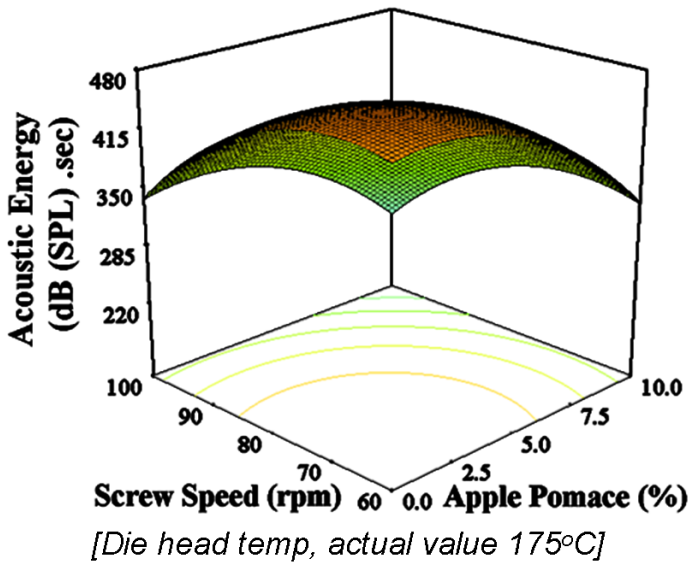


Figure 5-8: Acoustic Energy (dB (SPL) .sec) as a function of screw speed (rpm) & apple pomace (%).

Linear Distance: The 'jagged linear distance' or 'linear distance' is a useful parameter for investigating the crispy and crunchy characteristics of a snack. A longer distance with more obvious force points describes a crunchy snack whereas a crispy snack produces a line with many smaller and closer fluctuations as illustrated in Figure 5-9 (combination 21 (C21) (F3 (C21)) & combination 15 (C15) (F3 (C15)). The image shown in F3 (C21) is as a result of combination which included no apple pomace; a crunchier snack was created, whereas the combination illustrated in F3 (C15) which contains apple pomace showed a shorter line with an increased number of smaller fluctuations that are closer together indicating a crispier snack.

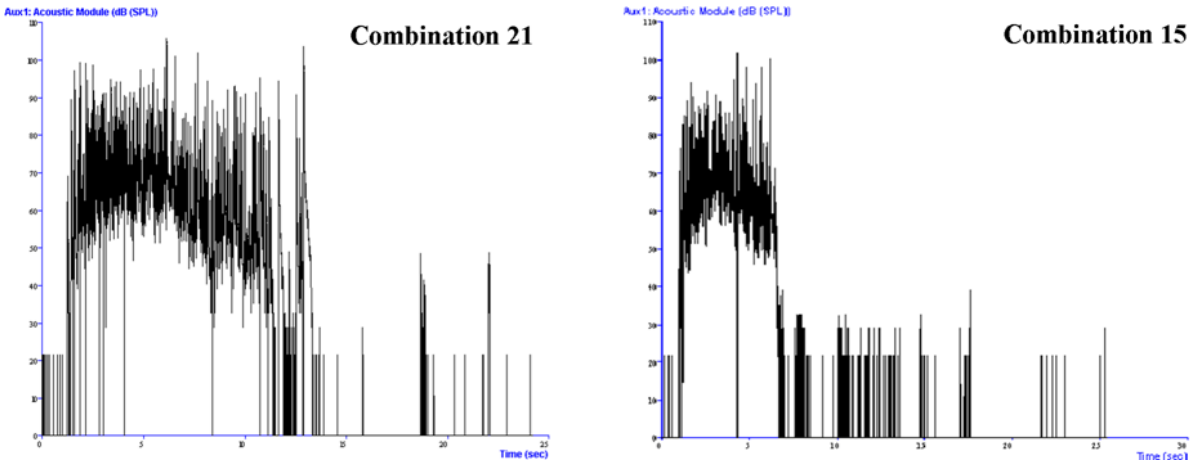


Figure 5-9: An example of a crunchy and crispy graph, Combination 21 & Combination 15.

From Figure 5-10, it can be seen that the linear distance parameter was significantly affected by apple pomace inclusions ($p<0.001$) and screw speed

($p<0.05$, Table 5-3). The effect of apple pomace was seen to create a saddle shape in Figure 2 (c), indicating its effectiveness over the other factors. An increase in linear distance (i.e. a crunchier product) was found when apple pomace was not present and screw speed was kept to a low speed.

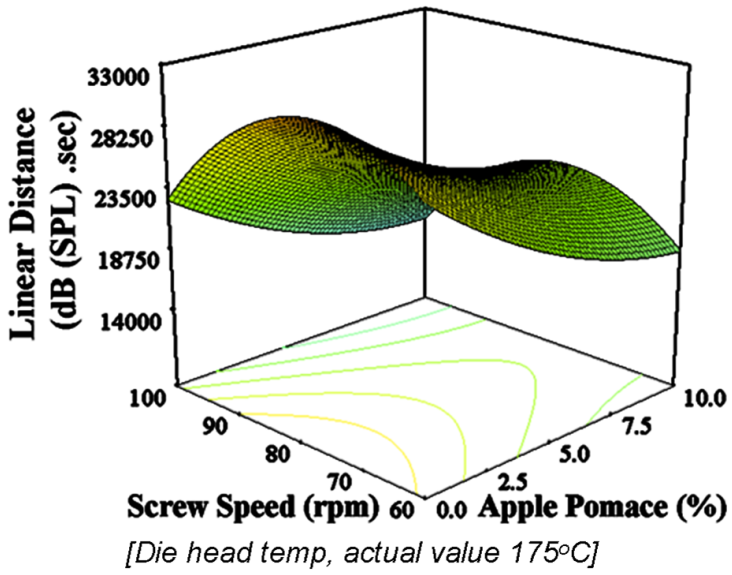


Figure 5-10: Linear Distance (dB (SPL) .sec) as a function of screw speed (rpm) & apple pomace (%).

5.4.3 Cooked starch properties:

5.4.3.1 Peak viscosity (PV):

The starch properties, post extrusion can give a good indication as to the degree of gelatinization and the rate of molecular degradation that occurred during extrusion as a result of high temperatures and high shear rates (Carvalho, Takeiti, Onwulata & Pordesimo, 2010). Higher PV values are usually as a result of the presence of un-gelatinized starch granules after extrusion, which are then allowed to swell and eventually amylose is allowed to leach out of the granule (Chang & Ng 2011).

The peak viscosity (PV) or cold peak viscosity results varied greatly (99-692 cP, table 5-2) demonstrating significant differences between experimental combinations. This response was found to be primarily effected by screw speed ($p<0.001$) and apple pomace ($p<0.05$, Table 5-3, Figure 5-11). Although the modelling for this particular response did not show die head temperature to have a significant effect, in general die head temperature (and particularly in combination with screw speed) can dictate starch functionality, where excess temperatures significantly affect the pasting properties of the degrading starch.

Screw speeds effect can be explained as an increase in shear rate from increasing screw speeds which create the onset of starch degradation and dextrinization during the extrusion process, ultimately reducing the starch pasting properties (Moraru & Kokini 2003). While increasing apple pomace levels (as previously described in the RER section) creates a starch diluting effect, the fragments of the fibrous apple pomace can adhere to the bubble cell walls eventually tearing the cell causing it to collapse, reducing the expansion rate of the end product and

a smaller tightly packed extrudate is created (Chang et al., 1998).

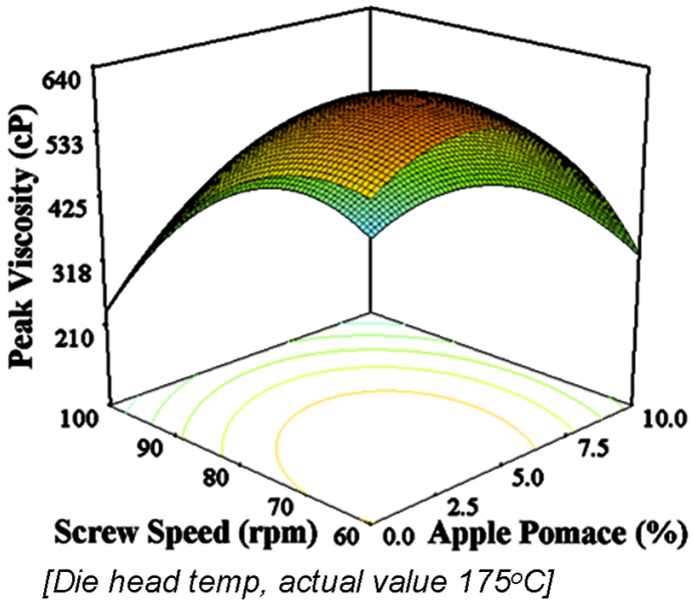


Figure 5-11: Peak viscosity (cP) as a function of screw speed (rpm) & apple pomace (%).

Chang & Ng (2011) illustrated similar results in their extrusion trial: as screw speed was increased, the PV of the resultant material was decreased. They reported this incidence as a result of starch degradation. Nascimento et al. (2012) discussed the effects of sesame oil cake (a highly fibrous ingredient) on the effects of corn extrudates. These authors proposed a decrease in RVA values as a result of the high insoluble fibre content of the sesame oil cake. It increased starch breakdown as its insoluble fibre fractions acted as a shearing material which damaged nucleating bubbles during extrusion (Nascimento, Carvalho, Takeiti, Freitas & Ascheri, 2012).

5.4.3.2 Final viscosity (FV):

The final viscosity of a sample describes the ability of the starch to form a viscous paste or a gel after it has been cooked at high temperatures and high shear rates (Ktenioudaki, O'Shea & Gallagher, 2012).

Figure 5-12, 5-13, illustrates how the final viscosity (FV) response was found to be effected by all three extrusion parameters, screw speed ($p<0.0001$) and die head temperature ($p<0.0001$), and apple pomace (AP) additions ($p<0.001$, Table 5-3).

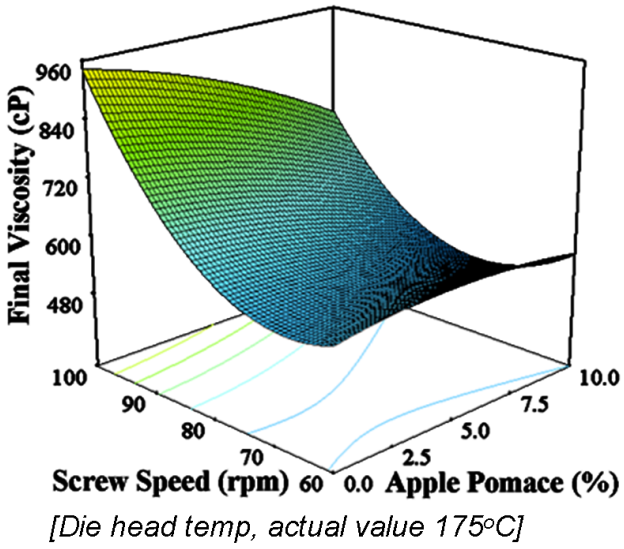


Figure 5-12: Final viscosity (cP) as a function of screw speed (rpm) & apple pomace (%).

High final viscosity values were particularly noticed in combinations where no apple pomace was included and the extrusion variables were at their extreme levels e.g. Combination 16 in Table 5-2. The authors hypothesise that this effect may be explained as a result of highly degraded or melted starch, in particular the

amylopectin fraction (released after high shear rates during the extrusion process) which re-associated during the final cooling stage to form a firm gel. Ozean & Jackson (2005) proposed that uncoiled amylopectin branches re-associated with extruded starch fragments blocking water absorption sites, therefore reducing its ability to absorb water resulting in an increased final viscosity.

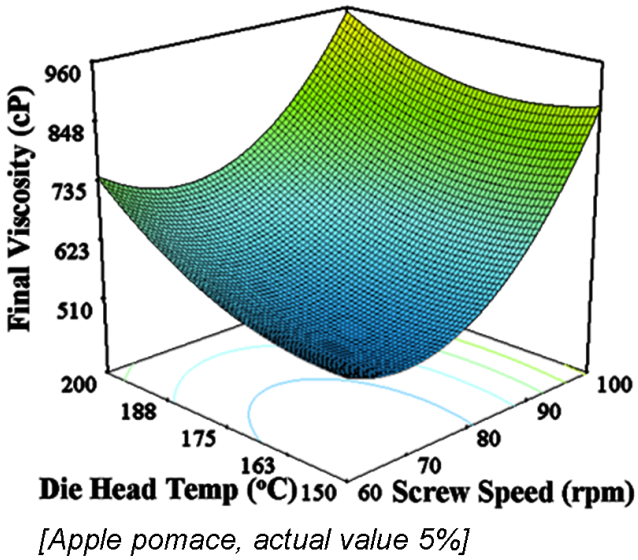


Figure 5-13: Final viscosity (cP) as a function of die head temperature (%) & screw speed (rpm).

AP inclusion was found to have an interesting effect on the FV parameter. At low screw speeds (60 rpm) and high apple pomace levels (10 %) e.g. Combination 10, AP was found to decrease FV of the resulting extrudate (600 cP). The occurrence of a reduction in FV can be attributed to the effects of the high level of insoluble fibre found in AP. As previously discussed, AP has a weakening effect on the starch present, reducing its pasting potential.

At high AP levels (10 %) and high screw speed (100 rpm) e.g. Combination 9,

the FV was increased (800 cP); the effect of screw speed would appear to take predominance over apple pomace addition resulting in an increased FV.

The final viscosity parameter has been directly related to the physical characteristics of the final product in particular with bulk density (R^2 = 0.68) and specific volume (R^2 = -0.80, Table 5-4). As previously considered, this can be recognised as a result of degraded starch which reassembles to form a gel on cooling to 25 ^{o}C to increase the FV. Therefore, this degraded starch cannot absorb more water reducing its potential to expand when extruded resulting in a dense extrudate with a decreased specific volume.

5.4.4 Optimisation and validation:

5.4.4.1 Optimisation of formulation and extrusion process:

The optimisation tool from the design expert software was utilized to derive the optimal formulations based on response results evaluated. This was implemented by choosing the maximum limits for both the favourable factors and desirable responses i.e. apple pomace (factor), radical expansion ratio (response), porosity (response) and specific volume (response). Undesirable responses (e.g. hardness) were minimized to produce formulations with a more pleasing texture. The remaining factors were left in range and all outstanding responses were left out of this procedure. Seven formulations with predicted quality response values were generated based on these entered parameters. Depending on the best desirability ratings, two formulations (F1 & F2) were selected for analysis and the most favourable formulation was chosen. Images of these formulations can be seen in Figure 5-14; the combinations were F1 (desirability 0.77) – 7.7 % apple pomace, die head temperature 150 ^{o}C and screw speed 69 rpm and F2 (desirability 0.77) – 7.9 % apple pomace, die head temperature 150 ^{o}C and screw speed 71 rpm.

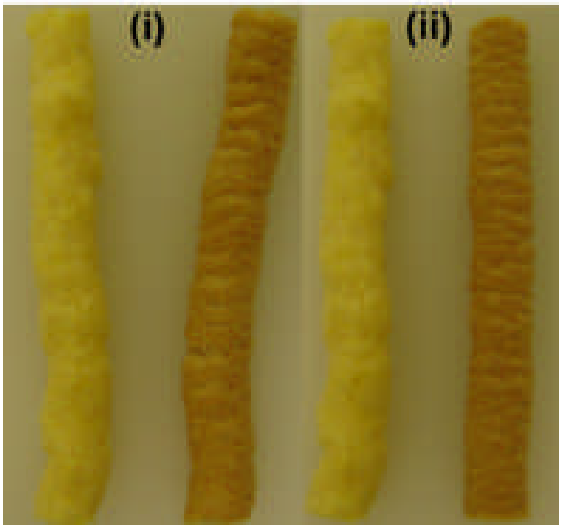


Figure 5-14: Optimised extruded snacks – formulation 1 with the control (i) & formulation 2 with the control (ii).

5.4.4.2 Validation of optimised formulation and extrusion variables:

It is crucial to validate the model in order to evaluate the precision of the findings. This was carried out by investigating the accuracy factor, bias factor and average mean deviation of each optimised response result. Accuracy and bias factors give an estimation of the overall performance of the model. The average mean deviation calculates the error of fit of the data to the model.

The predicted and experimental results for the optimised responses are illustrated in Table 5-5 and the performance indices are shown in Table 5-6. Even though both formulations have a similar desirability (0.77), overall formulation 1 performed the best in terms of its physical characteristics and texture and gave the best fit.

Table 5-5: Predicted and experimental values for formulation 1 & 2.

	Formulation 1			Formulation 2		
Variables	**Predicted values**	**Experimental values**	**St. Dev (+/-)**	**Predicted values**	**Experimental values**	**St. Dev (+/-)**
Porosity	0.602	0.586	0.01	0.604	0.588	0.01
Expansion Ratio	4.477	4.391	0.061	4.44	4.24	0.144
Specific volume (mL/g)	6.796	6.23	0.397	6.75	6.05	0.497
Hardness (N)	145.609	132.567	9.222	144.72	123.24	15.189

Table 5-6: Accuracy factor (AF), Bias factor (BF) and average mean deviation (Σ %) values for formulation 1 & 2.

	Formulation 1			Formulation 2		
Variables	**AF**	**BF**	**Σ (%)**	**AF**	**BF**	**Σ (%)**
Porosity	0.97	1.03	2.73	0.97	1.03	2.82
Expansion Ratio	0.98	1.02	1.98	0.95	1.05	4.79
Specific volume (ml/g)	0.92	1.09	9.00	0.90	1.12	11.62
Hardness (N)	0.91	1.10	9.84	0.85	1.17	17.43

It revealed results close to 1 which is ideal, except for hardness and specific volume which demonstrated a percentage error of 9 % and 8 % respectively. Roessle et al. (2011) demonstrated how the AF adequacy is governed by each additional predictive factor; i.e. acceptable AF findings vary depending on the number of additional factors. For example, this model contained three predictive variables which would give an acceptable error base of 0.20-0.30 which corresponds to 20 – 30 % error; therefore the error range is acceptable.

A similar tendency was found with the bias factor, which displayed results close to 1 apart from hardness and specific volume. This demonstrates a good relationship between the experimental and predicted results.

From Table 5-6, calculated results for the average mean deviation (AMD) illustrate the best for physical characteristics and texture AMD were produced for formulation 1 compared to formulation 2. As formulation 1 produced a lower level of error it is the more acceptable formulation.

5.5 Conclusion:

Using response surface design as a tool, the effects of three factors on the physical characteristics, texture, starch properties and moisture contents of a snack were highlighted.

The design demonstrated the ability of screw speed to affect all responses. In particular, when screw speed was set to its maximum speed it was found to have detrimental effects on the responses. It produced such negative effects as a high bulk density, a low expansion ratio, decreased porosity and a small specific volume. Severe screw speeds also decreased the moisture content and was found to degrade starch which was illustrated in high final viscosity values.

Die head temperature affected the physical properties, starch properties and the moisture content of the extrudates. As temperature is the main element in driving moisture to create a porous and well expanded structure, this is to be expected. At the maximum temperature and in combination with screw speed and AP inclusion it significantly decreased the quality of the snack producing a dense and tightly packed extrudate.

Apple pomace affected the radical expansion ratio, texture, acoustic properties, starch properties and moisture. This is attributed to the high fibre content present in AP. As discussed, fibre is well documented as having hydrophilic properties; beyond a certain limit it began to have a negative impact on the responses due to its ability to absorb excess water and damage aerated bubble structures.

Optimisation of these three factors was crucial for the development of a high quality puffed snack. The optimal extrusion conditions and AP inclusion to

produce a high quality snack were calculated with the aid of the optimisation tool to be; die head temperature at 150 ^{o}C, screw speed approximately 69 rpm and AP addition of 7.7 %.

The study successfully demonstrates how apple pomace can be utilised in an extruded puffed snack. It highlighted how an under-utilized by-product may be substituted for corn flour and incorporated into an expanded puffed snack. A high level of apple pomace was included into the extruded snack without compromising the expansion characteristics of the snack while potentially improving its nutritive properties.

5.6 References:

AACC. (1991a). Method 44-15A, Moisture: air oven methods, approved methods of the American Association of Cereal Chemists International, St. Paul, Minnesota, USA.

AACC. (1991b). Method 62-05 Preparation of sample: bread Brabender moisture oven, manufacturer's handbook, approved methods of the American Association of Cereal Chemists International, St. Paul, Minnesota, USA.

Altan, A., McCarthy, K.L., & Maskan, M. (2008a). Evaluation of snack foods from barley-tomato pomace blends by extrusion processing. *Journal of Food Engineering*, 84(2), 231-242.

Altan, A., McCarthy, K.L., & Maskan, M. (2008b). Twin-screw extrusion of barley–grape pomace blends: Extrudate characteristics and determination of optimum processing conditions. *Journal of Food Engineering,* 89(1), 24-32.

Altan, A., McCarthy, K.L., & Maskan, M. (2009). Effect of Extrusion Cooking on Functional Properties and in vitro starch digestibility of barley-based extrudates from fruit and vegetable by-products. *Journal of Food Science,* 74(2), E77-E86.

Asare, E.K., Sefa-Dedeh, S., Sakyi-Dawson, E., & Afoakwa, E.O. (2004). Application of response surface methodology for studying the product characteristics of extruded rice-cowpea-groundnut blends. *International Journal of Food Sciences and Nutrition,* 55(5), 431-439.

Brennan, M.A., Derbyshire, E., Tiwari, B.K., & Brennan, C.S. (2013). Ready-to-

eat snack products: the role of extrusion technology in developing consumer acceptable and nutritious snacks. *International Journal of Food Science & Technology,* 48(5), 893-902.

Carvalho, C.W.P., Takeiti, C.Y., Onwulata, C.I., & Pordesimo, L.O. (2010). Relative effect of particle size on the physical properties of corn meal extrudates: Effect of particle size on the extrusion of corn meal. *Journal of Food Engineering,* 98(1), 103-109.

Chang, Y.K., Silva, M.R., Gutkoski, L.C., Sebio, L., & Da Silva, M.A.A.P. (1998). Development of extruded snacks using jatobá (Hymenaea stigonocarpaMart) flour and cassava starch blends. *Journal of the Science of Food and Agriculture,* 78(1), 59-66.

Chang, Y.H., & Ng, P.K.W. (2011). Effects of extrusion process variables on quality properties of wheat-ginseng extrudates. *International Journal of Food Properties,* 14(4), 914-925.

Chessari, C.J. & Sellahewa, J.N. (2000). Effective process control. In: Guy, R. (Eds.), *Extrusion cooking Technologies and applications*. Woodhead Publishing Ltd, Cambridge, pp. 83-107.

De Mesa, N.J.E., Alavi, S., Singh, N., Shi, Y.-C., Dogan, H., & Sang, Y. (2009). Soy protein-fortified expanded extrudates: Baseline study using normal corn starch. *Journal of Food Engineering,* 90(2), 262-270.

Dehghan-Shoar, Z., Hardacre, A.K., & Brennan, C.S. (2010). The physico-chemical characteristics of extruded snacks enriched with tomato lycopene. *Food*

Chemistry, 123(4), 1117-1122.

Frame, N.D. (1994). Operational characteristics of the co-rotating twin-screw extruder. In: Frame, N.D. (Eds), *The technology of extrusion cooking.* Blackie Academic & Professional, an imprint of Chapman & Hall, Glasglow, pp. 1-51.

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M., Weisz, M., Trakhtenberg, S. & Martin-Belloso, O. (2001). Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. *Journal of Agricultural and Food Chemistry,* 49(2), 952-957.

Hossain, M.B., Brunton, N.P., Martin-Diana, A.B., & Barry-Ryan, C. (2010). Application of response surface methodology to optimize pressurized liquid extraction of antioxidant compounds from sage (Salvia officinalis L.), basil (Ocimum basilicum L.) and thyme (Thymus vulgaris L.). *Food & Function,* 1(3), 269-277.

Hossain, M.B., Brunton, N.P., Patras, A., Tiwari, B., O'Donnell, C.P., Martin-Diana, A.B., & Barry-Ryan, C. (2012). Optimization of ultrasound assisted extraction of antioxidant compounds from marjoram (Origanum majorana L.) using response surface methodology. *Ultrasonics Sonochemistry,* 19(3), 582-590.

Karkle, E.L., Alavi, S., & Dogan, H. (2012). Cellular architecture and its relationship with mechanical properties in expanded extrudates containing apple pomace. *Food Research International,* 46(1), 10-21.

Ktenioudaki, A., O'Shea, N., & Gallagher, E. (2012). Rheological properties of wheat dough supplemented with functional by-products of foodprocessing:

Brewer's spent grain and apple pomace. *Journal of Food Engineering,* 116(2), 362-368.

McCarthy, D.F., Gallagher, E., Gormley, T.R., Schober, T.J., & Arendt, E.K. (2005). Application of response surface methodology in the development of gluten-free bread. *Cereal Chemistry,* 82(5), 609-615.

Meng, X., Threinen, D., Hansen, M., & Driedger, D. (2010). Effects of extrusion conditions on system parameters and physical properties of a chickpea flour-based snack. *Food Research International,* 43(2), 650-658.

Moraru, C.I., & Kokini, J.L. (2003). Nucleation and expansion during extrusion and microwave heating of cereal foods. *Comprehensive Reviews in Food Science and Food Safety,* 2(4), 147-165.

Nascimento, E.M.D.G.C.D., Carvalho, C.W.P., Takeiti, C.Y., Freitas, D.D.G.C., & Ascheri, J.L.R. (2012). Use of sesame oil cake (Sesamum indicum L.) on corn expanded extrudates. *Food Research International,* 45(1), 434-443.

O'Shea, N., Arendt, E.K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science & Emerging Technologies,* 16, 1-10.

Ozcan, S., & Jackson, D.S. (2005). Functionality behavior of raw and extruded corn starch mixtures. *Cereal Chemistry Journal,* 82(2), 223-227.

Pastor-Cavada, E., Drago, S.R., González, R J., Juan, R., Pastor, J.E., Alaiz, M., & Vioque, J. (2011). Effects of the addition of wild legumes (Lathyrus

annuus and Lathyrus clymenum) on the physical and nutritional properties of extruded products based on whole corn and brown rice. *Food Chemistry,* 128(4), 961-967.

Pérez, A.A., Drago, S.R., Carrara, C.R., De Greef, D.M., Torres, R.L., & González, R.J. (2008). Extrusion cooking of a maize/soybean mixture: Factors affecting expanded product characteristics and flour dispersion viscosity. *Journal of Food Engineering,* 87(3), 333-340.

Rosell, C.M., Santos, E., & Collar, C. (2009). Physico-chemical properties of commercial fibres from different sources: A comparative approach. *Food Research International,* 42(1), 176-184.

Rossle, C., Ktenioudaki, A., & Gallagher, E. (2011). Inulin and oligofructose as fat and sugar substitutes in quick breads (scones): a mixture design approach. *European Food Research and Technology,* 233(1), 167-181.

Saeleaw, M., Dürrschmid, K., & Schleining, G. (2012). The effect of extrusion conditions on mechanical-sound and sensory evaluation of rye expanded snack. *Journal of Food Engineering,* 110(4), 532-540.

Singh, B., Sekhon, K.S., & Singh, N. (2007). Effects of moisture, temperature and level of pea grits on extrusion behaviour and product characteristics of rice. *Food Chemistry,* 100(1), 198-202.

Stojceska, V., Ainsworth, P., Plunkett, A., & İbanoğlu, Ş. (2009). The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products. *Food Chemistry,* 114(1), 226-232.

Stojceska, V., Ainsworth, P., Plunkett, A., & İbanoğlu, Ş. (2010). The advantage of using extrusion processing for increasing dietary fibre level in gluten-free products. *Food Chemistry,* 121(1), 156-164.

Taniwaki, M., & Kohyama, K. (2012). Mechanical and acoustic evaluation of potato chip crispness using a versatile texture analyzer. *Journal of Food Engineering,* 112(4), 268-273.

Van der Sman, R.G.M., & Broeze, J. (2013). Structuring of indirectly expanded snacks based on potato ingredients: A review. *Journal of Food Engineering,* 114(4), 413-425.

Wijngaard, H.H., Rößle, C., & Brunton, N. (2009). A survey of Irish fruit and vegetable waste and by-products as a source of polyphenolic antioxidants. *Food Chemistry,* 116(1), 202-207.

Yagci, S., & Gogus, F. (2008). Response surface methodology for evaluation of physical and functional properties of extruded snack foods developed from food-by-products. *Journal of Food Engineering,* 86(1), 122-132.

Yagci, S., & Gogus, F. (2009). Development of extruded snack from food by-products: a response surface analysis. *Journal of Food Process Engineering,* 32(4), 565-586.

Yang, S.-h., Peng, J., Lui, W.-B., & Lin, J. (2008). Effects of adlay species and rice flour ratio on the physicochemical properties and texture characteristic of adlay-based extrudates. *Journal of Food Engineering,* 84(3), 489-494.

Chapter 6

General discussion

6 General discussion:

Prior to the incorporation of new ingredients into food formulations it is important to understand their properties as raw materials. In this thesis, apple and orange pomaces from the cultivars “Karmijn de Sonnaville” and “Valencia” were studied as potential new ingredients.

Although both by-products had a high proportion of fibre, they performed differently when their gelling ability was studied. Apple pomace developed highly viscous paste post-heating. Pastes were also prepared using the Rapid-Visco-Analyser (RVA) (without heating) and the fundamental rheological properties of the by-products were determined. The apple pomace developed a visco-elastic paste which became more robust as the oscillation frequency increased. The effect of frequency on the orange pomace flour slurry was not as evident; only a slight increase in G' and G* were observed as the frequency was increased.

The heated viscous and rheological properties of the above pomace flours demonstrated the differences in the functional properties between the by-products; this is a result of a combination of factors. As the by-products originate from the exterior of the fruit it was thought they would contain a high level of soluble fibre in the form of pectin. Using a quick, reproducible and short time preparation NMR method, the pectin present in the by-products was quantified and characterised.

The pectin present in apple pomace had a high degree of methylation, which is known to affect the viscosity of pastes. As apple pomace also contains

significant levels of sugar and starch, it shows potential as a possible 'structure-forming' ingredient, where the viscous properties of the products are important; e.g. yoghurt, beverages and some snack foods.

The orange pomace contained a high level of fibre and pectin. The pectin had a low level of methylation; the pomace also contained low levels of starch and sugar. This would suggest the insoluble fibre fraction of orange pomace to be more dominant. Insoluble fibre, when used as an ingredient, can be beneficial as a bulking agent in products. As it retains more moisture it could also be useful in reducing moisture loss during staling. Therefore it could be an ingredient to assist with an improved crumb texture and staling properties in gluten-free bread. Gluten plays a key role in slowing down the staling process via moisture retention. It is also associated with increasing loaf volume and reducing crumb hardness. Hence, with more research, it may be possible that orange pomace could perform a similar structural role in gluten-free bread.

The application of orange pomace as an ingredient in gluten-free bread was examined. A response surface design was devised to determine the optimal orange pomace addition. Due to the material being fibrous in nature, water addition was the second parameter studied in the design. Unlike wheat bread production, where proofing can be standardized easily, proofing time was the final variable examined in this study.

Orange pomace was the dominant factor involved in formulating the bread. Lower inclusions of orange pomace resulted in loaves with higher specific

volumes and a softer texture, particularly at levels of addition greater than 2 %. The results revealed an interesting relationship between orange pomace addition and proofing time. An optimal proofing time of 50 minutes coupled with 4 % addition of orange pomace resulted in loaves with a reduced hardness and an increased loaf volume.

Incorporating too high a level of orange pomace (above 6 %) into the formulation resulted in unfavourable effects on the loaf volume, crumb structure and crumb texture. This was discussed as a result of orange pomace having hydrophilic properties, which competed with starch for the water, reducing its ability to create a viscous structure to retain carbon dioxide (CO_2). It was also suggested that its presence diluted the methylcellulose/starch matrix, developing a weak structure which cannot withstand proofing and ultimately collapses. Below 5 % orange pomace addition, the ingredient was proposed to support the starch/matrix structure, resulting in higher loaf volumes. Using the design expert optimisation tool, the optimised parameters were calculated to be 5.5 % orange pomace, 94.6 % water addition and 49 minutes. The optimised formulation doubled the total dietary fibre content of the final breads in comparison to the control.

To further understand the fundamental workings of orange pomace as an ingredient, a trial to study its influence on batter properties and structure was undertaken. The role of orange pomace in gluten-free batter pre-baking, and the sensory acceptance of the bread post-baking were investigated.

The starch pasting properties of the batter which contained orange pomace were

lower in comparison to the control. This illustrated the high water binding content of orange pomace. The greater levels of starch present in the control formulation resulted in a batter with a higher final viscosity. When orange pomace was not present, the free water was absorbed by the starch and upon heating the starch granules swelled and eventually burst, creating a gel on cooling.

Fundamental rheology revealed how batter containing orange pomace had a more robust structure, with a higher viscoelasticity (G' & G*), when compared to the control batter. During the bread study it was suggested that orange pomace could have a supportive role to the methylcellulose/starch matrix. This, coupled with enhanced viscoelastic batter properties, may have been a contributing factor to the higher loaf volumes which were found.

Sensory analysis was performed; panellists scored orange pomace favourably in the majority of categories, apart from "texture while chewing", where the control was preferred. A preference test between the two breads was also completed; overall the orange pomace bread was scored comparably to the control bread.
The previous two studies laid a good foundation for producing novel results in relation to the pre-baking (rheology, viscous properties and microstructure) and post baking (baking and sensory properties) of orange pomace flour as a potential novel ingredient in a gluten-free bread system.

A further trial was then completed, whereby the effects of apple pomace were studied during the extrusion process and also on the resulting extrudate.

Extrusion is a low cost, high temperature, short time and high pressure process (Frame, 1994). Pre-moistened starchy material is converted into a puffed snack via the transformation of mechanical energy to thermal energy (Chessari & Sellahewa 2000).

The objective of this study was to develop an optimised formulation containing apple pomace; one which did not compromise on the expansion and textural characteristics of the extruded snack. Extrusion contains numerous extruder variables, the most significant of which need to be optimised. Therefore, a response surface design was performed to optimise the levels of apple pomace addition, the die head temperature and the screw speed of the extruder. Analysis consisted of parameters which are considered crucial in the development of a puffed snack (e.g. radical expansion ratio, bulk density and porosity) while also gathering a comprehension of the practical effects (e.g. texture and snack moisture) of apple pomace addition. (An ideal puffed snack should be highly expanded, aerated and have a porous structure.)

Unlike the gluten-free bread system where orange pomace was the dominating variable studied, the screw speed and die head temperature of the extruder had the most significant effect on expansion characteristics of the extrudate. Excess screw speeds degraded starch and limited its ability to expand upon being extruded, producing a dense tightly packed snack. The hilum is the centre of the starch granule where nucleation begins (Moraru & Kokini 2003). Extreme screw speed can cause damage to the hilum, reducing the ability of starch to absorb and carry water to begin bubble formation, resulting in an under-expanded and dense

snack.

Super-heated water is the driving force behind bubble growth. When high screw speeds were coupled with excess die head temperature, the extreme temperatures were believed to have driven the moisture out of the melt too quickly, reducing the expansion capabilities of starch. As expected, a negative correlation was found between bulk density and radical expansion ratio; snacks with a high bulk density will show poor expansion characteristics.

Apple pomace, when added in excess quantities, had a negative impact on expansion characteristics of the extrudate. The fibre present in apple pomace potentially reduced the expansion for a number of reasons. The fibre in apple pomace is hydrophilic; therefore it would bind water in competition with the starch, reducing the ability of starch to expand. If less moisture is present in the melt, the viscosity of the melt is increased, therefore restricting expansion properties. The arrangement of fibre can be explained as being linear in structure; hence it could align itself linearly within the barrel preventing the ability of starch to expand once extruded. The higher fibre present following higher additions of apple pomace may have pierced and burst the bubble structure, thus reducing expansion.

Positive implications of using apple pomace in an extruded snack were noted, in particular with regard to the texture and acoustic properties. Increasing the levels of apple pomace addition reduced the mechanical hardness of the snack and increased the crispiness of the snack. This was hypothesised to be related to the makeup of the fibre present in the apple pomace. The presence of pectin would

also have been a contributing factor. Pectin is known to have a lubricating effect within the extruder barrel, producing a crispier snack instead of a harder snack (van der Sman & Broeze 2013).

The optimised and validated snack combination was calculated to be 7.7 % apple pomace addition, die head temperature of 150 ^{o}C and a screw speed of 69 rpm. This combination of parameters resulted in a puffed product with positive expansion and textural characteristics.

6.1 Final remarks:

Disposal of fruit and vegetable "waste" can be quite costly for the producer, and also wasteful. Seeking an alternative use (i.e. as a novel ingredient) could be economically beneficial for the producer.

This thesis has studied the nutritive properties and physicochemical characteristics of two fruit by-products. It also investigated their application in two food systems (gluten-free bread and extruded products).

The chemical composition of the by-products distinguished fibre as the main functional constituent. Understanding the type of fibre in each by-product was significant in explaining their application and function in the different food products.

Orange pomace flour proved to be a feasible ingredient in a gluten-free bread formulation, producing an increased loaf volume and comparable texture to the control. It also improved the total dietary fibre content of the bread compared to the starchy control. Its use in combination with other anti-staling agents e.g. ascorbic acid and enzymes such as cellulose, could further improve its impact on reducing hardness. Properly elucidating the role of orange pomace in a gluten-free batter proved significant in understanding how it reacts with the other components of the batter system.

Apple pomace was applied to an extruded snack formulation. The soluble fraction of fibre (pectin) was theorized to play a major role in developing a crispier snack. This supports the earlier statement of the importance of gaining a

greater understanding of the application of fibre present in the by-products ingredients.

This thesis highlights that it is possible to include fruit by-products into food products. Including the by-products can improve texture, nutrition and sensory properties of products. It also highlights the importance of understanding the raw materials prior to product development. These by-products have the potential to be applied to a selection of cereal and sweet products i.e. cakes, pretzels, pastries and confectionary.

6.2 References:

Chessari, C.J. & Sellahewa, J.N. (2000). Effective process control. In: Guy, R. (Eds.), *Extrusion cooking Technologies and applications*. Woodhead Publishing Ltd, Cambridge, pp. 83-107.

Frame, N.D. (1994). Operational characteristics of the co-rotating twin-screw extruder. In: Frame, N.D. (Eds), *The technology of extrusion cooking.* Blackie Academic & Professional, an imprint of Chapman & Hall, Glasglow, pp. 1-51.

Moraru, C.I., & Kokini, J.L. (2003). Nucleation and expansion during extrusion and microwave heating of cereal foods. *Comprehensive Reviews in Food Science and Food Safety*, 2(4), 147-165.

Van der Sman, R.G.M., & Broeze, J. (2013). Structuring of indirectly expanded snacks based on potato ingredients: A review. *Journal of Food Engineering*, 114(4), 413-425.

Appendix: Publications and awards.

Peer reviewed publications:

O'Shea, N., Arendt, E. K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science and Emerging Technologies*, 16(0), 1-10.

Ktenioudaki, A., O'Shea, N., & Gallagher, E. (2013). Rheological properties of wheat dough supplemented with functional by-products of food processing: Brewer's spent grain and apple pomace. *Journal of Food Engineering*, 116(2), 362–368.

O'Shea, N., Arendt, E., & Gallagher, E. (2013). Enhancing an extruded puffed snack by optimising die head temperature, screw speed and apple pomace inclusion. *Food and Bioprocess Technology*, 1-16.

O'Shea, N., Doran, L., Auty, M.A.E., Arendt, E., & Gallagher, E. (2013). The rheology, microstructure and sensory characteristics of a gluten-free bread formulation enhanced with orange pomace. *Food and Function*, 4(0), 1856-1863.

O'Shea, N., Arendt, E., & Gallagher, E. (2013). The coeliac position: state of the art in gluten-free research. *Concise Reviews and Hypotheses in Food Science*. (Accepted).

Manuscripts under review:

O'Shea, N., Roessle, C., Arendt, E., & Gallagher, E. (2013). New application of a fruit by-product: a gluten-free approach. *Cereal Foods World.* (manuscript under review).

O'Shea, N., Ktenioudaki, A., Smyth, T. P., McLoughlin, P., Doran, L., Auty, M.A.E., Arendt. E., & Gallagher, E. (2013). Nutritional and physicochemical examination of apple pomace (Malus domestica Cv. "Karmijn de Sonnaville") and orange pomace (Citrus sinensis L. Cv. "Valencia"). *Industrial Crops and Products.* (manuscript under review).

Oral presentations:

O'Shea, N., Doran, L., Auty, M.A.E, Arendt, E., & Gallagher, E (2013). Utilization of orange pomace as a novel and low cost ingredient in the optimisation of gluten-free bread – rheology & baking study. *12th European Young Cereal Scientists and Technologists Workshop, Nottingham, UK.* (10^{th} - 12^{th} April).

O'Shea, N., Arendt, E., & Gallagher, E (2013). Investigating the effects of extrusion parameters and apple pomace on the physical attributes of an extruded puffed snack. *Annual Food Research Conference, Ashtown Food Research Centre, Dublin, Ireland.* (27^{th} - 28^{th} of June).

O'Shea, N., Arendt, E., & Gallagher, E (2013). Organoleptic enhancement of a puffed corn snack using Irish apple pomace. *Annual Walsh Fellowships Seminar, Royal Dublin Society (RDS), Dublin, Ireland.* (28^{th} of November).

Poster presentations:

O'Shea, N., Doran, L., Auty, M.A.E., Arendt, E., & Gallagher, E (2012). Orange pomace as a novel, low cost ingredient to improve the structure of gluten-free bread. *41st Annual Food Research Conference, UCC, Cork, Ireland.* (26^{th} - 27^{th} of April).

O'Shea, N., Doran, L., Auty, M.A.E., Arendt, E., & Gallagher, E (2012). A by-product from the Irish fruit processing industry as a novel ingredient in gluten-free bread. *Annual Walsh Fellowships Seminar, RDS, Dublin, Ireland.* (22^{nd} of November).

Awards:

Best Poster Award:

O'Shea, N., Doran, L., Auty, M.A.E., Arendt, E., & Gallagher, E (2012). A by-product from the Irish fruit processing industry as a novel ingredient in gluten-free bread. *Annual Walsh Fellow Seminar, RDS, Dublin, Ireland.* (22nd Novemember).