Manipulating Starch Digestibility by Influencing Molecular and Microscale Starch Structure in 3rd Generation Extruded Snack Foods

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Abstract

Pulses are high in several nutritional properties. Prior to consumption, pulses must undergo time consuming pulse preparation methods. These are necessary to minimise anti-nutritional factors intrinsic in legumes and improve functionality. In comparison to cereal and tuber starches, most pulse starches have greater thermal stability and can withstand more mechanical shear. This results in increased resistance to digestion.

This study explores the effects of extrusion processing and formulation moisture on the starch digestibility and structure of chickpea and red lentil flours. It is hypothesised that extrusion of pulses will result in increased starch digestibility.

Red lentil and chickpea flour were extruded (80°C; 30 rpm) at 10%, 40% and 80% potato starch substitution using 25% and 35% formulation moisture. The degree of starch gelatinisation was quantified using DSC (Differential Scanning Calorimetry) and XRD (X-ray Diffraction). Starch digestibility of raw flours, extrudates and expanded final products was quantified using static in vitro digestion methods using PAHBAH (4-hydroxybenzoic acid hydrazide) assay as an endpoint to obtain starch digestibility kinetics.

A complex non-linear relationship was observed between formulation moisture content and pulse incorporation. Increasing pulse content resulted in decreased starch gelatinisation (4.2 J/g in 80% chickpea flour incorporated vs 1.4 J/g in 10% chickpea flour incorporated extrudates) during extrusion, conversely increasing starch crystallinity (22.9% starch crystallinity in 80% chickpea flour incorporated, vs 20.0% starch crystallinity in 10% chickpea flour incorporated extrudates). Formulation moisture did not significantly affect gelatinisation, but

high formulation moisture was found to increase retrogradation 0.4 J/g in 10% chickpea flour incorporated extrudates with low moisture formulation vs 2.4 J/g in 10% chickpea flour incorporated extrudates with high moisture formulation. The degree of gelatinisation correlated strongly with the extent of starch digestibility in expanded snacks (P-value (Pearson correlation) < 0.00001), but not with overall crystallinity (P-value (Pearson correlation) = 0.51, indicating that the presence of ungelatinised starch was the main factor limiting starch digestion in these systems. These insights provide knowledge on the design of nutritious third generation snacks with the aim of lowering starch digestibility.

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1. Introduction

1.1. Background and project overview

There is a growing interest to increase the consumption of pulses within the human diet. This is due to a number of factors – popularity of plant-based diets (where pulses provide a good source of protein) and a move towards diets higher in fiber which pulses are also rich in (Edwards et al., 2020; McCrory et al., 2002).

In addition to their high fibre content, pulses are also generally considered to be low glycaemic index ingredients. The starch in pulses is entrapped within thick plant cell walls (shown in Figure 1.1) which limits the access of amylolytic enzymes, while the starch itself may be intrinsically more resistant to digestion when incorporated into food products. Due to the low post-prandial glycaemic responses observed after consuming many pulse based foods, including pulses into food formulations may be viewed positively from a nutritional perspective (Edwards et al., 2020).

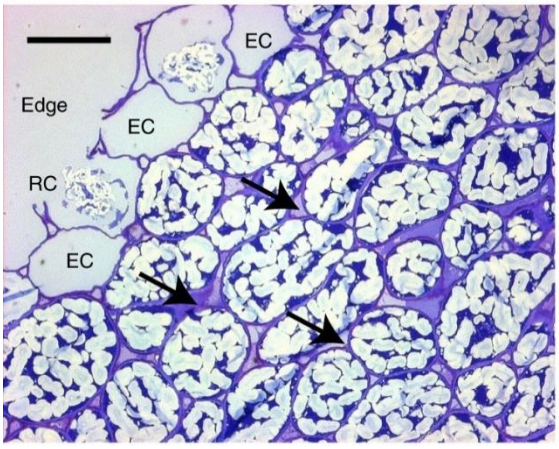

Figure 1.1: Micrograph showing the cellular structure of chickpea (stained with 1% wt/vol toluidine blue). Starch granules can be seen entrapped within thick cell walls. Adapted from (Edwards, Ryden, et al., 2021)

Despite these health benefits, uptake of pulse-based ingredients by the food industry has been limited. Pulses have a range of undesirable processing characteristics related to their physico-chemical functional properties. Pulse ingredients may impart undesirable textural properties to products such as tough or chewy textures and grain swelling in processed foods as well as ‘off’ or ‘beany’ flavours. Compared to other widely used starch-based ingredients they have very high water holding properties and require longer cooking at higher temperatures.

Despite the prevalence of knowledge regarding extruded snacks including those containing pulses there is a gap in understanding the digestibility and physical characterisation of extruded pulse snacks. In this project, I will address this knowledge gap through applying innovative 3rd generation extrusion technologies to generate pulse enriched snack products. The physico-chemical and techno-functional properties of these snacks will be explored and used to understand the digestion behaviour of these products in simulated *in vitro* digestions.

1.2. Snacks

Savoury snack food ingredients are commonly sourced from cereals and tubers, of which starch is the major component. Specifically, extruded snack foods require isolated food starches to be incorporated as ingredients which provide positive textural attributes to the finished product (Guy, 2001). The gelatinisation of isolated starch granules is key to the expansion properties of extruded snack foods. Ungelatinised starch granules act as nucleation points for bubble formation during expansion giving rise to even expansion throughout the finished product. Snacks prepared using potato starch such as savoury snacks

can be highly processed (Monteiro et al., 2013). The raw materials to produce these snacks are first milled to a fine powder. The granule size of potato starch is variable, ranging from 1 to 110 μm depending on the source. Potato starch is composed of approximately 21% amylose, 75% amylopectin, 0.1~% protein and 0.08% phosphorous (Lisińska & Leszczyński, 1989) Extrusion causes the starch gelatinise and partially depolymerise (depending on extrusion conditions), which in combination to the high temperatures present during extrusion produces starch that is more readily available to amylolytic enzymes when undergoing digestion (Brennan et al., 2013b).

When heavily processed food items like snacks are consumed, the starch contained within them are easily broken down into simple sugars, resulting in a high glycaemic response (Brennan et al., 2013a). Glycaemic response refers to the increase in blood glucose which is observed post-prandially as a consequence of consuming carbohydrate rich meals. The gylcaemic response is commonly measured using the glycaemic index (GI). GI is defined as the increase in post-prandial blood glucose following consumption of a meal containing a defined quantity of available carbohydrate compared to a reference (normally glucose or white bread). Regular consumption of high GI foods has been linked to a range of negative health consequences such as obesity, cardiovascular disease and diabetes (Brand-Miller et al., 2013). While conventional potato crisps have a medium GI (Atkinson et al., 2008), the high degree of processing involved in producing extruded snack products can dramatically increase the starch digestibility and therefore the GI of the products (Brennan et al., 2013a). In addition, snack foods often contain a high percentage of fat through the frying process. It has been found that these

snacks which are high in energy are not very satiating, and have in fact been linked with a decrease in satiety (Rolls et al., 2006).

The prevalence of chronic conditions, such as diabetes and obesity, as well as non-communicable diseases such as cancer and cardiovascular diseases has risen globally over the years (Bigna & Noubiap, 2019; Budreviciute et al., 2020; Koopman et al., 2016). There is extensive literature indicating that obesity and being overweight are main contributors to non-communicable diseases (Anis et al., 2010; Clark & Brancati, 2000; Taghizadeh et al., 2015). Kearns et al. (2014) examined the associations between the prevalence of chronic disease and BMI, and found that achieving a modest reduction in BMI - by as little as one unit - within the population would result in a 4% decrease in chronic disease in both males and females. Regular consumption of high GI and high fat snack products may be linked to the prevalence of these conditions (Brennan et al., 2013a; McCrory et al., 2002).

1.3. Pulses

1.3.1. Overview of pulses

The Fabaceae or Leguminosae family, commonly known as legume family is the third largest family in the plant kingdom with over 20,000 species and is the second most important for the human diet after Graminae/Poaceae (Gepts et al., 2005). Grain legumes, also known as pulses are the dry edible seeds of leguminous plants, which according to the Codex Alimentarius Commission (2007), are distinguished from leguminous oil seeds by their low fat content. Legume crops have the ability to fix nitrogen from the atmosphere using root nodules instead of requiring it from the soil (Biswas & Gresshoff, 2014). This

makes pulses and legumes more economical and environmentally friendly than cereal crops as they do not require expensive nitrogenous fertilisers and can increase soil fertility (Tiwari & Singh, 2012). Pulses are high in a number of nutritional properties, and are an excellent source of protein, vitamins, minerals, dietary fibre and carbohydrates as well as antioxidants and polyphenols in the human diet (Hall & Moraru, 2022; Rebello et al., 2014).

Chickpeas (*Cicer arietinum L.*) are one of the eight Neolithic founder crops in the Fertile Crescent of the Near East. Desi (microsperma) and Kabuli (macrosperma) are the two primary cultivated chickpea types. Chickpea grains are composed of 63.0% carbohydrate, 20.5% protein, 6.0% total lipid, 12.2% total dietary fibre, and 10.7% total sugars (United States Department of Agriculture, 2019). Chickpeas are considered a good source of protein, as they contain all eight essential amino acids: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Chickpeas contain relatively low amounts of sulphur-rich amino acids (methionine) in comparison to animal-based protein sources. The glycaemic index of chickpeas is low in comparison to other high-carbohydrate foods such as wheat bread and rice, which may be attributed to their high fiber content and complex carbohydrate structure. Chickpea starch granules are 10 – 20 μm, which is a relatively small granule size. Chickpea starch contains a high amount of amylose (36 - 41%), gelatinisation temperature is high 69 - 72°C (Ghoshal & Kaushal, 2020).

Red lentils (*Lens culinaris*) are another popular legume with a rich macronutrient composition. Red lentils contain 63.1% carbohydrate, 23.9% protein, 2.17 % total lipid, 10.8 % total dietary fibre, and 15.5% total sugars (United States Department of Agriculture, 2019). In comparison to chickpeas,

red lentils are slightly higher in protein, but contain less dietary fiber. Similarly to chickpeas, red lentils contain all of the essential amino acids, with lower amounts of sulphur rich amino acids, and also classified as a low glycaemic index food. Red lentil starch granules are smaller in comparison to chickpea starch granules, at 2 – 10 µm. Red lentil starch also contains a high amount of amylose 32.52 ± 3.52%, with a similarly high gelatinisation temperature of 70°C (Frias et al., 1998).The complex carbohydrates in pulses include dietary fibres, resistant starch and oligosaccharides which produce a low glycaemic response (Rizkalla et al., 2002). As a source of protein, pulses contain a good amino acid profile – being rich in lysine, they complement lysine deficient cereals whereas cereal proteins contain high amounts of sulphur containing amino acids such as methionine and cysteine (Belitz et al., 2009). They can be grown in a wide range of climate and soil conditions, which makes them economically ideal for consumption in developing low-income countries, as an alternative to animal protein sources.

1.3.2. Current consumption of pulses

Pulses are currently widely used as a protein source in developing countries. Based on agricultural importance, Leguminosae are second to only cereal crops regarding area harvested and total production (FAO, 2016).

Pulses are traditionally consumed primarily as whole or split pulses, as an addition to stews, curries and soups. When consumed in this manner, the dish usually requires thermal processing for an extended period of time (De Almeida Costa et al., 2006). Pulse flour such as chickpea flour is also heavily used for making breads. Pulses may also be soaked and ground into a paste before being used as an ingredient for recipes using a fermented batter (idli or dosa).

Germinated beans may be eaten raw when sprouted following a series of soaking and draining.

These methods of pulse preparation are very time consuming stages in advance of pulse ingestion, but the steps are necessary when using raw pulses, due to the presence of anti-nutritional factors. These food processing methods improve the palatability of foods in addition to increasing the bioavailability of nutrients and bioactive compounds.

1.3.3. Pulse proteins

Proteins are made up of sequences of amino acids. Some are essential amino acids that the body cannot synthesise, so they must be obtained externally from the diet. The amino acid profile of food proteins is an indicator of the nutritional quality of the food, and the amino acid profile can be very different between proteins from different sources. This can also be reported as the biological value or protein digestibility (Boye et al., 2010).

Amino acid composition is an important factor in the nutritional indication of proteins. However, protein bioavailability, or digestibility must also be taken into account when determining protein quality (Boye et al., 2012). The Protein Digestibility Corrected Amino Acid Score (PDCAAS) is a traditionally used (for the past 20+ years) method of assessing protein quality and is determined by the most limiting amino acid of a food in comparison to a reference amino acid pattern specifying the needs of a human(Food and Agriculture Organization/World Health Organization, 1991) . Using this measure, protein sources such as milk, beef and eggs score highly with a PDCAAS of 100. In comparison, pulses score lower with a PDCAAS of 52 for chickpeas, 51-63 for

lentils and 50-68 for peas. Pulse scores vary depending on cultivar, variety and processing method (Vaz Patto et al., 2015).

The major proteins fractions contained in pulses are globulins (approximately 70%) and albumins (10 – 20%) (Roy et al., 2010). Also present in minor proportions are prolamins and glutelins. Pulse proteins can be classified according to their solubility in different solvents in reference to the Osborne classification system (Osborne, 1924). Using this classification, globulins are soluble in dilute salt solution, albumins in water, prolamins in 70% ethanol solution and glutelins in dilute alkali solution (Oomah et al., 2011).

Globulin proteins are usually classified as 7S or 11S according to their sedimentation coefficients (S – Svedberg unit). In peas, the 7S and 11S globulin proteins are respectively named vicilin and legumin, so the equivalent proteins of other legume seeds are often specified as vicilin-like and legumin-like globulins.

Legumin proteins are the main storage protein of most legumes with a molecular mass (MM) of 340-360 kDa. They are composed of 6 subunits in a quaternary structure, each with a MM of approximately 60 kDa which are linked by non-covalent interactions. Subunit pairs are made up of acidic (MM of approximately 40 kDa) and basic (MM of approximately 20 kDa) subunits linked by a single disulphide bond. Legumins frequently sediment with vicilin as a single component.

Vicilins are trimeric proteins with a molecular mass of 175 – 180 kDa, although protein molecules have also been reported with various subunits of 75, 43, 33,

56, 12 and 25 kDa. Unlike 11S globulins, vicilins do not form disulphide bonds, due to a lack of cysteine residues. (Boye et al., 2010).

Convicilins are a third type of globulin found in legumes in lesser amounts in comparison to vicilin and legumin. Like vicilin, it is a 7S protein, with molecular weight ranging from 220 – 290 kDa. Each molecule consists of 3 to 4 subunits which each have a molecular weight of 70 kDa. Similarly to 7S vicilin, convicilin is low in carbohydrate, however unlike vicilin, convicilin does contain sulphur-containing amino acids (Boye et al., 2010; Croy et al., 1980).

Albumins can vary greatly in molecular mass, with a range of 16 to 483 kDa (Papalamprou et al., 2010) They encompass different protein types such as enzymatic proteins, lectins and protease inhibitors as well as metabolically active compounds which are important during seed germination. Albumins are high in lysine and sulphur containing amino acids such as cysteine and methionine.

Prolamins and glutelins make up a small proportion of legume protein fractions (10-20%). Prolamins are characterised by a high concentration of proline and glutamine whereas glutelins are also high in methionine and cysteine.

1.3.4. Pulse antinutritional factors

The hull of pulses, particularly peas and lentils, are high in antinutrients, lipids which tend to cause off flavour and odour, and most of the insoluble fiber content (Wood and Malcolmson, 2011). Antinutritional factors are compounds named as antinutrients due to their ability to reduce the bioavailability of nutrients. Common antinutritional factors found in pulses and legumes are phytates, lectins and protease inhibitors.

Phytates are salts of phytic acid and are commonly found in plant seeds such as nuts or pulses/legumes as the main storage form of phosphorous. Phytates are identified as antinutritional factors due to their ability to complex with minerals such as calcium, zinc, iron and magnesium, causing these minerals to become unavailable for absorption. Phytates may also interact with proteins to form complexes such as protein-phytate or protein-mineral-phytate, which may decrease solubility, digestibility and functionality of proteins. Phytates have also demonstrated the ability to inhibit the action of digestive enzymes α-amylase, pepsin and trypsin (Deshpande & Cheryan, 1984; Knuckles et al., 1985; M. Singh & Krikorian, 1982).

In addition, phytates have been found to inhibit the action of enzymes involved in digestion. Phytate inhibits the activity of α-amylase by 16% at 0.5 mM and 95% at 9.0 mM at pH 7 after 15 minutes of preincubation (Deshpande & Cheryan, 1984). This may be due to the interaction of phytates with α-amylase, or formation of a complex with minerals such as calcium as calcium halogen salts are known to catalyse α-amylase activity.

Knuckles, Kuzmicky, & Betschart (1985) studied that effect of sodium phytate and partially hydrolysed sodium phytate (0-82% hydrolysed) on *in vitro* pepsin digestion of casein and bovine serum albumin and found that the inhibitory effect increased with increased phytate concentration and varied according to the substrate. It is thought that pepsin inhibition by phytates occurs by the binding of phytate to the protein substrates via positively charged protein groups, proceeding at a pH lower than the isoelectric point of proteins (Okubo et al., 1975; Vaintraub & Bulmaga, 1991).

Trypsin proteolysis is also inhibited, with Singh & Krikorian (1982) observing that preincubation of 0.1M phytate with 2.5% casein solution at 37°C resulted in diminished enzyme activity of over 40%. They suggest that this is caused by the binding of phytates to calcium. Calcium ions are required for the formation of trypsin from trypsinogen, therefore inhibiting the conversion of this reaction leads to a lack of trypsin molecules which in turn hinders proteolysis by trypsin.

Lectins are hemagglutinating glycoproteins which make up a portion of the albumin fraction. They exhibit carbohydrate binding specificity and stimulate intestinal cells, interfering with absorption of nutrients within the intestine (Gonzalez de Mejia et al., 2003; Y. Li et al., 2010).

Protease inhibitors such as trypsin and chymotrypsin inhibitor also belong to the albumin protein fraction. Similarly, to lectins, protease inhibitors interfere with enzyme activity during digestion. They may belong to either the Kunitz or Bowman-Birk family, with Kunitz type inhibitors specifically inhibit trypsin and Bowman-Birk type inhibitors acting on both trypsin and chymotrypsin simultaneously at independent binding sites (Lajolo & Genovese, 2002). These proteins are resistant to pepsin, and the low pH present during the gastric phase so are available to inhibit to trypsin and chymotrypsin through irreversible binding.

Although these factors interfere with the digestibility of starch and proteins, lowering the nutritional value of pulses, this effect is usually only manifested if the seed or the flour is consumed uncooked; processing through soaking or cooking reduces or eliminates enzyme inhibitor activity.

1.3.5. Pulse starches

Pulse starches typically contain a higher concentration of amylose in comparison to cereal and tuber starches with 28.6-34.3% amylose content reported for chickpea starch (Singh et al., 2004). They are good sources of both slowly digestible starch and resistant starch (Bajaj et al., 2018).

Obtaining high purity starch from certain pulses such as lentils, lima beans or white navy beans may be difficult due to the presence of highly hydrated fine fiber fractions and insoluble flocculent protein (Schoch & Maywald, 1968). Pulse starches are isolated by using wet or dry milling techniques, with wet milling processes obtaining higher purity than dry milling (N. Singh et al., 2011).

In comparison to cereal and tuber starches, the majority of pulse starches have greater thermal stability and can also withstand more mechanical shear. Native (uncooked) pulse starches are more readily digested than native tuber or high amylose maize starch, but are less digestible than native cereal starches (Hoover et al., 2010).

1.4. Starches and starch structure

1.4.1. Overview of starches

Starches are polymeric carbohydrate glucose units joined by glycosidic bonds. They are made up of a mixture of two different α-glucans: amylose and amylopectin.

Amylose is a sparsely branched or linear polymer containing long chains of several hundreds to thousand glucosyl units. Amylose consists predominately of α-(1,4)-D-glucose residues which bond to each other to form a tightly packed helical structure (Hizukuri et al., 1981; Takeda et al., 1987). When in the

presence of iodine, a complex is formed, which produces an intense blue colour with a λ_{max} = 620 nm. Because of this characteristic light microscopy can be used to assay qualitatively for amylose in starch (Knutson & Grove, 1994).

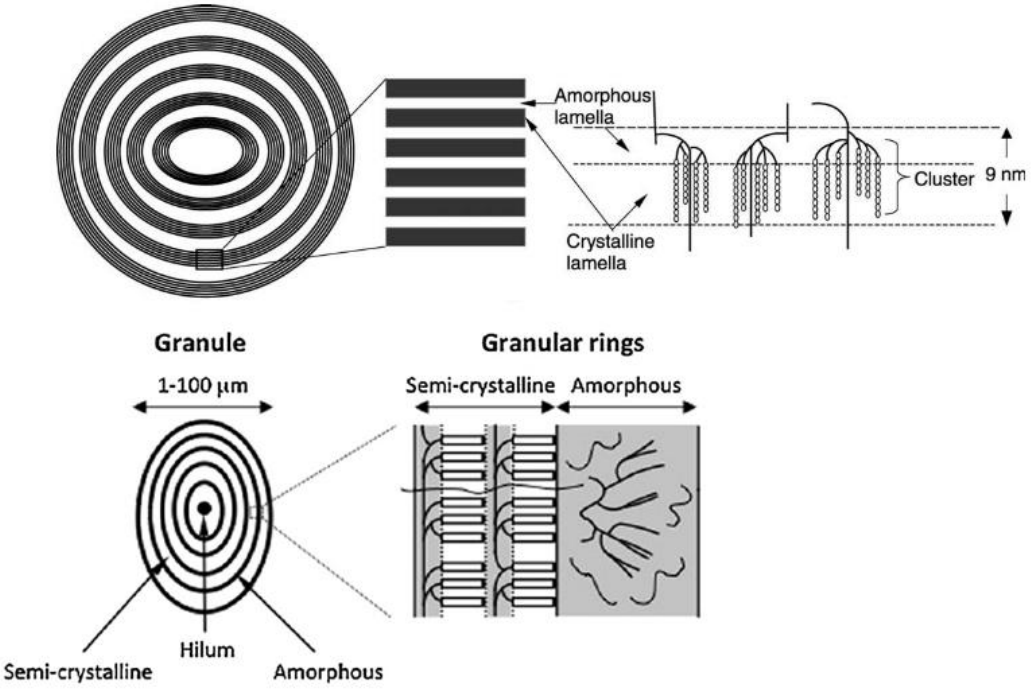

Figure 1.2: Graphical representation of the starch granule (left), featuring its centre of growth (a.k.a. the granular hilum) and its characteristic alternating semi-crystalline and amorphous growth rings (right), adapted from (Vamadevan & Bertoft, 2014).

Amylopectin is the major component of starch by weight (~75%) and is one of the largest molecules found in nature. It is a highly branched molecule of relatively short chains in comparison to amylose, consisting of three types of branch chains. Each amylopectin molecule contains chains which are referred to as A, B and C chains. The A-chains are terminal chains and do not have further branches coming from them. The B-chains have A-chains branching off of them, while each amylopectin molecule contains only a single C-chain which holds the unique reducing end of the molecule (Vamadevan & Bertoft, 2014).

The shorter A-chains form double-helices which form the crystalline parts of the starch granule (Figure 1.8), while the longer B- and C- chains span the amorphous parts of the starch granule. Due to the short length of the unit chains which amylopectin contains, iodine does not form a stable complex. The complex that does form results in the development of a purple colour with a λ_{max} = 530 nm (Knutson & Grove, 1994).

Plant starches are typically classified into three types: A, B and C depending on the x-ray diffraction pattern they produce. The diffraction pattern differs according to the amylopectin crystalline lattice and packing density of the double helices, with B-type structures found to be more spacious than A-type. The conformity of amylose and amylopectin within a starch granule differ according to the various species of starch. Starch granule structures are part crystalline and part amorphous (Figure 1.2). The crystalline component is largely composed of short chains of amylopectin molecules which form bundles of double helices (Imberty et al., 1991). Both polymorphs are composed of double helices in ordered arrays, A-type structures closely packed into a monoclinic unit cell containing 8 water molecules and B-type structures packed in a hexagonal unit cell containing 36 water molecules (Imberty & Perez, 1988; Popov et al., 2009). A-type starches are commonly found in cereal endosperms, whereas tuber and root starches typically exhibit B-type diffraction patterns.

Pulses are usually C-type starches. It has been suggested that C-type diffraction patterns arise from a mixture of both A and B type crystals within a single or distinct granule, and in fact do not define a distinct and separate structure. Bogracheva, Morris, Ring, & Hedley (1998) investigated the structure and properties of C-type starches from pea seeds and found that these

granules contained polymorphs of both A- and B-type. B-type polymorphs were found in the centre of starch granules whereas A polymorphs were located on the periphery. The A and B structures were also found to melt at different temperature, with the B type polymorph melting at a lower temperature than the A type in the presence of 0.6M KCl. This characteristic shift in melting behaviour in the presence of KCl has been used to characterise the presence of C-type crystalline structures in legume starches (Bogracheva et al., 1998).

1.4.2. Starch functional properties

The functional properties in pulse flours or pulse isolates refer to the physical and chemical properties which can influence behaviour during processing, storage, preparation and consumption. These properties may include solubility, gelatinisation, water binding, gelation and emulsification (Hoover et al., 2010). Essentially functionality is the behaviour of the material (starch or protein) under various water, pH, heat, and time conditions during each of the processing operations required to produce a food product.

Water holding capacity (WHC), also known as water absorption capacity (WAC) is the ability described as the amount of water that can be absorbed and held per gram of sample material (Farooq & Boye, 2011). In flours, this property indicates how some nutrients and bioactive compounds interact with water (Boucheham et al., 2019). Pulse flours have a higher WHC in comparison to semolina, rice and potato starch (Boucheham et al., 2019; Chandra et al., 2015). Water holding capacity of pulses increases upon cooking (Elhardallou & Walker, 1993).

1.4.3. Starch gelatinisation

When starch is heated in the presence of excess water it undergoes a distinctive thermal transition, referred to as gelatinisation, which is one of the most important steps during the processing of starch (Figure 1.3). Gelatinisation can occur at different temperatures depending on the botanical origin of the starch, although it will generally occur between 50 and 80°C. At the onset of gelatinisation, water enters into the amorphous parts of the starch granule, causing them to rapidly swell. This triggers the melting of the crystalline regions of the granule as a cooperative process (Perry & Donald, 2000). Following melting of the crystalline parts of the starch granule, the granule rapidly swells to between 5 and 20 times its original volume, and it also begins to leach polymers, mainly amylose, into the surrounding solution resulting in a rapid increase in viscosity. The swollen remnants of the granules, termed ‘granule ghosts’ may remain intact, although their fragile structure is easily disrupted by shear forces resulting in further leaching of polymers and viscosity changes (Zhang et al., 2014).

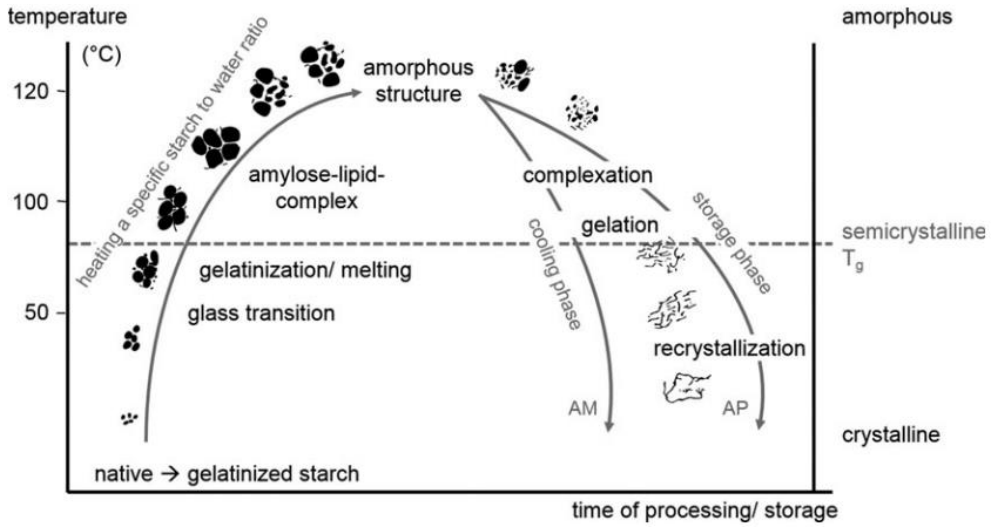

Figure 1.3: Phase diagram showing the state and phase transition of starch when applying a temperature profile (T_g, gelatinisation temperature; AM, amylose; AP, amylopectin). Adapted from (Schirmer et al., 2015)

The gelatinisation of starch is highly dependent on water content during the gelatinisation process. For starches with a normal amylose content (15-30% amylose) under excess water conditions, gelatinisation will occur completely and at a relatively low temperature. Water is necessary for gelatinisation, as it allows swelling of the granule and it is able to form hydrogen bonds with free hydroxyls on the residue glucose moieties of the starch chain which become available following melting of the crystalline regions of the granule. Under water limiting conditions, starch gelatinisation temperatures will be shifted higher as there is a greater endothermic barrier to overcome in order to achieve complete gelatinisation (Perry & Donald, 2002). In addition, at lower water contents the granules will not swell and will retain their compact granular structure. At very low water contents (below ~20%) no gelatinisation will occur at all, and instead the starch crystallites will melt at around 160°C. Therefore, water content and temperature are the two key variables which impact on the extent of starch gelatinisation during food processing (Bogracheva et al., 2002; Perry & Donald, 2002; Roder et al., 2009).

There are a number of different methods which can be used to measure gelatinisation of starch. Add an overarching statement of the different methods before jumping into the various methods since they all measure different aspects of gelatinization to provide mechanistic insight. Polarised light microscopy reveals birefringence, which is an indicator of crystalline areas that have an ordered arrangement. When examining native starch under polarised light, maltese crosses are recognisable within starch granules, demonstrating the presence of crystalline structures. The maltese cross pattern arises as a result of the ordered repeating helices in the crystalline regions of the starch granule which rotate the plane of polarised light (Figure 1.4). The cross pattern reflects the radial arrangement of chains within a starch granule. Upon gelatinisation of starch granules, these maltese crosses are no longer visible when viewed under polarised light. This shows that the structure of the starch granule is no longer an ordered structure which is capable of rotating the plane of polarised light (Pérez et al., 2009).

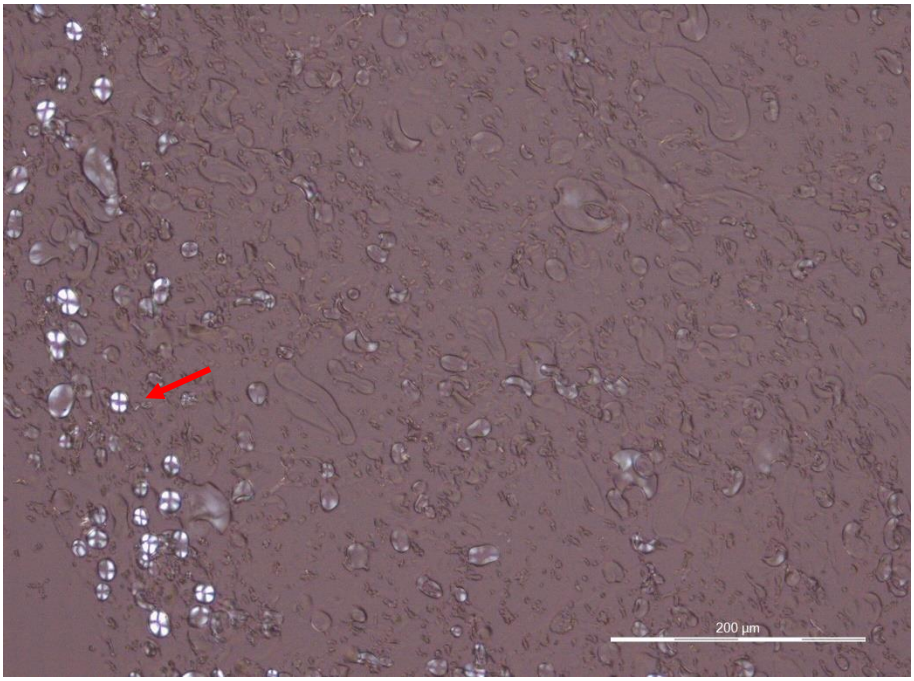

Figure 1.4: Polarised light microscopy of third generation extruded half product containing 40% red lentil flour, low formulation moisture. Red arrow indicates native starch granule, signified by the distinct maltese cross.

Differential scanning calorimetry (DSC) is a technique used to measure enthalpy change of a material by heating or cooling a sample in tandem with a reference pan and measuring the direction and degree of heat flow in the sample. Through this, changes in physical state and structure can be inferred, as well as interactions of starch with other components of model systems and composite food matrices (Liu et al., 1991). DSC can detect both first order transitions such as melting or crystallisation as well as second order transitions, such as glass transitions. When starch gelatinises, an endothermic change occurs which is visible as a peak. This occurs at different temperatures according to the starch source. Cereal and tuber starches produce a narrow peak at differing temperatures whereas legume starches produce a single peak which splits into a broad double peak in the presence of 0.6M KCl (Bogracheva et al., 1998). The temperature of the gelatinisation peak which is observed for starch analysed using DSC is related to the stability of the crystalline regions of the starch granule, with more perfect crystalline regions melting at higher temperatures, which is reflected in the differences in melting temperature observed between starches from different botanical origins (Genkina et al., 2007; Tester et al., 1998).

X-ray diffraction (XRD) is a non-destructive analytical technique used in the determination of material crystallographic structures. XRD can be used to determine the crystallinity pattern through packing of helices. Starch granules exhibit X-ray diffraction patterns associated with A-type (cereal starches) and B-type (tuber and amylose rich starches) crystalline polymorphic forms. C-type polymorphs can also be distinguished, arising from coexisting A-type and B-type crystal structures within the starch granule and is characteristic with pulse

starches (Buléon et al., 1998; Zobel, 1988). V-type crystal structures are characteristic of amylose complexed with fatty acids and monoglycerides, and can appears when starch gelatinises, and appears natively in some cereal starches (Gernat et al., 1993) (Figure 1.5).

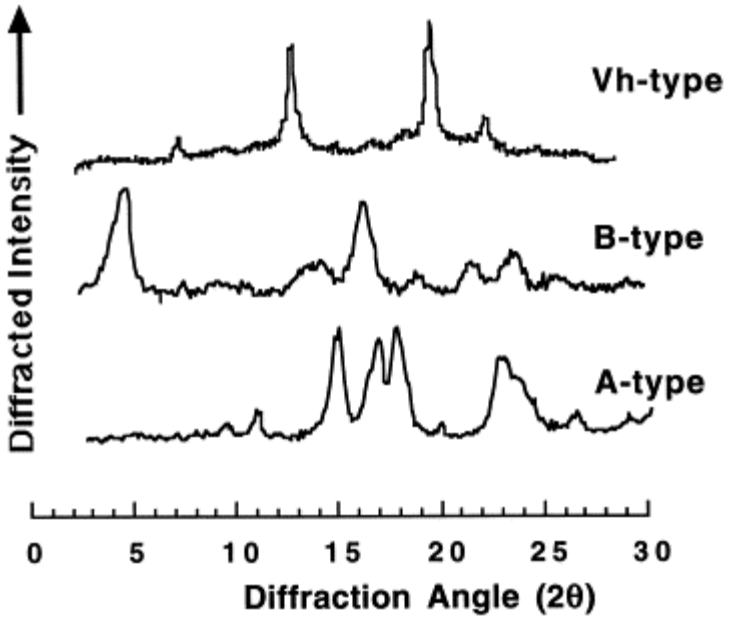

Figure 1.5: X-ray diffraction diagrams of A-, B- and Vh-type starch. Adapted from (Buléon et al., 1998)

XRD analysis may also be used in the characterisation of starch gelatinisation, as the melting of starch crystallites can be examined (Zobel et al., 1986), and to calculate the degree of crystallinity in different starch sources (Figure 1.6) (Lopez-Rubio et al., 2008).

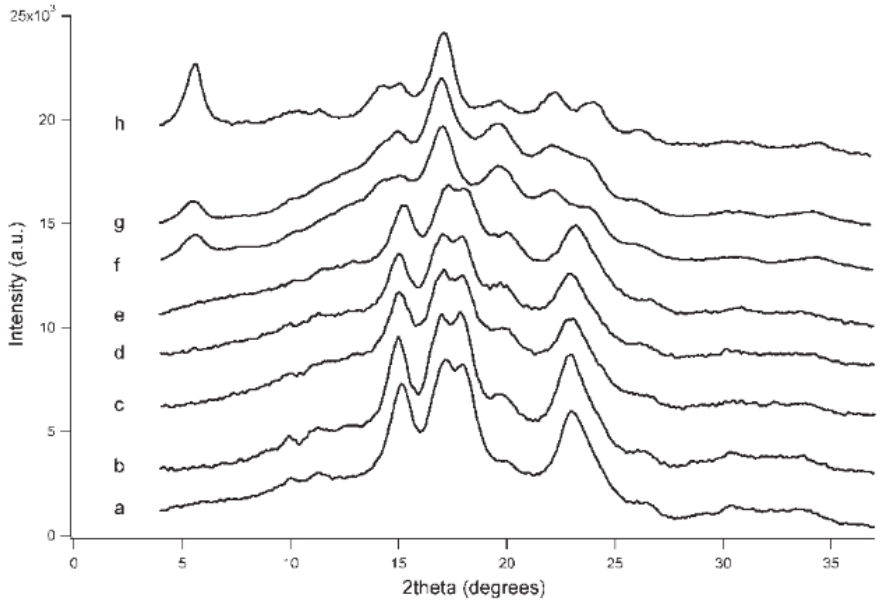

Figure 1.6: X-ray powder diffractogram of a) Penford waxy maize, b) rice, c) NB1 wheat, d) Penford wheat, e) Golden Promise barley, f) Hylon, g) Gelose80, and h) Potato starches. Peak positions occur where the X-ray beam has been diffracted by the crystal lattice. Data have been offset for clarity. Adapted from (Lopez-Rubio et al., 2008)

1.4.4. Starch retrogradation

Starch retrogradation occurs upon cooling of gelatinised starch. This reaction involves the interaction amylose and amylopectin chains, leading to the formation of a more ordered structure. Retrogradation is accompanied by physical changes such as increased viscosity and turbidity of pastes, formation of a gel, an increase in the degree of crystallinity and exudation of water (syneresis) (Wang et al., 2015). Starch retrogradation also results in the starch structure forming a B-type X-ray diffraction pattern over time (Miles et al., 1985). Factors which can affect degree and rate of retrogradation include amylopectin branch chain length distribution, amount of amylose leaching out of granules during gelatinisation and the extent of interaction between amylose-amylose (AM-AM) and amylose-amylopectin (AM-AP) chains (Ambigaipalan et al., 2013). Pulse starches show a higher degree of retrogradation in comparison to A-type

starches. This higher tendency to retrograde may be linked to the higher amount of amylose present in pulse starches in comparison to cereal starches.

DSC can be used to characterise starch retrogradation depending on the change in enthalpy following storage at 4°C (storage conditions which accelerate retrogradation). The degree of retrogradation (DR) is described as the gelatinisation enthalpy change of starch granules divided by the enthalpy change of reheated retrograded starch gels (Wu et al., 2009). The quantity of water present during starch gelatinisation and the following retrogradation is found to have a significant effect on DSC measurements, with Wang, Li, Zhang, Copeland, & Wang (2016) observing a greater enthalpy change for retrograded starch with a water content of 33 to 50% than that of native starch. This may be caused by melting of residual crystallites remaining after gelatinisation in addition to crystallites formed by retrogradation.

The changes that the starch granules undergo during gelatinisation and retrogradation can have an effect on digestibility. Native starch is slowly digested by digestive enzymes due to the highly ordered structure found within granules. Gelatinisation through processing or cooking disrupts the ordered structure into an amorphous mass, which highly increases susceptibility to enzymatic breakdown. The transition of a disordered-to-ordered structure including double helices and crystallites, due to retrogradation causes starch to become less easily broken down by digestive enzymes, and results in a lower glycaemic response.

1.5. Starch based snack processing techniques

First generation snacks are a popular and commonly known snack type. These snacks require minimal mechanical processing, such as sliced and deep fat fried potato crisps, nuts or popped popcorn, and are the most uncomplicated and basic in terms of production. Second and third generation snacks utilise the innovate technique known as extrusion (Riaz, 2016).

1.5.1. Extrusion

1.5.1.1. Overview of extrusion

When using pulses and legumes for manufacture of food products, they are rarely used in their raw form. Instead, pulses such as lentils and chickpeas undergo several industrial processes before they are prepared for use.

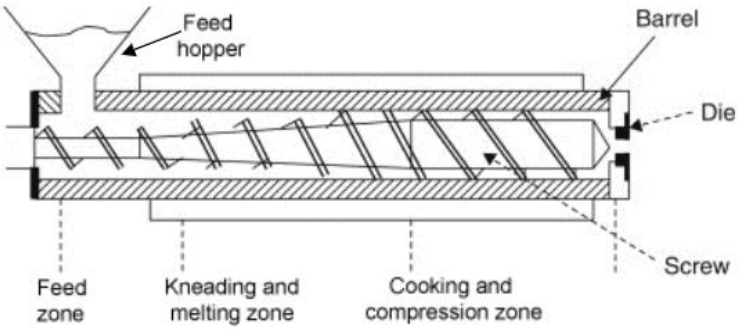

Figure 1.7: Schematic representation of a single screw extruder for food production. Adapted from (Pasqualone et al., 2020)

Extrusion is an efficient procedure which combines several other processes, such as mixing, shearing, kneading and cooking (Figure 1.7). Ingredients are blended together with specific water content, then fed into an extruder barrel which contains one or two screws. These screws spin and push the formulation through the barrel which may be heated to different temperatures according to barrel section. Pressure is imparted onto the mixture as it gets pushed towards

the narrow die head which along with high temperatures causes starch to get cooked.

This process is commonly used in the manufacture of various food products, such as pasta, fruit gums and expanded snack foods. It is a popular method of producing foods in industry due to it being a high productivity, low labour process. In addition, the high temperatures and short cooking times facilitate significant nutrient retention (Guy, 2001).

Extrusion can be used to alter the digestibility of food components. Pulse protein sources are more difficult to digest in comparison to animal sources, due to the closed compact structure of the proteins, which prevents easy access of digestive enzymes. The intense mechanical shearing that extrusion imparts on food is able to open up the structure, exposing sites for enzyme attack. In addition, high heat causes protease inhibitors to become denatured, increasing protein digestibility (Alonso et al., 2000a).

1.5.1.2. Types of extrusion

Second generation snacks are those that are formed through direct extrusion and make up the majority of snacks. Products such as puffed corn snacks and cereals fall under this category. Direct extrusion requires specific extrusion parameters and dough formulations to produce snacks with appealing sensory properties. High shear and pressure is produced through screw speed being relatively high (150 - 300 rpm), and high temperatures (130 - 200°C) cooks contained starch fully such that starch granules are completely gelatinised. Product formulations are high in feed moisture (13–15 % (wb)), and the combination of pressure and heat as it moves through the screw generates flash moisture evaporation as it exits the die head, along with a swift drop in

pressure. These events result in the rapid expansion of the formulation, giving rise to a finished product with desirable characteristics such as a light and crisp texture (Grasso, 2020; Korkerd et al., 2016).

Third generation snacks are also processed using extrusion technologies, however unlike second generation snacks which are directly expanded as they leave the extruder, these third generation extrudates exit the extruder in a glassy state and require a second processing step in order for expansion to occur. The difference in extrudate structure is due to differences in extrusion parameters. In contrast to second generation technology which utilises high temperature and high shear, third generation extrusion is carried out at lower pressure and temperature, resulting in lower shear. A reduction in formulation moisture also ensures that moisture flash as the extrudate exits the screw does not occur, therefore the resulting half product retains a glassy structure, with the starch within the dough not fully cooked and partially gelatinised. These half products, also known as pellets can be stored at a low moisture of 10 – 12%. Due to the low volume of product, these extrudates are far more easily stored than their second generation counterparts. However they then require a further heating step to facilitate expansion.

1.5.2. Expansion

Expansion is an important process for improving sensory attributes of third generation snacks such as appearance and texture (Mazumder et al., 2007). Extruded snack half products are usually either oven baked or deep fat fried, which involves heat transfer as well as water evaporation (Fellows, 2000). This process produces highly expanded snacks which have desirable textural properties such as crunchiness and crispiness (Meng et al., 2010).

Extruded snack pellets containing low moisture content may be stored at room temperature. In order for expansion to occur, the extrudates are heated to high temperatures. As the temperature increases, the glass transition temperature is reached, and the pellet enters the rubbery state at ~50°C (Boischot et al., 2003; Moraru & Kokini, 2003). Beyond this temperature, water may be absorbed by starch granules, resulting in gelatinisation (van der Sman & Bows, 2017). Vaporisation of water within the starch matrix is purported to drive extrudate expansion (Varnalis et al., 2001). While in the rubbery state, expansion begins with heterogeneous nucleation. Nucleation sites may be trapped air bubbles, free volume or hydrophobic ingredients such as fibres (Moraru & Kokini, 2003; van der Sman et al., 2018). As the temperature continues to increase, bubbles expand until either they collapse, or expansion ends when the extrudate moves back into a glassy state (Boischot et al., 2003). The solid matrix will remain in the expanded form as it is cooled to room temperature.

Deep fat frying is the traditional and popular method used in the expansion of third generation snacks. The heat transfer that takes place by this method is a combination of convection (within oil) and conduction (within food) (Alvis et al., 2009). To increase the nutritional quality of snacks as well as reduce the calorific content, modern methods use infrared heating, microwave or hot air to pop snacks (Moraru & Kokini, 2003). Using hot air in order to expand third generation potato starch pellets, (Norton et al., 2011) found that no expansion occurred below a lower bounding temperature limit of 160°C, and proposed that this was due to the glass transition temperature of starch (165–175°C in these extrudates) limiting expansion.

The Maillard reaction is a browning reaction which results from a reaction between reducing sugars and amino acids (Tamanna & Mahmood, 2015). This non-enzymatic reaction leads to changes in food colour, organoleptic properties and protein digestibility (Lund & Ray, 2017). The Maillard reaction in pulses has been shown to enhance the inhibition of α-amylase activity (Moussou et al., 2017).

1.6. Digestion of starches/proteins

1.6.1. Digestion in the human body

When food is consumed, the first process encountered in the breakdown of food is within the oral cavity. Food is mechanically broken down and mixes with saliva until a food bolus is formed, and is then transferred down the oesophagus to the stomach. Salivary α-amylase, is present at the oral stage. α -Amylase is an enzyme that plays a crucial role in the digestion of carbohydrates. α -amylase breaks down complex carbohydrates, such as starch and glycogen, into smaller molecules, primarily maltose and dextrins. This enzyme exhibits substrate specificity towards starches and related polysaccharides. It specifically acts on the α-1,4-glycosidic bonds in both amylose and amylopectin. It is most efficient in hydrolysing soluble starch and can also act on other starch sources, such as gelatinised starch, but with slightly reduced efficiency. Crystalline starch is less susceptible to breakdown by α -amylase, due to its highly organized structure. α-Amylase functions optimally under slightly acidic to neutral pH conditions, typically around pH 6 to 7.5. Calcium ions (Ca^{2+}) play a significant role in the activity, stability, and optimal function of α-amylase. Calcium ions help maintain the enzyme's tertiary structure and promote its activity. Magnesium ions (Mg^{2+}), are also necessary and act as a cofactor for α-amylase (Anitha Gopal & Muralikrishna, 2009; Butterworth et al., 2011).

Within the gastric phase, food encounters strong mixing and grinding arising from stomach contractions, as well as a drop in pH, from a neutral pH to pH 3-5. Pepsin, responsible for the breakdown of proteins, is the major digestive enzyme secreted during gastric digestion. Gastric lipase is also present to initiate lipid digestion This combination of high mechanical forces as well as

lowered pH and enzymatic breakdown causes food to possess the consistency of a liquid or paste which is delivered to the small intestine.

The small intestine is a coiled tube made up of three sections: the proximal duodenum, middle jejunum, and distal ileum. Food is further broken down within the small intestine due to enzymes secreted from the pancreas such as proteases, lipase and amylase. These digestive enzymes break down proteins, fats and starch respectively. Bile is also secreted into the small intestine by the liver, and aids in the breakdown of fats. The surface of the small intestine contains small finger like tissue projections called villi, which increases surface area significantly. Through the villi, nutrients are absorbed and transported to the bloodstream. The remaining food is then transported to the large intestine where it may be fermented.

1.6.2. In vivo digestion models

When researching human nutrition and digestibility, it is not often that humans are the subject. This is due to a number of reasons such as the inability to keep a controlled environment and reproducibility, as well as the ethics involved, low throughput and high costs. Instead, animal models are extensively used. Rodents, such as mice are traditionally used as an *in vivo* animal model due to their relatively low cost, ease of maintenance and rapid rate of reproduction (Low, 2012). In addition, rodent models offer a variety of strains and genetically modified lines are available to target different diseases (Zhang et al., 2013). However, food intake in rodents is far higher relative to body size and is digested more rapidly. Gut physiology also varies greatly between rodents and humans, due to their propensity for practising coprophagy and the presence of colonic separation mechanisms that rodents use to obtain a greater supply of

essential nutrients (Bjornhag & Snipes, 1999; Graham & Åman, 1987; Soave & Brand, 1991).

Rather than using rodents as a research animal model, pigs are also popular. A porcine model has the advantage of being more anatomically and physiologically similar to humans than a rodent model, making it a lot more nutritionally relevant (Patterson et al., 2008). Like humans, pigs are omnivores and have similar metabolic and physiological processes take place within the digestive system. Additionally, the nutritional requirements of pigs are similar to that of humans (Gonzalez et al., 2015). However, pigs are more costly to keep than rodents, and require specific housing facilities.

1.6.3. In vitro digestion models

Although in vivo animal models or human nutrition studies are important in digestibility research, they are usually very expensive to run. In addition, the data received can be difficult to interpret and draw conclusions between food structure and digestion. In vitro protocols are inexpensive, and a lot more easily manipulated to focus research on specific areas of digestion by changing different enzyme concentrations as well as allowing for far easier sampling. Both in vivo and in vitro methods give complementary data which are essential when studying digestion.

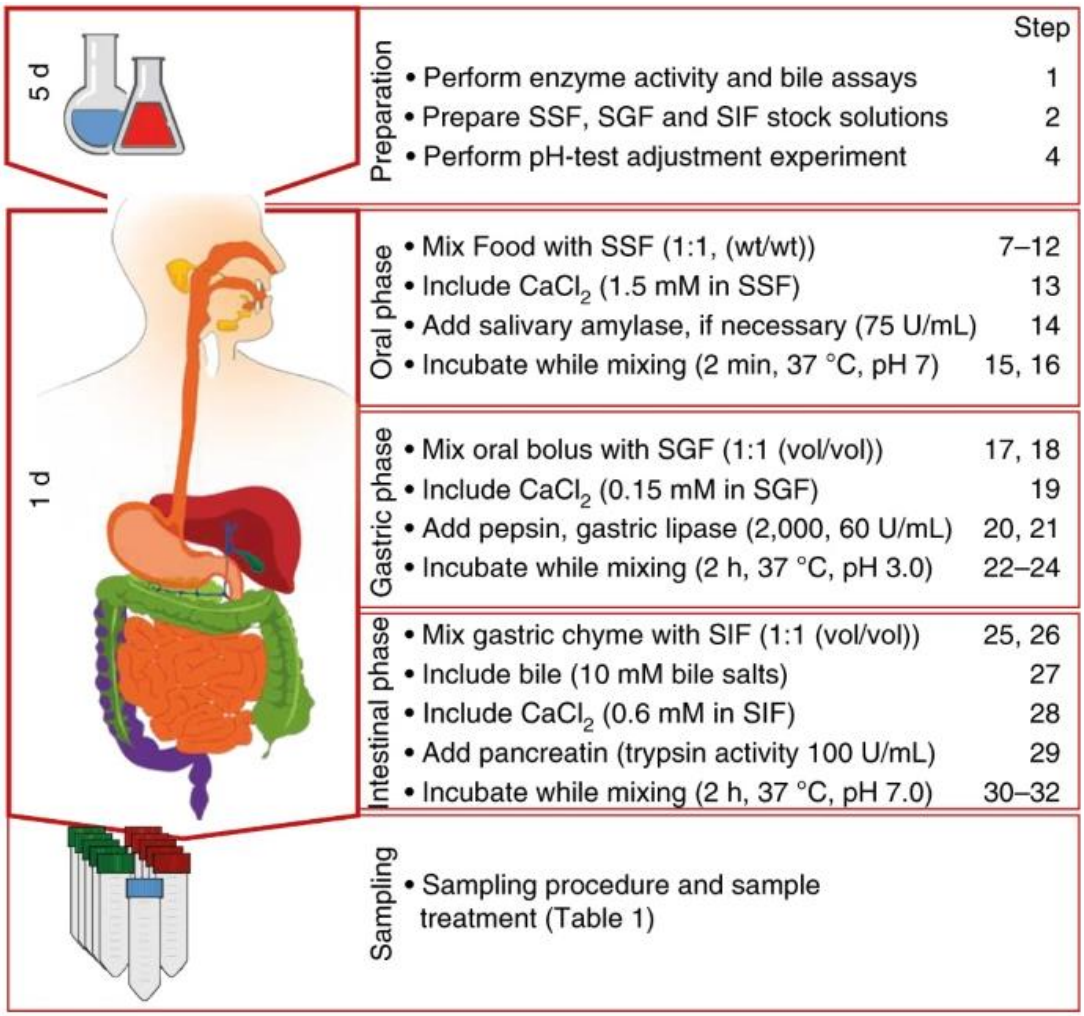

Figure 1.8: Flow diagram of the INFOGEST 2.0 in vitro *digestion method. SSF, simulated salivary fluid; SGH, simulated gastric fluid; SIF, simulated intestinal fluid. Adapted from* (Brodkorb et al., 2019)

The COST action Infogest network is a global network of scientists set up in 2011 across 32 countries in order to find a method of simulated gastro-intestinal digestion so that data between lab groups could be more comparable in the future (Minekus et al., 2014).

In vitro methods of gastro-intestinal digestion traditionally use static (also known as biochemical) models of simulating physiological conditions such as controlling temperature and addition of digestive enzymes as well as changing pH to mimic the specific conditions (Figure 1.8).

Oral phase

The first stage is the oral step, which simulates the processing of food which occurs in the mouth. As this stage can last from seconds to minutes, and food samples are not predicted to chemically change to a significant extent, it is not always a required step and is dependent on what is being researched (Intawongse & Dean, 2006). For example, liquid foods would not undergo any significant oral processing so could straight to gastric phase, although salivary amylase may be added. Also in the case of analysing protein digestion, enzyme action occurring in the oral cavity is unrelated to protein breakdown, therefore not required for protein analysis (Wickham et al., 2009). Solid foods are slightly more complicated to simulate than liquids when bypassing the oral phase. A homogenisation step is usually required, although this does not form a food bolus.

When an oral phase when used, it begins with a mastication step to homogenise the food sample and is usually simulated using a mincer, food processor or mortar and pestle. Prewarmed simulated salivary fluid (SSF), made up of electrolyte stock solutions in deionised water is then added to create a thinner paste like consistency. If required, the SSF can be added during the mastication step to assist with the homogenisation. Human salivary α-amylase is then added along with calcium chloride solution. α-amylase is the major digestive enzyme catalysing starch breakdown during digestion. This glycoprotein specifically catalyses the hydrolysis of α-1,4 glycosidic bonds in starch, yielding a mixture of maltose and glucose. Maltose molecules are formed when the bond cleaved is second from the end of a starch molecule, whereas a glucose molecule arises when the bond cleaved is terminal. Incubation of food sample during the oral phase is for 2 minutes at 37°C.

Gastric phase

In the human body, the main events that occur in the gastric phase are mechanical shearing through stomach contractions, a drop in pH and digestion through gastric enzymes. These events cause digesta to breakdown significantly prior to being delivered to the duodenum.

During in vitro digestion, samples are taken from the oral phase, or directly after food homogenisation to proceed to the gastric phase. This phase simulates the change in pH and contact with pepsin – the principle gastric enzyme present at this stage of digestion which is active in very acidic conditions (pH<3). Pepsin breaks down proteins into smaller peptides. It exhibits broad specificity, favouring peptides with linkages with aromatic or carboxylic L-amino acid linkages. It prefers to cleave after bulky, hydrophobic amino acids such as phenylalanine and leucine as well as Glu linkages to a lesser extent. Pepsin does not cleave at valine, alanine or glycine (Fruton, 1970).

This stage begins with mixing of the sample, either directly homogenised sample or sample from the oral phase with simulated gastric fluid (SGF) and lowering the pH using HCl to pH 1-2. Pepsin is then added, and the sample is incubated for 1-3 hours at 37°C. To replicate mechanical disruption via in vitro digestion, it is difficult to simulate mechanical shearing using a static model. Instead, mixing can be achieved by using a shaking incubator, rotary mixer within an incubator or water bath with integrated mixer.

When sampling at the gastric stage, it is important for digestion to be completely halted. This can be achieved by increasing the pH to neutral immediately after

sampling using sodium bicarbonate, or through the addition of a protease inhibitor.

Intestinal phase

When a food sample reaches the intestinal phase in vivo, the resulting chyme from the gastric compartment gets neutralised by secretion of carbonate. This causes the pH to rise to approximately pH 6.5, depending on factors such as sample type and gastric emptying rate. The pH then increases during digestion to approximately pH 7.5 over time.

The intestinal phase of gastro-intestinal digestion usually encompasses the processes occurring in the small intestine. To begin this phase, sample from the gastric phase is mixed with simulated intestinal fluid (SIF). Digestive enzymes are then added, either in the form of pancreatin from porcine pancreas or as individual enzymes (porcine trypsin, bovine chymotrypsin, porcine pancreatic α-amylase, porcine pancreatic lipase and porcine pancreatic colipase) along with bile and calcium chloride solution. Trypsin and chymotrypsin enzymes are proteases which breakdown proteins, whereas lipase catalyses the breakdown of fats. The resulting solution is then neutralised using sodium carbonate or sodium hydroxide to pH7. A pH of 7 is used as an average of 6.5 – 7.5 which the sample goes through in the course of duodenal digestion. pH of the sample may require readjustment during the digestion process and can be attained by manually altering the pH or using an automated titrator. The standard incubation time for digestion at the intestinal stage is 2 hours at 37°C.

Similarly to sampling at the gastric phase, samples taken at the duodenal phase must have enzyme activity stopped immediately after sampling. This is achieved by either increasing the pH or using a protease inhibitor.

Egger et al. (2017) compared a porcine *in vivo* digestion model to the static harmonised *in vitro* digestion model in order to test the physiological relevance of the *in vitro* model. Using skimmed milk powder as substrate, protein hydrolysis of *in vitro* and *in vivo* models was compared using gel electrophoresis, mass spectrometry, high performance liquid chromatography and spectrophotometric o-phthaldialdehyde determination of free amino acids. They found that there was correlation between peptides identified at the gastric and intestinal phase of the *in vitro* digestion with those identified at the gastric to distal jejunal *in vivo* samples. It was concluded therefore that the harmonised *in vitro* digestion model is representative of *in vivo* digestion of skim milk powder in pigs, specifically at gastric and intestinal endpoints.

Sanchón et al. (2018) evaluated the harmonised *in vitro* digestion model in comparison to *in vivo* jejunal stage of milk protein digestion in humans. Peptide release and protein patterns of milk casein and whey of *in vitro* gastric and intestinal phase samples was compared to *in vivo* samples taken through a nasogastric tube positioned at the proximal jejunum. It was found that peptide pattern and protein degradation was comparable between *in vivo* and *in vitro* methods, demonstrating that the harmonised static in vitro model presents a good assessment of physiological digestion of milk proteins.

1.7. Project aims and objectives

Snacks are a part of the human diet that is here to stay, therefore it is beneficial to have healthier snacking choices. Current popular snacks are not healthy due to their high fat content and easily digestible starch. Healthy snacks are a current trend to combat this through the production of snacks that are more nutritionally dense. The incorporation of ingredients such as pulses into crisps is an advantageous method to do this as they contain sources of fiber and protein. Extrusion is an innovative and efficient method of processing the snacks. There is a good amount of research on extruded snacks which contain novel ingredients, however all of it is carried out on second generation snacks produced through directly expanded extrusion, and unfortunately there is a lack of understanding on the starch digestibility of third generation extruded snacks, and how processing and formulation affect the digestibility.

In this project, a sample set of snacks in which pulse sources of chickpea and red lentil are incorporated will be formulated and produced using a single screw extruder at a pilot scale. *In vitro* starch digestibility experiments will be carried out and digestibility kinetics will be analysed. The influence of processing on starch digestibility will be assessed by analysing the starch digestibility at various stages of third generation snack production: raw materials, extrudates and final expanded product. The impact of the pulse ingredients and water content will be evaluated through different snack formulations containing various concentrations of either chickpea or red lentil flour, at low and high moisture. The study will aim to relate how each part influences starch digestibility with the aim to be able to set out parameters for the production of

healthy, slowly digesting snacks through controlling starch gelatinisation (by ingredients – difference in water holding, and by moisture content).

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

The chemicals used in this thesis were as follows: *p*-hydroxybenzoic acid hydrazide (CAS: 5351-23-5) Merck, Dorset, UK; porcine pancreatic α-amylase (CAS: 9000-90-2) Merck, Dorset, UK; soluble potato starch (CAS: 9005-25-8) Merck, Dorset, UK; D-(+)-Maltose mono-hydrate (CAS: 6363-53-7) Merck, Dorset, UK; PBS (P4417) Merck, Dorset, UK; Na_2CO_3 (CAS: 497-19-8) Merck, Dorset, UK; KOH (CAS: 1310-58-3) Merck, Dorset, UK; $CaCl_2.2H_2O$ (CAS: 10035-04-8) Merck, Dorset, UK; HCl (CAS: 7647-01-0) Merck, Dorset, UK; NaOH (CAS: 1310-73-2) Merck, Dorset, UK; NaCl (CAS: 7647-14-5) Merck, Dorset, UK; CH_3CO_2H (CAS: 64-19-7) Merck, Dorset, UK; CH_3CH_2OH (CAS: 64-17-5) Merck, Dorset, UK; I_2 (CAS: 7553-56-2) Merck, Dorset, UK; KI (CAS: 7681-11-0) Merck, Dorset, UK; Toluidine blue (CAS: 6586-04-5) Merck, Dorset, UK.

The total starch analysis kit was purchased from Megazyme (K-TSTA-100A) Wicklow, Ireland.

2.1.2. Food materials

The raw materials native potato starch (Superior potato starch, KMC, Denmark), pre-gelatinised potato starch (Paselli WA4, Avebe, Netherlands) and rice flour (FL4097, Whitworth Bros Ltd., Wellingborough, UK) were provided by PepsiCo Int. Ltd. (Leicester, UK). Red lentil flour (SRLF-100) and chickpea flour (WCPF) was purchased from AGT Poortman, London, UK. Black rapeseed for Specific Volume analysis was purchased from Food4wildbirds, Morley UK.

2.2. Sample preparation

A standard formulation is important for quality control purposes. A standard dough was prepared using 92% native potato starch, 5% rice flour, 2% pregelatinized potato starch and 1% sodium chloride to be used as a control.

Table 2.1: Extrudate recipe for standard and pulse incorporated (10, 40 & 80%) formulations

Ingredient	*Standard*	*10% pulse*	*40% pulse*	*80% pulse*
Potato starch	92	82	52	12
Pre-gelatinised potato starch	2	2	2	2
Rice flour	5	5	5	5
Salt	1	1	1	1
Pulse flour	0	10	40	80

Water heated to 80°C was added to the dough in order to make up either 25% (30 mL water added to 100 g dry mix) or 35% (54 mL water added to 100 g dry mix) moisture by formulation water. The ingredients were pre-mixed in a KitchenAid stand mixer for 2 minutes to disperse the water evenly into the mix.

The low moisture (25%) and high moisture (35%) sample doughs were prepared with chickpea flour or red lentil flour at incorporation levels of 0%, 10%, 40% and 80% by replacing potato starch (Table 2.1).

2.3. Third generation extrusion processing of pulse incorporated doughs at a range of different conditions

The doughs manufactured in 2.2 were extruded using a single screw extruder (HAAKE™ Rheomex OS Extruder for the HAAKE™ PolyLab™ OS System, Thermo Scientific, UK) at 30rpm, 80°C with a 1 mm diameter die block attached, then cut to approximately 2 cm and dried to approximately 10 – 12% moisture before being stored.

Extrusion conditions were optimised according to a standard recipe for screw speed (20, 30 & 40rpm), temperature (80°C & 100°C) and moisture level (25%, 30% & 35% by total moisture) with optimum results based on expansion capacity. Barrel temperature, screw speed and die block pressure were monitored using HAAKE PolySoft OS Monitor software (Version 3.2.0.1, Thermo Fisher Scientific, UK).

Post extrusion, pellets were cut to a uniform length of 2 cm and cooled on drying racks at room temperature overnight. Extrudates were transferred to plastic bags and stored at room temperature away from sunlight until further use.

2.4. Expansion of third generation half products

Extrudates were expanded using an air fryer (Salter EK1950, UK). Pellets were fried at specific temperatures (180°C, 210°C and 240°C) and time lengths (60s, 90s, 120s) and the expansion conditions were then optimised (Chapter 3) on the basis of expansion ratio and browning index. Expanded samples were stored at ambient temperature in sealed plastic bags. Extrudate and expanded samples were powdered using a coffee grinder to pass through a 45 µm aperture size sieve prior to all subsequent analyses.

2.5. Total moisture content of raw materials, extruded half products and expanded snacks

Moisture content (%) of raw potato starch, rice flour, pre-gelatinised potato starch, red lentil flour and chickpea flour was determined using thermogravimetric analysis (TGA). Analysis of raw materials was performed using a Leco TGA 701 (Leco, UK).

Sample (2.5 ± 0.2 g) was weighed out in triplicate into high capacity aluminium TGA pans. Samples were equilibrated at 30°C and scanned at 9°C min^{-1} to 130°C until the weight reached constancy.

Moisture content (%) of extruded potato starch control half products, chickpea or red lentil flour incorporated (10%, 40% & 80%) half products with low or high formulation moisture, and equivalent expanded final products were determined using TGA analysis. Analysis of processed materials was performed using a TA Instrument (TA Instruments Ltd., New Castle, USA) thermogravimetric analyser (TGA 550) equipped with aluminium pans.

Sample (5 – 10 mg) was weighed out into aluminium pans.

2.6. Water holding capacity of raw materials and processed samples

The water holding capacity of raw materials, extruded half products and expanded products was assessed.

Sample (1 g) was weighed into 50 mL Corning tubes then 10 mLs deionised water was added to each tube. Sample tubes were vortex mixed and centrifuged at 3500 rpm for 20 minutes, left to settle overnight then centrifuged again at 3500 rpm for 20 minutes. As much water was pipetted from the top of the samples as possible, then tubes were re-weighed.

2.7. Starch isolation of chickpea and red lentil flours

Starch was isolated from raw chickpea and raw red lentil flour. Chickpea and red lentil flour samples (5g) were weighed out into 50 mL tubes. 40 mL of 70% ethanol was added and tubes mixed on rotary mixer. After sufficient mixing, tubes were centrifuged at 3000xg for 10 mins, then the non-starch layer was

scraped off and the process repeated until the sample was white in colour. When the pellet was pure white, a final cycle using absolute ethanol was carried out then sample was spread flat in a thin layer onto petri dishes to dry at room temperature for 2 days before storage at ambient temperatures in plastic containers.

2.8. Specific volume of extruded half products and expanded products and calculation of expansion ratio

Expansion of extrudates was measured using specific volume analysis. This method is based on the measurement of volume by displacement of a sample from a material of known density. Black rapeseeds were used as standard material (AACC 10-05.01).

Extrudate or expanded sample (10 g) was weighed out (as intact samples, without grinding). Rapeseed (100 mL) was measured out separately using a measuring cylinder. The volume of rapeseed that was displaced by 10 g of sample was determined.

The specific volume measurement of volume was calculated using the equation as follows:

$$specific\ volume = \frac{volume}{mass}$$

Where higher specific volume indicates a lighter, crisper snack and a low specific volume indicates a more dense snack.

2.9. Colour analysis of expanded products

Expanded snacks were scanned using Epson Perfection V850 Pro to produce images under consistent conditions. Images were analysed using Adobe Photoshop CC 2015 to calculate L* a* b* values, with 8 replicates taken. Browning index was calculated using L* a* b* coordinates as follows (Maskan et al., 2002; Mohapatra et al., 2010):

$$BI = 100 \times (\frac{X - 0.31}{0.17})$$

where

$$X = \frac{(a* + 1.75L)}{(5.645L + a* - 3.012b*)}$$

2.10. Total starch content of raw materials, extrudates and expanded snacks

The total starch content of the raw materials, extrudates and expanded snacks (0%, 10%, 40% and 80% chickpea or red lentil flour incorporated, at 25% and 35% formulation moisture) was determined using an assay adapted from Megazyme (K-TSTA, Megazyme International Ireland Ltd., Wicklow, Ireland) and performed in triplicate. This assay converts starch to D-glucose using the enzymes thermostable α-amylase and amyloglucosidase, producing a colourimetric reaction which can be quantitatively measured at 510 nm (McCleary et al., 2020).

Total starch was initially measured using potassium hydroxide (KOH) to solubilise starch samples. This was modified due to the KOH method no longer being recommended by the Megazyme Total Starch Assay Kit (K-TSTA-50A).

Instead, the recommended method using NaOH was optimised and utilised. The results from using the NaOH method was comparable to that of the KOH method. Therefore, previous experiments were repeated using the new method. This method was scaled down 5x by volume, with results found to be comparable to the full-scale assay.

Approximately 20 mg of sample was wetted with 40 µL of 80% ethanol, vortex mixed, then 400 µL of 1.7 M NaOH added with stirring in an ice water bath for approximately 15 minutes. 1.6 mL of 600 mM sodium acetate buffer (pH 3.8) was added to the solubilised sample which was then pH adjusted to pH 5. Starch was digested using 20 µL of thermostable α-amylase and 20 µL of amyloglucosidase, and incubated at 50°C for 30 minutes before being centrifuged at 13,000 rpm for 5 mins. Supernatant (200 µL) was added to 1.8 mL of 100 mM sodium acetate buffer (pH 5.0). The quantity of glucose released was determined using 1.5 mL of glucose oxidase/peroxidase (GOPOD) reagent, incubated at 50°C for 20 minutes. Absorbance was measured at 510 nm against a reagent blank of 100 mM sodium acetate buffer (pH 5.0) and glucose controls consisting of D-glucose standard solution (1 mg/mL).

2.11. Differential Scanning Calorimetry for gelatinisation analysis

DSC measures changes in enthalpy of a sample through thermal events in relation to a reference sample pan. The sample pan and reference pan are maintained at the same temperature throughout analysis, this may be heating, cooling or isothermal. Exothermic or endothermic processes induced by changes in heat transfer are quantified by converting the difference between the

sample pans and the reference pan to energy (J/g). Physical changes of the sample such as gelatinisation may be observed.

2.11.1 Effect of change in formulation moisture on sample starch gelatinisation

Mixes hydrated to specific moisture levels were analysed using DSC to determine the optimum formulation moisture for extrusion. Pre-extrusion mixes were produced containing potato starch (92%), pre-gelatinised potato starch (2%), rice flour (5%) and salt (1%). Dry mixes were hydrated to specific moisture levels ranging from 35% to 70% moisture by total moisture (including inherent moisture of raw material).

Doughs containing a single raw material only, such as potato starch, red lentil flour or chickpea flour were combined with specific amounts of water to analyse low and high moisture levels. Moisture was calculated as formulation water, not taking into account the intrinsic moisture present in the samples. Therefore for low formulation moisture (25%), 25 mL water was added to 75 g of dry ingredient, and for high formulation moisture (35%), 35 mL water was added to 65 g dry ingredient.

Hydrated doughs were analysed using a TA Instruments DSC Q100 (TA Instruments, UK). 5 – 10 mg of sample was accurately weighed into aluminium pans which were hermetically sealed before loading. Samples were equilibrated at 10°C and kept isothermal for 10 minutes before being heated to 120°C at 5°C min-1 and kept isothermal for 1 minute. The furnace was continuously flushed with dry nitrogen at a rate of 50 $mL.min^{-1}$. Analysis was carried out using TA Universal Analysis (Gelatinisation enthalpy, peak, onset and conclusion temperatures).

2.11.2 Effect of processing and pulse incorporation on starch gelatinisation

The processing conditions were optimised through DSC analysis for the different compositions of the pulse ingredients, focusing on gelatinisation temperature and hydration level of dough. These changes are also expected to have an influence on starch digestibility.

DSC experiments on extrudates were conducted using a TA Instruments (TA Instruments Ltd., New Castle, USA) multicell differential scanning calorimeter (MC-DSC), equipped with three sample high capacity (1 mL) Hastelloy ampoules and one reference high capacity Hastelloy ampoule, where the reference pan was filled with deionised and degassed H_2O. The furnace was continuously flushed with dry nitrogen at a rate of 50 $mL.min^{-1}$.

Raw materials or extrudates were weighed into Hastelloy DSC pans with excess liquid (~200 mg of sample in ~800 µL of degassed, deionised H_2O) which were then hermetically sealed. The samples were scanned under nitrogen purge with equilibration at 10°C before being heated from 10°C to 150°C at a heating rate of 1°C min^{-1} and then cooled to 10°C at a cooling rate of 2°C min^{-1}, followed by a second heating cycle at 1°C min^{-1}. Analysis was carried out using TA Universal Analysis (Gelatinisation enthalpy, peak, onset and conclusion temperatures).

2.12. X-ray Diffraction (XRD)

X-ray diffraction (XRD) patterns of extrudate samples made up of potato starch only, and 10%, 40% & 80% chickpea or red lentil flour enriched with low or high

formulation moisture were measured on an ARL™ X'Tra Powder Diffractometer (Thermo Scientific™).

Extrudate samples were powdered using a coffee grinder to pass through a 45 µm aperture size sieve, then equilibrated in an atmosphere of 70% relative humidity for 1 week using a saturated NaCl solution prior to analysis.

All samples were scanned with Cu Kα radiation (λ =0.154 nm), and reflections were detected via a scintillation detector over the angular range of 2θ = 5.0–54.99°, with a step interval of 0.01°, and step duration of 0.6 s. The X-ray generator was set at 45 kV and 40 mA. Approximately 600 mg of each sample were packed into the loading dish to a depth of 4 mm and levelled with a razor blade.

The total crystalline content of raw materials and extrudate samples was calculated following the method as described by Lopez-Rubio et al., (2008) which was adapted for this specific set of samples. Where Lopez-Rubio et al. used Gaussian line shapes to fit the amorphous peak for their sample of interest, it was determined that for raw potato starch, chickpea flour & red lentil flour, and for the extrudate samples, a Lorentzian function proved to be a more accurate fit for the amorphous peak. The Gaussian function provided the best results for crystalline peak fitting. Peaks were manually fitted using the PeakFit (SigmaPlot, Systat Software Inc.©) software package according to selected B- C- and V-type crystallinity peaks (Table 2.2 and Figure 2.1).

Table 2.2: Position of peaks selected from B-, C- and V- type crystal structures for the peak fitting procedure

Peak	B-type	C-type-B	C-type A	V-type
1	5.51		5.8	
2				7.5
3	10.01	8.4	8.6	
4	11.02	11.2	11.2	
5				13
6	13.85		14.0	
7	14.60	15.0	15.0	
8	16.85	16.7	17.0	17
9		18.0		
10	19.6	20.2	19.2	19.8
11	22.30		22.2	22
12		23.0		
13	23.71	24.1	23.7	
14	26.16	26.2	26.4	
15	30.61			
16	33.84			

The total starch crystallinity percentage in the raw materials and extrudates was calculated based on the ratio between the sum of the total area under the whole diffractogram, and the area under each crystalline peak.

$$X_c = \frac{\sum_{i=1}^{i=n} Ac_i}{At}$$

where Ac_i is the area under each crystalline peak with index *i* and *At* is the total area under the diffractogram.

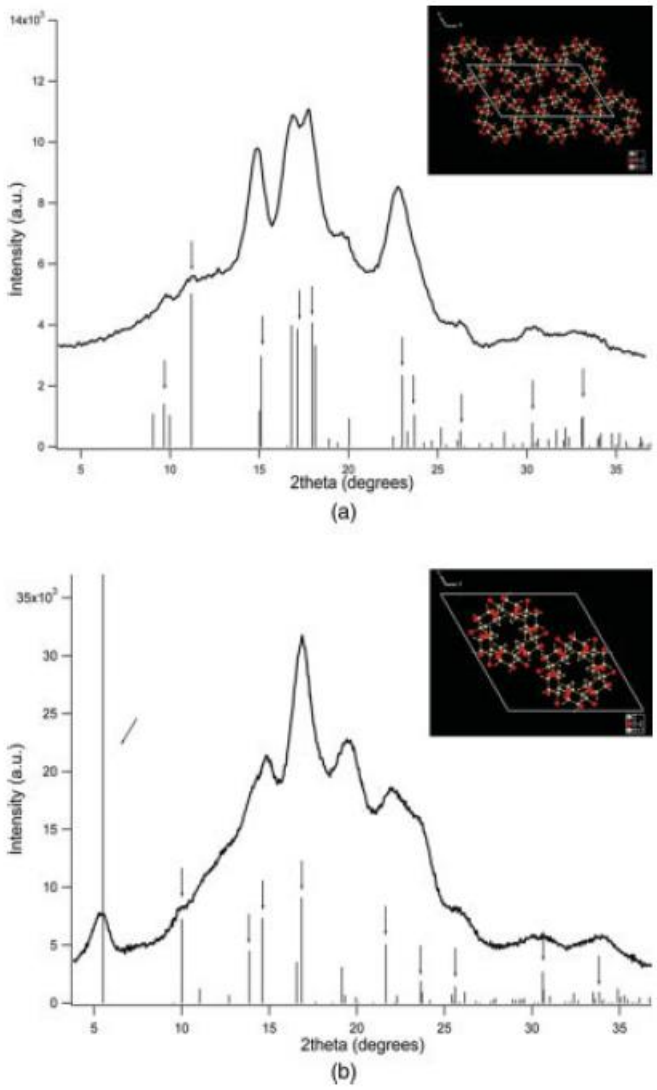

Figure 2.1: XRD pattern of starch samples a) A-type crystal powder pattern and unit cell b) B-type crystal powder pattern and unit cell. Arrows indicate the reflections selected for the fittings. Adapted from (Lopez-Rubio et al., 2008)

2.13. Starch digestion of raw materials, extruded half products and expanded snacks

In vitro starch digestibility kinetics of raw flours, extrudates and expanded snacks was quantified using a static *in vitro* starch digestibility assay (Butterworth et al., 2012; Edwards et al., 2014; Zou et al., 2015).

This method uses *p*-hydroxybenzoic acid hydrazide solution (PAHBAH) which measures non-specifically free reducing sugar groups released from starch hydrolysis catalysed by porcine pancreatic alpha amylase alone. The PAHBAH assay is a colorimetric assay which measures at 415 nm (colourless to yellow). The PAHBAH assay was chosen due to its high sensitivity as well as low potential for cross reactivity (Moretti & Thorson, 2008). Dinitrosalicylic acid (DNS) is another popular assay which produces an oxidation/reduction reaction resulting in colorimetric change from yellow to orange or red but has lower sensitivity in comparison to PAHBAH, therefore PAHBAH was selected ahead of the DNS assay for these studies (Figure 2.2) (Moretti & Thorson, 2008).

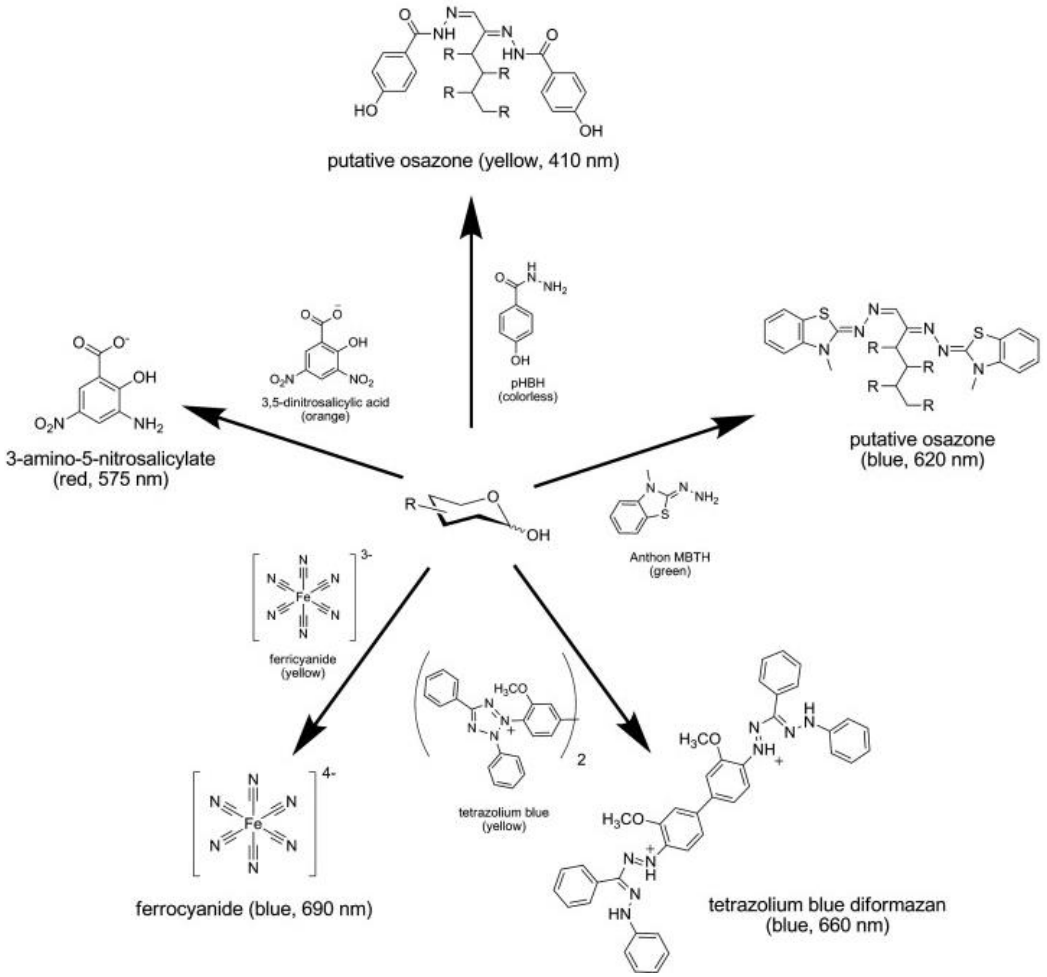

Figure 2.2 Comparison of the chemistry of different commonly used biochemical assays for glucose, including the DNS and PAHBAH (here referred to as pHBH) with coloured products shown. Adapted from (Moretti & Thorson, 2008)

2.13.1 Determination of enzyme activity

Enzyme activity of porcine pancreatic α-amylase was measured through the determination of starch conversion to maltose. Soluble potato starch was gelatinised by making up a 5 mg/mL suspension in PBS, pH 7.4 and heating at 90°C for 20 mins. 15 mL gelatinised potato starch samples were placed in triplicate onto a rotary mixer and 100 µL of 1mg/mL protein amylase working solution added to each sample to begin the assay. 100 µL aliquots were removed at predetermined timepoints (3, 6, 9 and 12 minutes) and added to Eppendorf tubes containing 0.3M Na_2CO_3 to stop the reaction. Maltose

conversion was quantified by colourimetric reaction with *p*-hydroxybenzoic acid hydrazide (Lever, 1977). Starch digestion samples were incubated with PAHBAH in a boiling water bath for 5 minutes before being cooled to room temperature and the absorbance measured at 405 nm using a spectrophotometer (Biochrom Libra S50, UK). To account for endogenous reducing sugars or enzyme activity, blanks were included for each sample excluding the addition of α-amylase.

2.13.2. Kinetic assay of starch digestion

Processed sample materials were weighed in triplicate into 15 mL Corning tubes so that each tube contained 100 mg starch, calculated by total starch assay. Samples were then incubated at 37°C with 70 U/ml porcine pancreatic α-amylase in 10 mLs of 0.01 M PBS on a rotary mixer. During the incubation period, 100 µL sample aliquots were collected into tubes at predetermined timepoints (0, 3, 6, 9, 12, 20, 30, 45, 60, 90, 120, 150 mins) containing 100 µL 0.3M NaCO3 to stop the reaction. When digesting unprocessed raw materials, the incubations were extended for a further 180 minutes, with aliquots collected at 180, 210, 270 and 330 minutes. Aliquots were centrifuged at 13000 x G for 5 minutes to exclude any starch remnants.

The starch hydrolysis products in the supernatant were quantified using the PAHBAH assay as an endpoint to obtain starch digestibility kinetics (as described in 2.13.1)

2.13.3. Calculations

The absorbance values of samples was converted to maltose concentration by plotting absorbance against concentration for standards and using the equation of the resulting straight line to substitute sample absorbance values.

Maltose concentration of samples was then converted to percentage of starch digested using the following equations:

$z = x \times dilution\ factor 1000$

$\%\ Starch\ digested = z((y/342) \times 0.9 \times 1000) \times 100$

Where x is the concentration (μM) and z is the corrected concentration of maltose in mM. y is the concentration of starch at the start of the reaction (normally 10 mg/mL). 342 is the molecular weight of anhydromaltose. This value is multiplied by 0.9 to correct for the addition of water during the condensation reaction catalysed by amylase, and multiplied by 1000 to correct from M to mM concentration.

Digestibility curves can be fitted to a first order equation:

$C_t = C_\infty(1 - e^{-kt})$

Where C_t is the concentration of the maltose at time t, C_∞ is the amount of starch digested at the end point of the reaction and k is a pseudo first rate constant.

By expressing the first derivative of the first order equation in logarithmic form, a Log of Slope plot can be obtained in which the values of digestibility constants *k* and C_∞ can be directly calculated from the slope (-*k*) and y-intercept (ln($C_\infty k$)) respectively (Butterworth et al., 2012).

$$\ln\left(\frac{\mathrm{d}C}{\mathrm{d}t}\right) = \ln(C\infty k) - kt$$

2.14. Microscopy

The extrudate samples were snapped using pliers to produce a piece approximately 3mm wide which could be held in a flat microtome chuck. A razor blade was used to trim the sample piece, so that the edges were sharp and block face was the thickness of the sample x approximately 2mm. A Leica Reichert-Jung Ultra Ultracut E microtome was used to cut 2µm thick sections, dry, using glass knife. The sections were collected with tweezers and placed onto a tiny drop of d.H2O on a glass slide. Multiple sections for each sample were cut so that different stains could be applied. The slides were then dried in an oven at 45°C.

The sections were observed and imaged on an Olympus BX60 microscope (Olympus, UK), using ProgRes® CapturePro camera and software (Jenoptik, Germany). Under brightfield imaging, IKI stain (1% iodine/2% potassium iodide) was diluted with d.H2O (approximately 1 part stain to 5 parts water) and used to stain starch. 0.1% toluidine blue was used to stain protein and cell wall structures. Polarising filters were used to identify crystalline starch by birefringence.

2.15. Statistics

Effects of moisture on pre-extrusion doughs, total starch content of samples, and effects of pulse incorporation in extrudates on starch digestibility were determined by one-way analysis of variance (ANOVA). Analyses were conducted on three replicates of single batches. Post hoc analysis was carried out using Tukey's test using statistical software (IBM SPSS).

3 Optimisation of dough formulations and the relationship between formulation and bulk properties of extrudates and expanded products

3.1. Introduction

Ready to eat snacks are conventionally produced using easily sourced starches such as potato starch or corn starch. These starches are well researched in physical characteristics for snack production (Kristiawan et al., 2016; Valle et al., 1995; Willett et al., 1997). Far less is known about the factors influencing expansion behaviour of products made using legume starches, which behave differently to potato and maize starches in a number of ways, including their molecular structure (molecular weight and chain length distribution), their granule structure and their gelatinisation temperatures (Pasqualone et al., 2020).

The expansion of a snack food is driven by bubble nucleation and growth, coalescence and setting, in a starchy matrix which is heated to a temperature where it is capable of undergoing viscous flow, before setting during cooling (Kristiawan et al., 2016). The blowing agent which expands the bubbles is water, which is vaporised and flash dried during the expansion process. Therefore, expansion of snack products requires that a temperature is achieved whereby the starchy matrix, even if not fully gelatinised, is capable of viscous flow and that the correct conditions are present for bubble nucleation. It has been hypothesised in the literature that there is an optimum degree of gelatinisation (DG) in the extruded half product of third generation snack products at which the best expansion is achieved (Kraus et al., 2014; Moraru &

Kokini, 2003; van der Sman & Bows, 2017). Below 50% DG, little expansion is observed due to limited viscous flow of the starch matrix and lack of matrix integrity leading to rapid drying/'blowing out' of bubbles. Above 66% DG, excessive bubble coalescence will occur due to the very high number of bubbles formed and lack of adequate water to support the bubbles leading to 'pillowing' or in extreme cases completely round snacks (van der Sman & Bows, 2017). It is therefore essential to optimise the DG of the extruded half product to allow for correct expansion. This can be achieved by altering the water content and extrusion conditions, however significant challenges are introduced by including legumes which have different melting temperatures and gelatinisation properties to potato or maize starches and therefore will require different extrusion conditions.

During expansion of snack products, an additional quality parameter which can affect consumer acceptance is non-enzymatic (Maillard chemistry) browning of the products. This occurs as a result of reactions between free amino acids and sugars. It may be expected to occur to a greater degree in legume enriched snacks due to the higher protein content (Parisi & Luo, 2018; S. Singh et al., 2007a).

In this chapter I aim to optimise the moisture content and degree of gelatinisation in a series of formulations of snack products produced with chickpea and red lentil flour by altering the moisture content and extrusion conditions. I will explore the impact of formulation of the snack products on the expansion properties, specific volume and browning during expansion of the final product.

3.2. Methods

3.2.1. Materials

All raw ingredients and chemical reagents used in this chapter are as described in Chapter 2.1.

3.2.2. DSC in moisture limited doughs

DSC was carried out in pre-extrusion doughs hydrated to different moisture levels using the method described in Chapter 2.11.2.

3.2.3. Extrusion processing

Low and high formulation moisture doughs incorporated with 0%, 10%, 40% or 80% chickpea or red lentil flour were extruded using a single screw extruder. Extrusion was carried out using the method as described in Chapter 2.3.

3.2.4. Expansion of extruded half products

Extrudates containing 0%, 10%, 40%, and 80% chickpea or red lentil flour using low or high formulation moisture pre-extrusion doughs were expanded using an air fryer (Salter EK1950, UK). Expansion was carried out using the method as described in Chapter 2.4.

3.2.5. Water holding capacity of raw materials and processed samples

The water holding capacity of raw potato starch, raw chickpea & red lentil flours, extrudates enriched with 0%, 10%, 40% and 80% chickpea or red lentil flour with low or high formulation moisture and the equivalent expanded products were determined using the method as described in Chapter 2.6.

3.2.6. Specific volume of extruded half products and expanded snacks

Specific volume analysis was carried out on extrudates containing 0%, 10%, 40% and 80% chickpea or red lentil flour formulated at low and high formulation

moisture, and their equivalent expanded products. This was carried out in order to assess the expansion capacity of different expansion parameters. Specific volume was determined as described in Chapter 2.7.

3.2.7. Colour analysis of expanded snacks

Expanded snacks containing 0%, 10%, 40% and 80% chickpea or red lentil flour formulated at low or high formulation moisture were analysed using Photoshop and the Browning index was calculated in order to investigate the effect of pulse incorporation and expansion temperature on snack colour. This was carried out using the method as described in Chapter 2.8.

3.3. Results

3.3.1. Optimisation of pre-extrusion dough formulation

The first stage in the production of third generation extruded snacks was the formulation of a standard recipe as a basis for all following extruded samples. A standard recipe for Quavers™ was modified according to the equipment available within a small-scale pilot plant set up. This formulation was predominantly made up of potato starch combined with a small quantity of pre-gelatinised potato starch, rice flour and salt (Table 2.1).

The requirement for an optimal final product was to achieve snacks that would have a high level of expansion. In order for good expansion to take place, the recipe needed to be manipulated to provide a specific level of starch gelatinisation. A level in which a portion of starch was gelatinised, but also contained ungelatinised starch granules was ideal as the gelatinised starch would provide an amorphous starch matrix, while native starch granules could serve as nucleation sites. As water is an important factor in starch gelatinisation and in third generation extrusion processing (Boischot et al., 2003), the impact of different hydration levels on the standard recipe was assessed.

Starch gelatinisation of standard base recipe doughs hydrated to specific formulation moistures (25%, 30%, 35%, 50% & 60%) was analysed using DSC (Figure 3.1). This was carried out in order to determine the optimal dough hydration level required to produce a partially gelatinised extrudate.

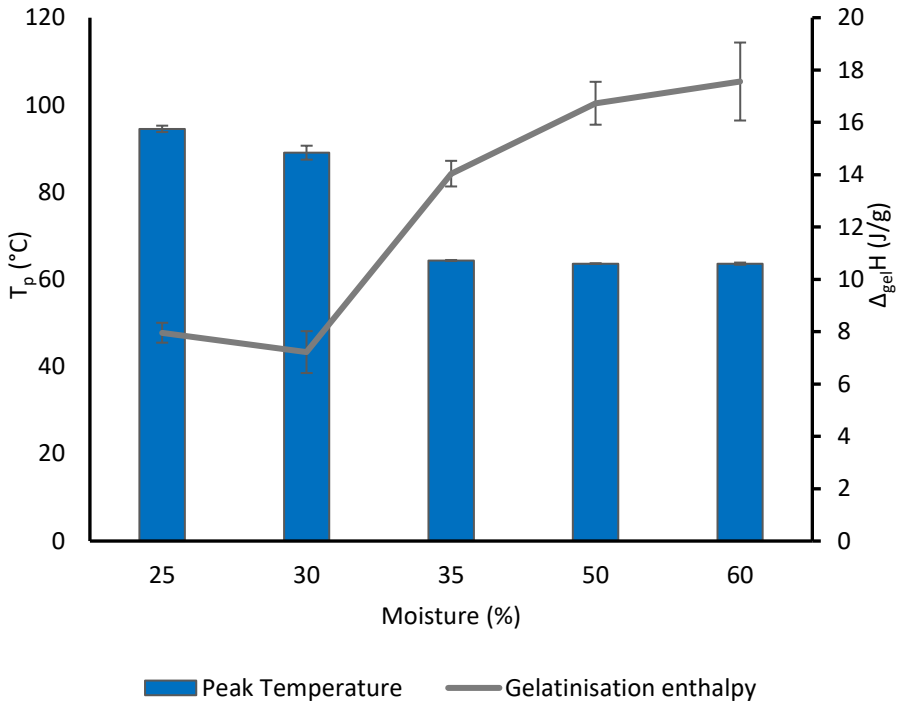

Figure 3.1: DSC analysis of pre-extrusion doughs with 25%, 30%, 35%, 50%, and 60% formulation moisture. T_p, peak temperature; $\Delta_{gel}H$, gelatinisation enthalpy. $\Delta_{gel}H$ of samples was calculated as Joules per gram of starch. Analysis was carried out in triplicate.

The gelatinisation temperature of hydrated doughs was found to decrease until a moisture level of 35% was achieved. Beyond this hydration level, the gelatinisation temperature remained at ~64°C. The lowest level of hydration (25%) resulted in the least gelatinisation. With increasing levels of formulation

moisture, the gelatinisation enthalpy was found to increase, with the 60% formulation moisture sample found to be the most gelatinised.

This suggests that water has an effect on crystalline disruption, and correlates with previous mechanisms proposed for starch gelatinisation in limited water systems (Biliaderis et al., 1980; Donovan, 1979; Nashed et al., 2003). They proposed that when excess water is present, starch gelatinisation is governed by starch crystallite melting due to extensive hydration and swelling of the amorphous region, characterised by a single endothermic transition with a narrow peak. Starch gelatinisation in limited water systems is hindered by the lower swelling capacity and crystalline disruption of the amorphous region. Starch crystallites undergo partial melting, followed by direct melting at higher temperature. This may give rise to a second endotherm peak at a higher temperature.

Using the DSC data of doughs hydrated to specific limited moisture contents, a standard mix hydrated to 35% moisture by total water was selected for further analysis due to the increased ability to gelatinise in comparison to lower moisture doughs. 25% formulation moisture was also selected as a low formulation moisture comparison. From this data, 80°C was determined to be the optimal temperature for extrusion to be carried out at, as this would ensure a portion of starch would be gelatinised and would allow for maximum expansion.

To further understand the gelatinisation behaviour of raw materials at the hydration levels of interest (25% & 35% formulation moisture), potato starch, chickpea flour and red lentil flour were hydrated and analysed individually using DSC.

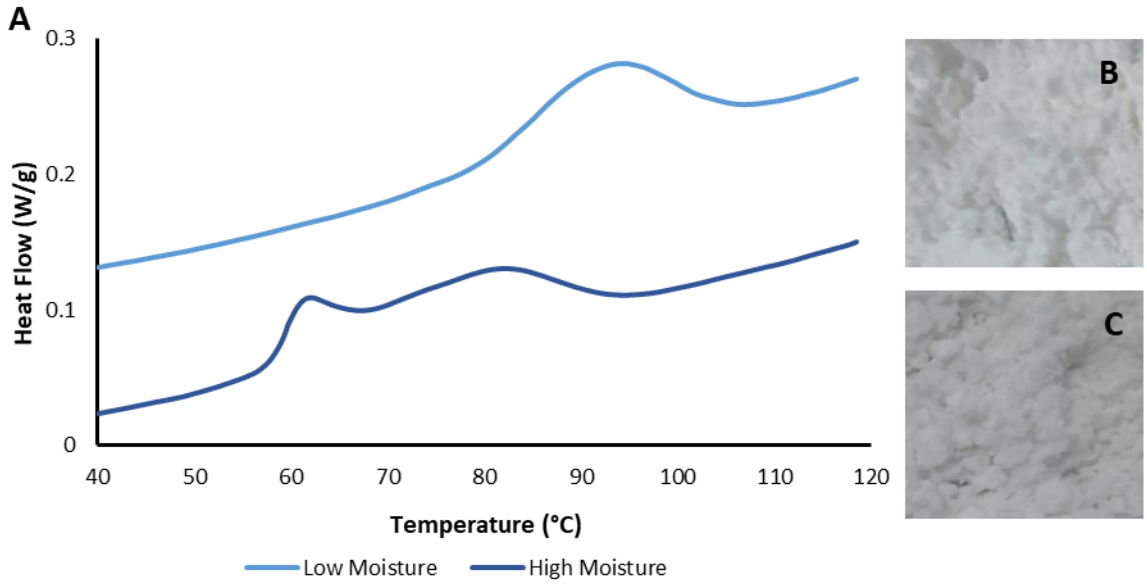

Figure 3.2: Limited water analysis of raw potato starch hydrated with low (25%) or high (35%) formulation moisture A) DSC thermogram B) Low formulation moisture pre-DSC dough C) High formulation moisture pre-DSC dough. Analysis was carried out in triplicate.

Potato starch at low and high formulation moisture was analysed using DSC (Figure 3.2 A). Similarly to standard hydrated mix data, a higher degree of gelatinisation was observed in the raw potato starch sample which was hydrated at a high formulation moisture. Gelatinisation was also shown to occur at a lower temperature in comparison to the low formulation moisture sample. When hydrated to 25% and 35% formulation moisture, there was very little visual difference observed between the appearance of the low and high formulation moisture dough (Figure 3.2. B & C).

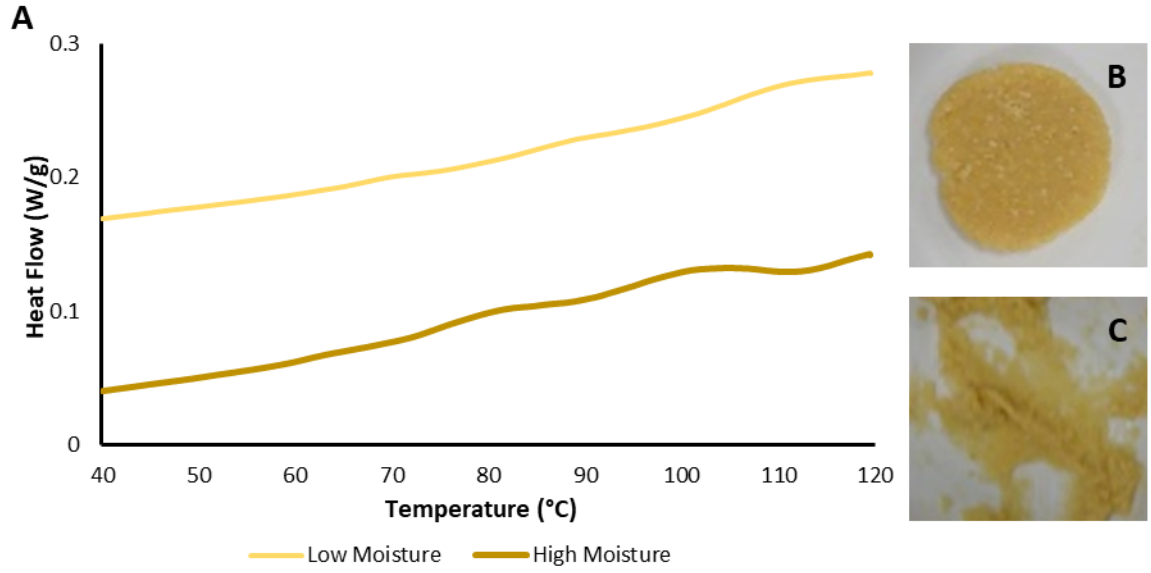

Figure 3.3: Limited water analysis of raw chickpea flour hydrated with low (25%) or high (35%) formulation moisture A) DSC thermogram B) Low formulation moisture pre-DSC dough C) High formulation moisture pre-DSC dough. Analysis was carried out in triplicate.

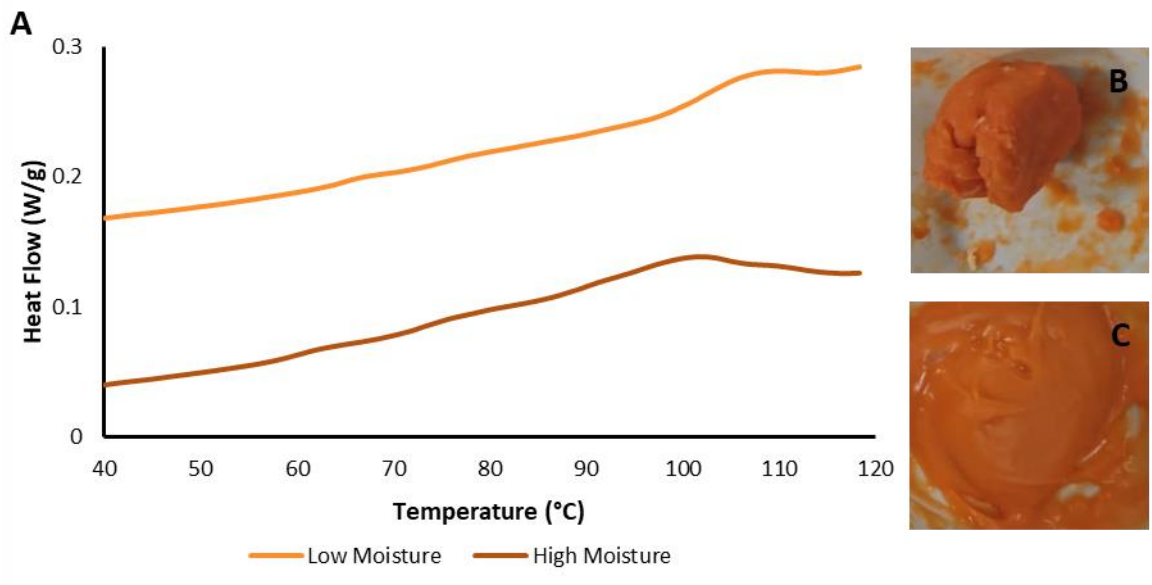

Figure 3.4: Limited water analysis of raw red lentil flour hydrated with low (25%) or high (35%) formulation moisture A) DSC thermogram B) Low formulation moisture pre-DSC dough C) High formulation moisture pre-DSC dough. Analysis was carried out in triplicate.

The same analysis was carried out on raw chickpea flour hydrated to 25% and 35% formulation moisture (Figure 3.3). The gelatinisation enthalpy value of chickpea flour was much lower in comparison to potato starch at both low and high formulation moisture. High formulation moisture chickpea dough resulted in increased gelatinisation in comparison to the low formulation moisture sample. The temperature of gelatinisation was also found to increase in chickpea flour in comparison to potato starch hydrated to low and high formulation moisture.

Table 3.1: Water holding capacity of raw potato starch, chickpea flour and red lentil flour

Sample	% water uptake
Potato Starch	12.1
Chickpea Flour	13.1
Red lentil Flour	12.9

Visually, hydration of chickpea flour to 25% and 35% formulation moisture resulted in a more dough like texture unlike the hydration of potato starch, which produced a consistency more similar to a crumb. An increase in formulation moisture of from 25% to 35% was observed to change the appearance of the chickpea dough. This correlates with the observed water holding capacity of chickpea flour, which was higher than that of potato starch (Table 3.1).

When raw red lentil flour was hydrated to 25% and 35% formulation moisture, the gelatinisation enthalpy was also found to decrease in comparison to hydrated potato starch. In comparison to chickpea flour hydrated to 25% and 35%, higher gelatinisation enthalpy was observed. Comparably to hydrated chickpea doughs, the temperature of gelatinisation occurred at a higher temperature than that of the hydrated potato starch. Upon visual observation, the red lentil flour sample hydrated to high formulation water sample (Figure 3.4

C) appeared to be wetter looking than the equivalent hydrated chickpea flour sample (Figure 3.3 C).

The next step in formulating third generation extruded snacks incorporating chickpea or red lentil flour was to incorporate the pulse flours into the standard potato starch based recipe. A range of incorporation levels was decided upon: 10%, 40% and 80%, which was incorporated by substitution of a portion of potato starch with either chickpea or red lentil flour (Table 2.1).

Figure 3.5: Pre-extrusion doughs incorporated with 10% or 80% chickpea or red lentil flour, with low (25%) or high (35%) formulation moisture.

25% (low moisture) and 35% (high moisture) sample doughs were prepared with chickpea flour or red lentil flour at incorporation levels of 0%, 10%, 40% and 80% by potato starch substitution. Increasing the formulation moisture of chickpea or red lentil flour incorporated mixes by 10% (from 25% formulation moisture to 35% formulation moisture) resulted in the formation of a dough as opposed to a crumb. 80% pulse incorporated hydrated mixes appeared wetter than their equivalent 10% pulse incorporated mixes.

3.3.2. Extrusion processing of optimised doughs

The doughs produced which contained 0%, 10%, 40%, or 80% chickpea or red lentil flour hydrated to low or high formulation moisture were extruded using a single screw extruder. Extrusion parameters such as speed (rpm) were optimised based on a visual assessment. Small sample sets of extrudate produced using different parameters were collected and deep fat fried at 185°C for 30 s. How well the dough travelled through the extruder was also taken into account. There was negligible change in extrudates when the screw speed was altered from 30 rpm to 40 rpm, whereas a screw speed of 20 rpm increased travel time and decreased in expansion capacity upon frying. Therefore, 30 rpm was chosen as the screw speed moving forth. When extruded at 100°C, the dough travelled through the screw well and the resulting half product was a good texture. Upon frying however, half products extruded at 100°C expanded at a far lower capacity in comparison to the equivalent product extruded at 80°C. This was hypothesised to be as a result of the half product being fully gelatinised. The optimised extruder parameters were used to produce half product extrudates containing 0%, 10%, 40% and 80% chickpea or red lentil flour with low or high formulation moisture (Figure 3.6). Incorporation of chickpea or red lentil flour resulted in a change in colour of increasing darkness/intensity with increasing pulse incorporation level.

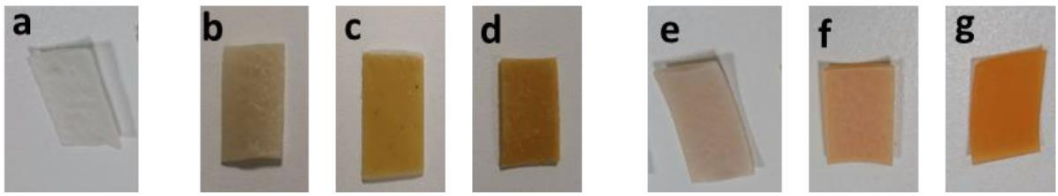

Figure 3.6: Extruded half products formulated with different pulse incorporation levels a) potato starch only b) 10% chickpea flour incorporation c) 40% chickpea flour incorporation d) 80% chickpea flour incorporation e) 10% red lentil flour incorporation f) 40% red lentil flour incorporation g) 80% red lentil flour incorporation.

3.3.3. Optimisation of the expansion process

The expansion capacity of the extruded half products was optimised to produce a snack that had a high expansion ratio and appealing organoleptic qualities. The expansion process of choice was through air frying. This was chosen over the traditional method of deep fat frying as this expansion method allows oil usage to be kept to a minimum (zero). This results in a reduction of the possibility of lipid interference with subsequent sample analysis and is also more nutritious in comparison to deep fat frying.

The standard control extrudates composed primarily of potato starch and lacking pulse flour, with low or high formulation moisture were air fried at three different time lengths (60 s, 90 s or 120 s) of interest to determine an optimal time scale by which all samples would be cooked to. 90 s was determined to be the optimum fry time due to the high and uniform expansion achieved upon visual analysis. Extruded half product samples containing different incorporation levels of pulse flour (10, 40, 80%) were then fried at this specific time length (90 s) at three different fry temperatures. The temperatures of interest were 180°C, 210°C and 240°C. In order to determine the optimal fry temperature, the degree of expansion and snack colour was assessed.

3.3.3.1. Browning index

Colour is a quality control measure frequently used to dictate the consumer acceptability of food products. The colour of the final expanded product was assessed for the level of browning which took place through processing. This was analysed by calculating the Browning index of samples expanded at 180°C, 210°C and 240°C in an air fryer. Browning index is commonly used to indicate browning in foods such as high sugar fruits that oxidise (Subhashree et al., 2017) and can be an indicator of acrylamide content in snacks (Verma & Yadav, 2022) which is not desirable for a snack product. Therefore, it was selected to characterise changes in browning with temperature changes.

Colour was analysed by taking images of each sample under uniform light settings and processing using photoshop to calculate l*a*b values and in turn determine the browning index of each expanded sample (Figure 3.7).

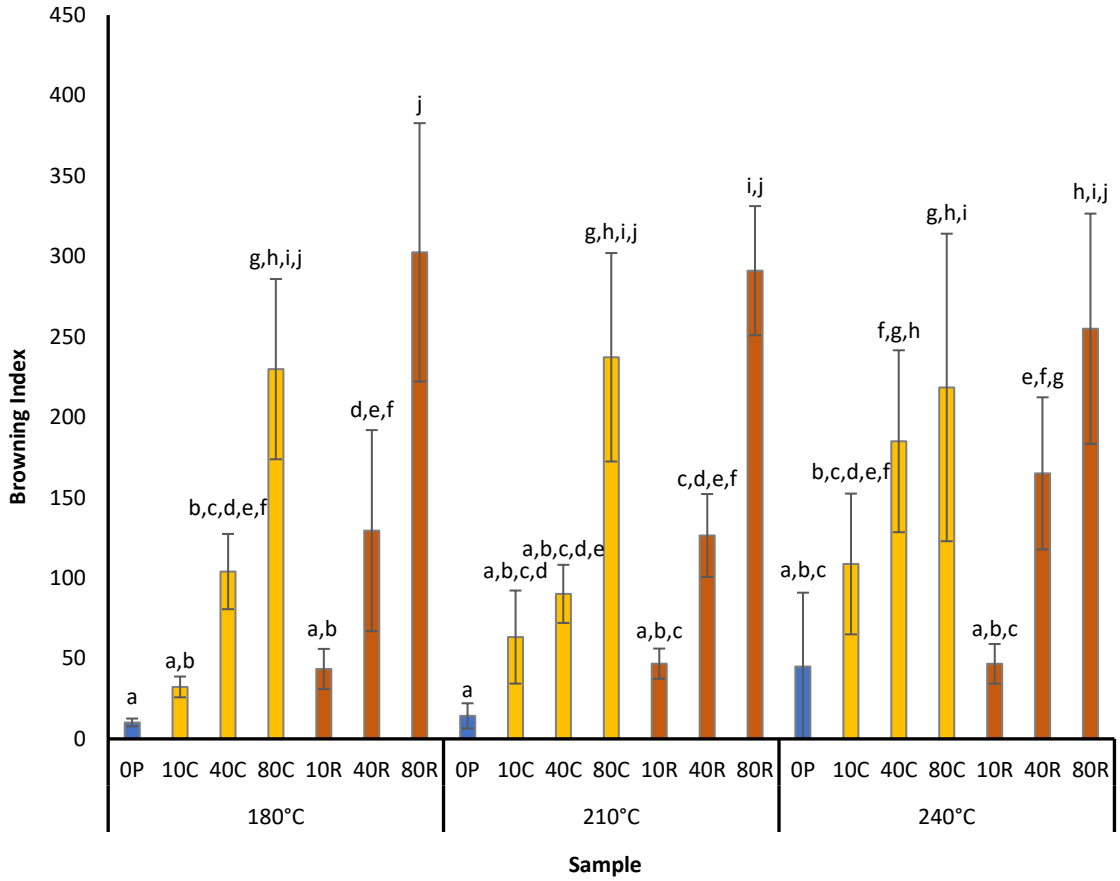

Figure 3.7: Browning index of expanded snacks incorporated with 0%, 10%, 40, & 80% chickpea (C) or red lentil (R) flour, air fried at 180°C, 210°C and 240°C. Analysis was carried out in triplicate. Different letters denote significance ($P < 0.05$) determined by one way ANOVA.

Browning index analysis showed that although temperature had an influence on browning, the increase in pulse incorporation had a higher effect. 10% chickpea or red lentil incorporation was not significantly different to potato starch only controls at 180°C, 210°C and 240°C. 40% pulse incorporation was significantly different to the potato starch only control sample in the majority of tested samples, with exception being the chickpea incorporated sample which was expanded at 210°C. 80% pulse incorporation was significantly different to 0%, 10% and 40% in all samples except the chickpea sample expanded at 240°C where the 80% was not significantly different to 40%. When comparing samples to their equivalents expanded at different temperatures, no significant difference was observed.

3.3.3.2. Specific volume

Specific volume data was assessed as a quantitative measure of extrudate expansion. Temperature was determined as a critical factor in the expansion process. It was hypothesised that an increase in temperature would result in higher expansion. Extrudates containing 0%, 10%, 40% & 80% chickpea or red lentil flour were air fried for 90 s at 180°C, 210°C and 240°C. Specific volume measurements of both the extrudate samples and the equivalent expanded snacks were recorded, and the ratio between the extrudate and equivalent expanded snack values calculated as specific volume change (%) (Figure 3.8).

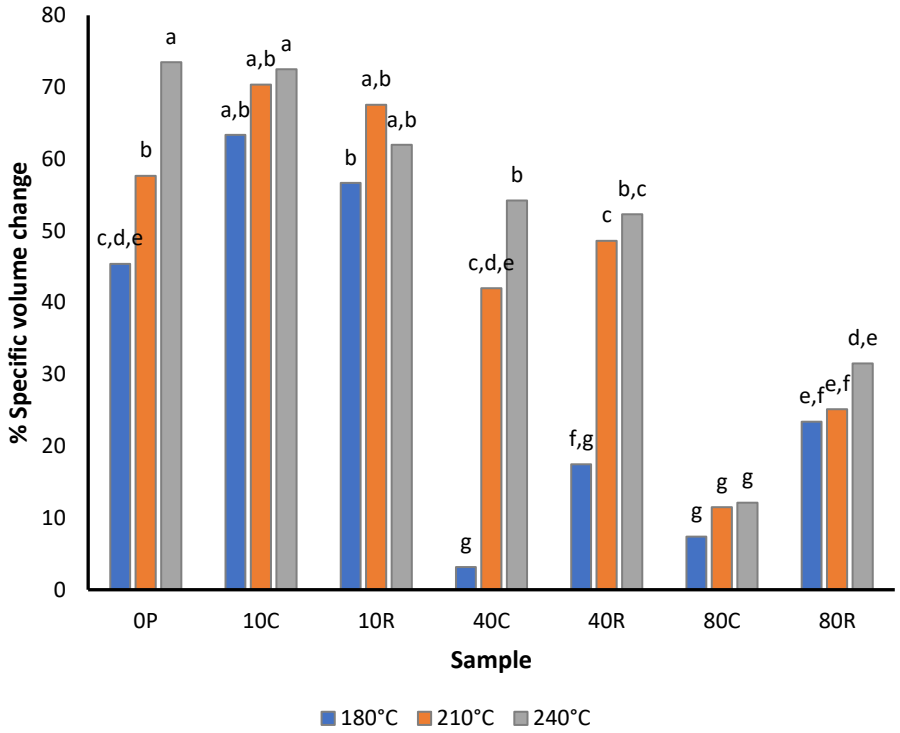

Figure 3.8: Specific volume change (%) of extruded half products containing 0%, 10%, 40% and 80% chickpea (C) or red lentil (R) flour, expanded at 180°C, 210°C and 240°C. Analysis was carried out in triplicate. Different letters denote significance ($P < 0.05$) determined by one way ANOVA.

The specific volume change of snacks expanded at 180°C, 210°C and 240°C in comparison to equivalent non expanded extrudates was used to determine the effect of temperature on expansion (Figure 3.8). Due to the amount of sample available, this was carried out on select samples with one replicate only. It was observed that an increase in temperature affected the expansion of extrudates, with higher temperatures resulting in an increased specific volume change. This difference in expansion with increasing temperature was significant for only the 0% pulse incorporated sample and the 40% chickpea incorporated sample.

Frying at 210°C was also found to provide a high amount of expansion. Addition of chickpea and red lentil flour resulted in a significant decrease in the expansion of the final products, with 40% & 80% pulse samples found to be significantly different to the potato starch only control sample at 210°C and 240°C. 80% chickpea or red lentil flour incorporated samples was also significantly different to both the 10% and 40% pulse incorporated at 210°C and 240°C. There was no significant difference between 40% and 80% chickpea or red lentil flour incorporated samples when fried at 180°C. A large and significant difference was observed between 180°C and 210°C in 40% chickpea or red lentil flour incorporated samples.

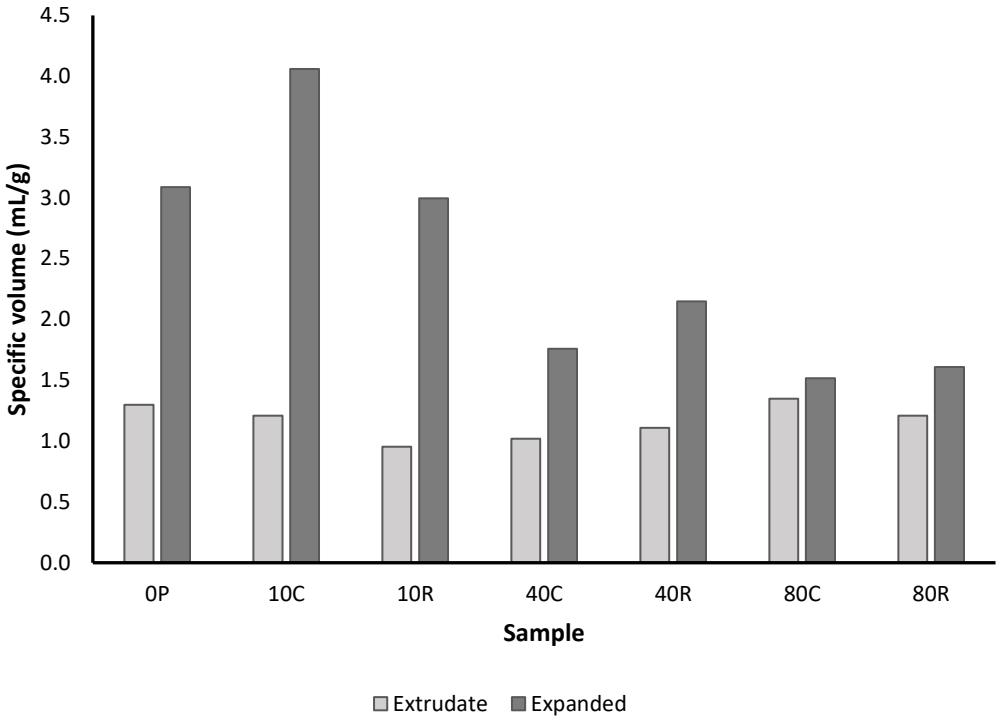

Figure 3.9: Specific volume of extrudate samples containing 0%, 10%, 40%, and 80% chickpea (C) or red lentil (R) flour and their equivalent air fried expanded products, expanded at 210°C. Analysis was carried out in triplicate.

The specific volume of extrudates and expanded snacks fried at 210°C was determined (Figure 3.9). When expanded at 210°C, the specific volume of extrudates was found to remain similar no matter the concentration level. In expanded snacks, an increase in the incorporation level of chickpea or red lentil flour resulted in a decrease in specific volume. A 10% red lentil flour incorporation resulted in a similar specific volume value in expanded products to the potato starch only control, whereas a higher specific volume value was observed in the expanded 10% chickpea flour incorporation product in comparison to both the 10% red lentil flour incorporated snack and the potato starch only expanded snacks. The difference between 10% and 40% pulse flour incorporation was increased for expanded chickpea in comparison to equivalent red lentil. At 40% and 80% pulse incorporation levels, a higher specific volume value was observed in red lentil incorporated expanded snacks.

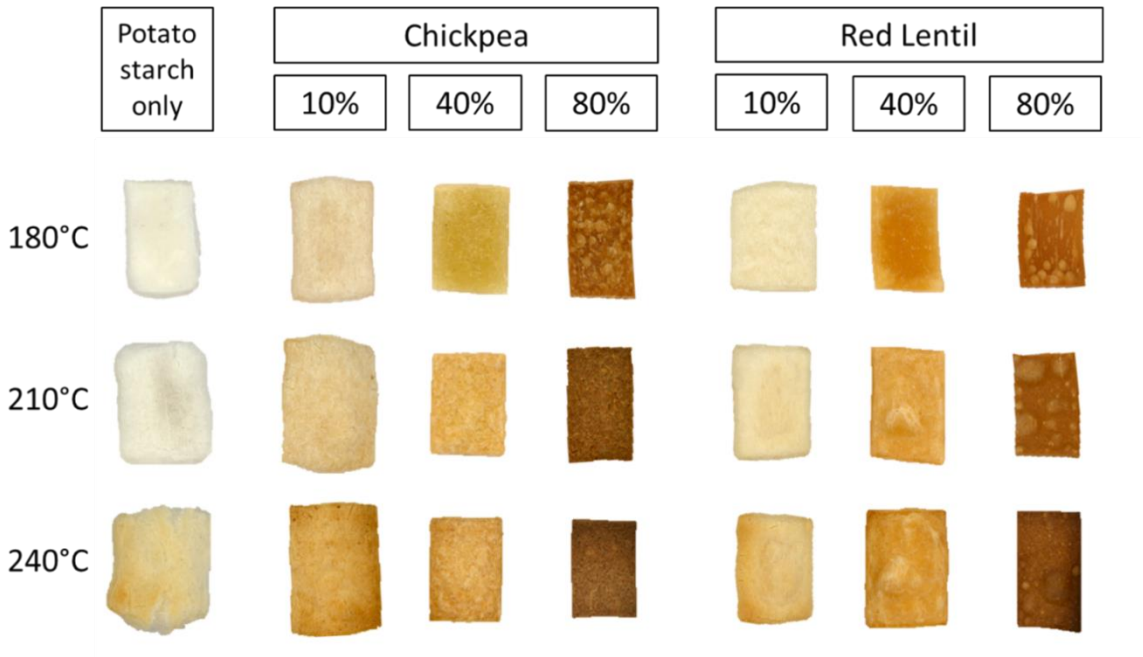

Figure 3.10: Expanded snacks made up of 0%, 10%, 40% or 80% chickpea or red lentil flour, air fried at 180°C, 210°C and 240°C.

In order to determine the optimal fry temperature, a combination of specific volume and browning index was assessed. The expansion of pellets was optimised for consistency and highest amount of expansion, whereas a lower browning index was determined as more favourable due less Maillard reaction. Visual evaluation of the final expanded product was also taken into consideration (Figure 3.10).

Pellets expanded at 180°C were frequently found to not fully expand at all, with uncooked areas detected particularly at higher pulse incorporation levels. This in turn resulted in unequal colour variation and caused inaccuracies in the Browning index as uncooked areas are visibly darker.

It was observed that frying at 240°C resulted in the highest amount of specific volume change for the majority of samples. The manner of expansion was not

uniform however, with the top and bottom of the snack breaking apart. Upon visual analysis, samples expanded at 240°C had a tendency to burn, resulting in unequal colour distribution and non-uniform browning.

Expansion at 210°C was found to give the most uniform product, both in expansion and colour, therefore moving forward was chosen as the optimum fry temperature.

3.4. Summary and conclusions

In this chapter, snacks containing specific amounts of chickpea and red lentil flour were formulated and produced, and processing steps were optimised.

The standard snack formulation was decided based on the Quavers recipe and optimised according to equipment availability. Starch gelatinisation was decided to be a main factor in snack acceptability in terms of texture/crispness. As this is highly dependent on the amount of water available, DSC was used to assess the effect of different dough mix hydration levels on starch gelatinisation. This showed that an increase in raw dough hydration resulted in a corresponding increase in starch gelatinisation, with doughs found to be fully gelatinised at moisture levels of >60%. Increased dough hydration was also linked with a decrease in gelatinisation onset temperature. Optimal hydration levels were decided based on samples that resulted in partial gelatinisation in order to have the most favourable expansion conditions. DSC data was also used to determine optimal temperature for extrusion processing.

Expansion is a leading factor for texture and sensory acceptability of snacks. Therefore, important to produce sample snacks that have high expansion. As health snack, it was decided that using the standard method of deep fat frying would not be advantageous as it produces a less nutritional snack. In addition, the use of oil would interfere with following investigations, such as total starch content and starch digestibility. Air frying, which utilises heated circulating air was instead chosen as the method of cooking. It was hypothesised that increasing temperatures would result in more expansion, particularly over 200°C according to literature (Norton et al., 2011) so in order to optimise expansion, temperatures of 180, 210 and 240°C were chosen for analysis. This

was investigated through specific volume tests of half product extrudates and air fried expanded snacks. It was found that higher temperatures did indeed lead to increased expansion, with samples which had been heated at 180°C not always fully expanded – middle sections of the extrudate were occasionally undercooked. However the difference in samples with any amount of pulse flour incorporated was usually not significant between 210°C and 240°C with exception being 40% chickpea. In addition, visual analysis showed that cooking at the highest temperature (240°C) resulted in uneven expansion and visible points of breakage. Expanding at 210°C was considerably more uniform.

(Kallu et al., 2017) studied expansion of fiber particle size incorporation and starch amylose content on expansion and found that smaller fiber particle size resulted in high expansion ratio. 23% amylose gave highest expansion with no fiber added. 50% amylose in combination with small particle size fiber resulted in greater expansion than starch on controls. These findings align with our results and may explain why 10% chickpea flour incorporation resulted in higher expansion than 0% pulse flour incorporation, as chickpea flour contains both a higher proportion of amylose in comparison to potato starch, and are smaller in particle size.

Snack appearance is also an important organoleptic property in the evaluation of final product snacks (Świąder & Marczewska, 2021). Appearance can be measured in ways such as colour and snack shape uniformity. It was determined that colour analysis should be carried out on snacks expanded at 180, 210 and 240°C. L*a*b* values were recorded using Photoshop and the Browning Index of each sample was calculated. It was hypothesised that as temperatures increased, there would be a correlating increase in Browning

Index. This hypothesis proved to be true for all samples but not significantly so. It was also observed that the addition of pulse flour gave rise to a higher Browning Index. This was true for either chickpea or red lentil flour. Of the temperatures investigated, snacks expanded at 210°C were found to be the most uniform in terms of a lack of burnt edges (240°C) or undercooked areas (180°C). For this reason, along with the consistency in expansion measured through specific volume, samples which had been cooked at 210°C were chosen for further analysis.

4. Impact of pulse incorporation on starch crystallinity, retrogradation and matrix structure in extruded and expanded pulse products

4.1. Introduction

Starch is the main material which forms the matrix of a snack, giving rise to the structural and functional properties of the snack, such as texture, organoleptic properties and digestibility (Sajilata & Singhal, 2005). Because the starch performs such an integral role in the techno-functional performance of a snack product it is important to understand how the chemical and physical structure of the starch changes through processing, storage and how the starch behaves in the final product.

Extruded snack products are formulated from mainly uncooked (raw) ingredients which are starch based (normally potato or maize, or in this thesis legume flours). In its raw, or native state, starch has a semi-crystalline granular structure (Huang & Rooney, 2001; Smith, 2001). It is in the form of water insoluble granules which are 1-100 µm in diameter, depending on botanical origin. Starch in its native form can have different crystalline forms, either A, B or C type, again depending on botanical origin with tuber starches displaying B-type crystallinity and legume starches displaying C-type crystallinity. The crystalline pattern of the starch is dependent on the form of the unit cell, with B-type starches having a hexagonal arrangement of starch double helices and A-type starches having a monoclinic orthorhombic unit cell. C-type starches contain a mixture of A- and B-type crystalline forms (Rodriguez-Garcia et al., 2021).

During extrusion, these dry native ingredients are mixed with water to make a dough, batter or crumb which is fed into the extruder. The extruder applies high pressure and temperature to the dough. This high energy input results in gelatinisation of the starch, whereby there is partial or complete loss of crystalline structure, and polymer leaching from the starch granule to form a glassy matrix which creates the structure of the extruded product. Extrusion cooking requires lower moisture levels (12-22%) in order for gelatinisation to occur in comparison to other processing technologies (S. Singh et al., 2007b). The starch exits the extruder as a pliable, rubber like material which then undergoes glass transition and forms a glassy matrix upon cooling. Depending on the extruder technology, this may be the final product, or in 3rd generation extrusion, a further expansion step will be required. The pellets formed following 3rd generation extrusion can be stored for a significant length of time and may undergo further structural changes such as retrogradation during storage which can impact functional performance of the product. To achieve good and even expansion, complete gelatinisation during extrusion is not desirable, as ungelatinized granules are required to act as nucleation points for expansion. During the expansion process the snack pellet undergoes further transitions whereby rapid heating leads it to undergo a glass-transition from glassy to rubbery, allowing gas bubbles to form, expand and be stabilised by the rubbery starch matrix. Upon cooling, the starch matrix reverts to a glass and fixes the bubbles formed during expansion into the product.

The incorporation of pulses into products traditionally made up of cereal or tuber starch sources has gained popularity in recent years. Legume starches have higher gelatinisation enthalpy when compared to maize starch, but lower than

potato (Chung et al., 2008). A higher gelatinisation enthalpy suggests that crystalline network formation within the starch granule is more stable (Chung et al., 2010). (Yağci & Evci, 2015) found that treatment by instant controlled pressure drop (Détente instantannée contrôlée in French, DIC), a high temperature short time (HTST) process increased the degree of starch gelatinisation and digestibility of chickpea-wheat snacks. In the production of cereal-legume pasta by twin screw extrusion processing (Rafiq et al., 2017), higher moisture and temperature parameters resulted in increased starch gelatinisation.

For 1st generation extruded snack products made up of 100% pulse flour (Costantini et al., 2021) found that a higher extrusion pressure induced a higher degree of starch gelatinisation. Red lentil extruded snacks were found to have a lower degree of gelatinisation in comparison to faba bean, brown pea and common bean. Across a broad range of different legume ingredients higher energy inputs and moisture contents are required to achieve complete gelatinisation during 1st generation extrusion processing (Pasqualone et al., 2020). The mechanical energy inputs and moisture contents to achieve gelatinisation during 3rd generation extrusion have not been explored in the literature.

Characterising changes that starch undergoes during processing and the impact of chickpea or red lentil flour incorporation is important to understanding and manipulate a final product. The aim of this chapter is to determine how altering the levels of pulse incorporation and dough moisture effects starch gelatinisation at different stages during 3rd generation snack extrusion, from raw material to finished product. We hypothesise that the addition of pulse flour will

result in reduced gelatinisation during the extrusion process as well as impact starch crystallinity in the final product. A difference in moisture in the starting batter is hypothesised to affect the starch crystallinity as a result of increased retrogradation of the extruded pellet during storage.

4.2. Materials & Methods

4.2.1. Materials

All raw ingredients and chemical reagents used in this chapter are described in Chapter 2.1.

4.2.2. Total starch

Total starch content in raw materials, extrudates and expanded products (0%, 10%, 40% and 80% chickpea or red lentil flour incorporated, at 25% and 35% moisture) was quantified using the Megazyme Total Starch Assay Kit (K-TSTA-50A). The NaOH method was used as described in Chapter 2.10.

4.2.3. Powder X-Ray diffraction analysis

XRD analysis was conducted to determine the degree of crystallinity in the raw materials and the residual crystallinity following processing in the extruded and expanded products. XRD analysis was conducted as described in Chapter 2.12.

4.2.4. Differential Scanning Calorimetry

DSC analysis was carried out to determine thermal transitions present in the raw materials, extrudates and expanded products under heating in excess moisture conditions. DSC analysis was carried out using an MCDSC Instrument (TA Instruments) as described in Chapter 2.11.2.

4.2.5. Microscopy

Light microscopy was carried out on sections (2 µm thickness) cut directly from extruded samples, and stained using IKI (starch), Toluidine Blue (Protein and cell wall components) or viewed under polarizing light conditions (starch

crystallinity). Microscopy sample preparation and imaging conditions are described in Chapter 2.14.

4.3. Results

4.3.1. Total starch

The total starch content of the raw materials (potato starch, chickpea flour and red lentil flour) was determined as well as the starch content of the extruded products. This was carried out in order to determine the impact of incorporating pulse flours on the starch content within the extruded products as well as to allow subsequent analysis to be carried out on a starch basis.

Sample	Starch % (w/w)	Moisture (%)	Starch % (d.w.b.)
Chickpea flour	37.64	13.07	43.30 ± 0.20^{a}
Red lentil flour	46.07	7.59	49.86 ± 0.20^{b}
Potato starch	76.63	19	94.48 ± 0.49^{c}

Table 4.1: Total starch percentage of raw potato starch, red lentil flour and chickpea flour

Analysis was carried out in triplicate. Significant difference is indicated by different letters ($P < 0.05$), determined by ANOVA.

The percentage of total starch in raw chickpea and red lentil flour and was found to be 43.30% (d.w.b.) and 49.86% (d.w.b.) respectively. This result is similar to the chickpea flour total starch percentage of 42.1% reported by Meares et al., (2004) and falls within the range of 35-53% reported for lentils (Reddy et al., 1984; N. Wang & Daun, 2006). The percentage of total starch was found to be significantly different between each of the raw materials.

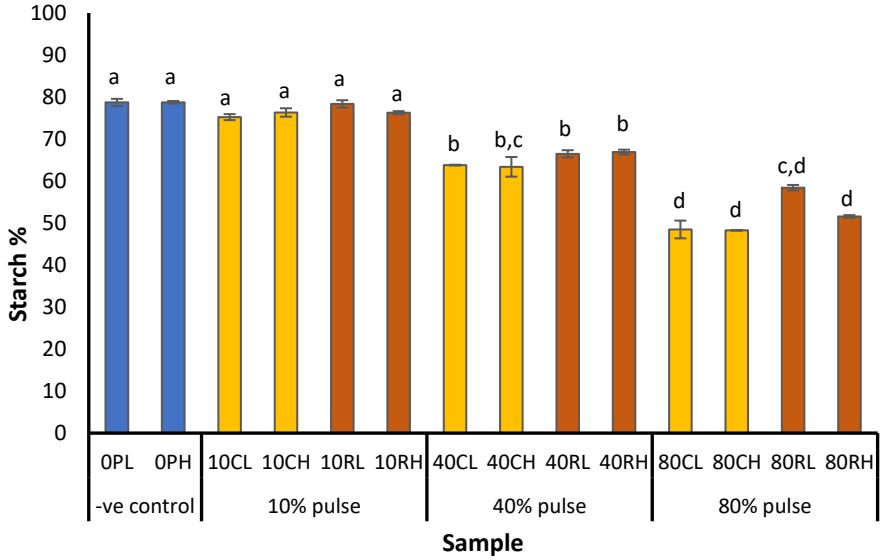

Figure 4.1: Total starch content (% w/w) of potato starch (P) only control and 10, 40 & 80% chickpea (C), and red lentil (R) flour enriched extrudates at low and high moisture formulations. Analysis was carried out in triplicate. Significant difference is indicated by different letters (P < 0.05), determined by ANOVA.

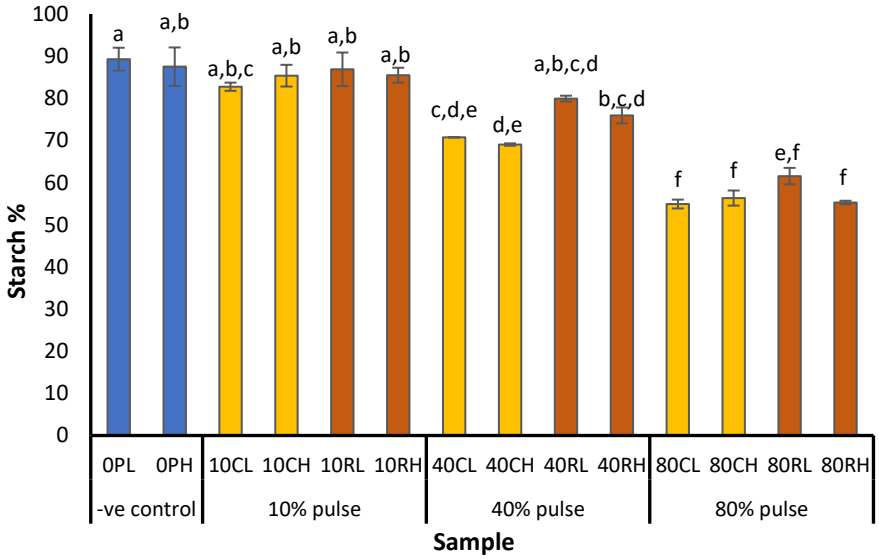

Figure 4.2: Total starch content (% w/w) of potato starch (P) only control and 10, 40 & 80% chickpea (C), and red lentil (R) flour enriched expanded snacks at low and high moisture formulations. Analysis was carried out in triplicate. Significant difference is indicated by different letters (P < 0.05), determined by ANOVA.

As expected, the percentage of total starch decreases with an increase in pulse flour incorporation. The potato starch control extrudates were found to have ~78% (w/w) starch content, and there was no significant difference observed in starch content between the high and low moisture levels. At 10% pulse incorporation, neither the red lentil nor the chickpea flours resulted in a significant reduction in the total starch content of the snacks. While a small reduction in total starch content would be expected with any addition of pulse flour due to the lower starch content than the potato starch ingredient, at 10% pulse incorporation this reduction was below the experimental error of the total starch method. With a 40% incorporation of pulse flour, a significant reduction was observed in total starch content compared to both the control and 10% incorporation products. This is as a result of the lower starch content in the pulse flour compared to the potato starch. The small difference observed between the red lentil and chickpea 40% extruded products was below the margin of error of the assay, and not statistically significant. The 80% pulse incorporated extruded products had a statistically significantly lower starch content than the control, 10% and 40% pulse incorporated extruded products. This is due to the very high level of pulse flour incorporation, where pulse flours have a lower starch content than potato starch. An interesting observation is the slightly higher starch content of the 80RL sample, which was not significantly different in starch content from the 40% pulse incorporation samples. This may reflect differences in water holding and water content between the different extruded products at this high pulse incorporation level.

4.3.2. Starch gelatinisation of raw materials

The gelatinisation behaviour of potato starch, chickpea flour and red lentil flour was determined using DSC. Raw uncooked materials were analysed in excess moisture conditions. This was to determine the extent of starch gelatinisation available to each of the raw materials to achieve baseline values for comparison in subsequent analysis of processed extrudates and expanded snacks made from these raw materials.

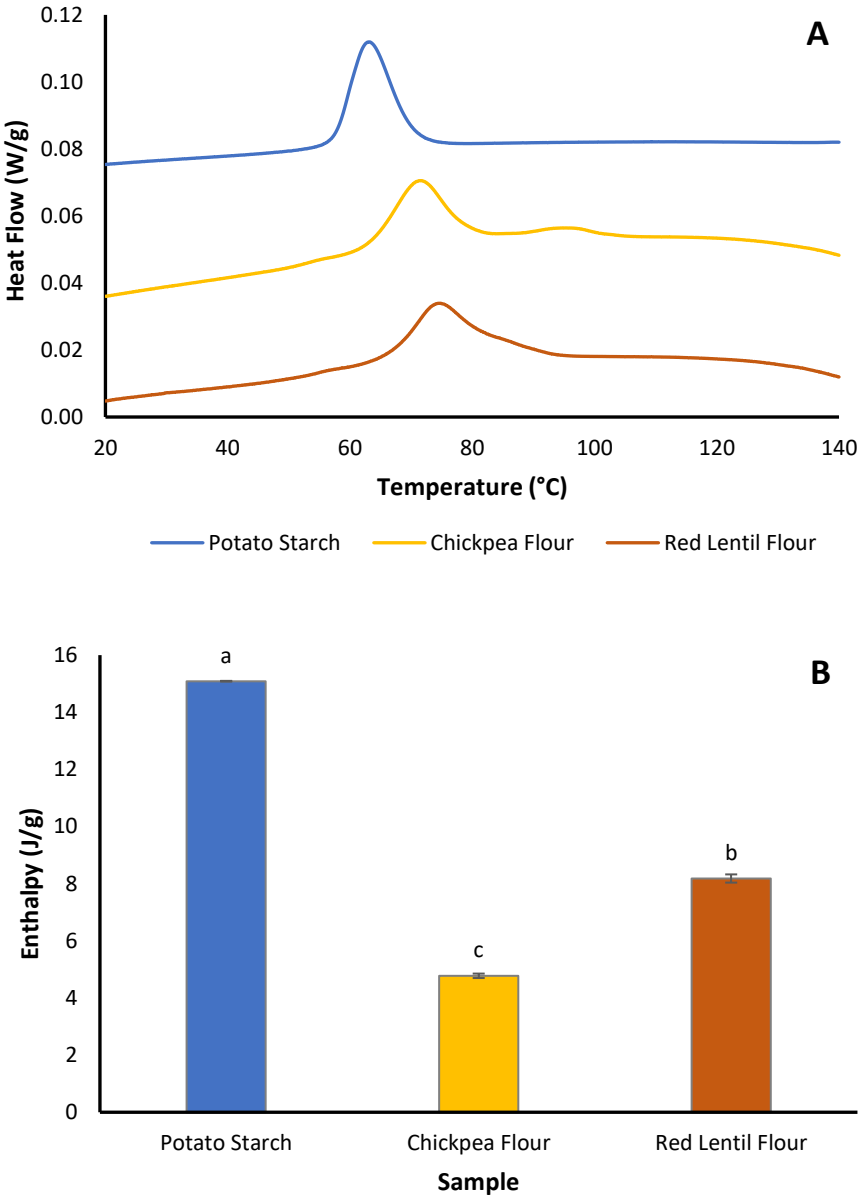

Figure 4.3: DSC thermograms A) and gelatinisation enthalpy B) of raw potato starch, raw chickpea flour and raw red lentil flour. Curves have been offset for visual clarity. Analysis was carried out in triplicate. Different letters denote significance ($P < 0.05$) determined by one way ANOVA.

Table 4.2: Starch gelatinisation temperature of raw materials

Raw sample	Temperature of Gelatinisation		
	T_o (°C)	T_p (°C)	T_c (°C)
Potato starch	57.4±0.03	63.0±0.04	76.0±0.63
Chickpea Flour	63.4±0.02	71.5±0.04	83.3±0.37
Red Lentil Flour	66.3±0.03	74.6±0.03	96.4±1.21

Analysis was carried out in triplicate. Values represent mean ± standard deviation.

The thermograms for potato starch were characterised by a single endothermic peak, chickpea flour displayed two distinct endotherms, one at around 70°C and a second peak at around 95°C, while red lentil displayed a broadened shoulder above the main gelatinisation endothermic peak at ~75°C. For all three samples the main endothermic peak can be assigned to the gelatinisation of starch and associated melting of starch crystallites. The second, higher temperature endotherm observed in chickpea, and to a lesser extent in red lentil, may be assigned to the melting of amylose-lipid complexes. Native potato starch has a very low lipid content, so amylose-lipid complexes are not observed, while the legume flours have a higher lipid content and a high amylose content, which both contribute to their tendency to form amylose-lipid complexes (Oyeyinka et al., 2021). The underlying molecular mechanisms explaining differences in gelatinisation temperatures of starches are still a topic of debate in the literature, however several mechanisms have been proposed. Starches with a higher gelatinisation temperature may demonstrate greater crystalline perfection or stability of crystallites, they may have different crystalline forms (A, B or C) or they may be more hydrophilic, for example the highly phosphorylated potato starch (Morris, 1990). The gelatinisation temperature of native potato starch was 63.0°C. This was similar to results reported by Przetaczek-Rożnowska et al. (2019) and Singh & Singh (2003). The gelatinisation temperature of chickpea flour was found to be 71.5°C. This is in agreement with (Kaur & Singh, 2005) who reported that gelatinisation temperatures ranged from between 70.6°C and 73.3°C depending on the variety of chickpea. The gelatinisation temperature of red lentil flour was 74.6°C. This is slightly higher than previously reported in literature (Kaur et al., 2010; Kaur & Sandhu, 2010).

The enthalpy of gelatinisation (ΔH_{gel}) represents the energy required for loss of molecular order and crystallinity within the starch granule (Cooke & Gidley, 1992). The higher temperatures required for gelatinisation of pulse flours in comparison to potato starch implies a more ordered crystalline structure, leading to greater resistance to gelatinisation. In Chapter 3 (Section 3.3.1.), it was observed that the potato starch underwent more complete gelatinisation during simulated restricted-moisture processing in the DSC instrument than the legume flours, despite the potato starches higher crystallinity. This may be as a result of the higher gelatinisation temperature observed for the legume starches (Figure 4.3) compared to potato starch, which reflects the greater crystalline stability during thermal processing of the legume starches relative to the potato starch.

Potato starch was found to have the highest gelatinisation enthalpy in comparison to pulse flours. This is consistent with the literature values for gelatinisation enthalpies of potato starch and legume starches (e.g. chickpea starch gelatinisation enthalpy values 3.9 – 4.9 J/g (Kaur & Singh, 2005)) and indicates that native potato starch has a greater degree of crystallinity than the legume starches tested int his study. These DSC results are consistent with the XRD data which is show in Figure 4.3 in which potato starch shows and XRD pattern with much sharper reflections than either of the legume starches, indicating that it has a greater degree of crystallinity, which is correlated with a higher gelatinisation enthalpy.

4.3.3. Starch gelatinisation and thermal transition of extruded and expanded products

The gelatinisation behaviour of extrudates containing 0, 10, 40 and 80% pulse flour incorporated at low and high moisture formulations was analysed using DSC in excess water conditions in order to determine the amount of gelatinisation that had occurred during extrusion and expansion processes. Post extrusion DSC enthalpy data indicates the amount of gelatinisation that did not occur during the extrusion process (i.e. it measures the amount of starch which remains ungelatinised following extrusion). The method is also sensitive to other thermal transitions, for example arising from retrogradation of starch during storage of the extruded pellets.

Table 4.3: Gelatinisation temperature of extrudates

Sample	Low Moisture			High Moisture		
	T_o (°C)	T_p (°C)	T_c (°C)	T_o (°C)	T_p (°C)	T_c (°C)
0% Pulse	64.1±0.7	70.0±0.8	78.3±1.1	69.4±0.9	73.1±0.9	80.9±2.0
10% Chickpea	64.9±0.7	71.4±0.8	82.9±1.7	69.2±0.8	76.5±0.8	83.8±1.6
40% Chickpea	67.2±0.8	74.6±0.8	85.4±1.4	73.1±0.7	77.6±0.8	87.3±1.0
80% Chickpea	69.5±0.7	74.8±0.8	87.1±1.0	73.5±0.7	78.0±0.7	88.0±0.6
10% Red Lentil	65.0±0.7	71.5±0.8	82.9±2.0	69.6±1.0	76.5±0.8	83.6±1.5
40% Red Lentil	64.8±0.7	74.1±0.7	94.9±0.7	71.3±0.7	77.3±0.8	94.8±1.8
80% Red Lentil	66.2±0.9	74.6±0.7	95.1±0.8	72.2±0.7	77.4±0.7	97.7±1.6

Analysis was carried out in triplicate. Values represent mean ± standard deviation.

The temperature of gelatinisation of extrudates was found to increase with increasing pulse incorporation (Table 4.3). The onset temperature of gelatinisation was higher in pulse incorporated extrudates in comparison to raw chickpea or red lentil flour. Gelatinisation temperatures of potato starch only extrudates were also higher than raw potato starch. This supports findings by (Xu et al., 2021) where potato starch samples of increasing degree of starch gelatinisation positively correlated with the onset temperature of gelatinisation. Extruded potato starch samples would also contain partially gelatinised starch. Increased onset temperature of partially gelatinised potato starch was postulated to be due to the colloidal molecular structure of the starch granules, the amylopectin chain length and the reordering of the crystalline structure following hydrolysis (Fu et al., 2012). An alternative hypothesis may be that during extrusion and partial gelatinisation, the less temperature stable granules are gelatinised, which leaves only the more stable granules with a higher gelatinisation temperature. The effect of moisture formulation positively correlated with temperature with a higher temperature required for gelatinisation in high moisture samples. This could be hypothesised to be due to the higher formulation moisture resulting in a higher amount of partial gelatinisation, and therefore higher melting temperatures. More complete gelatinisation is observed in the high moisture samples (Figure 4.4), which would support this hypothesis.

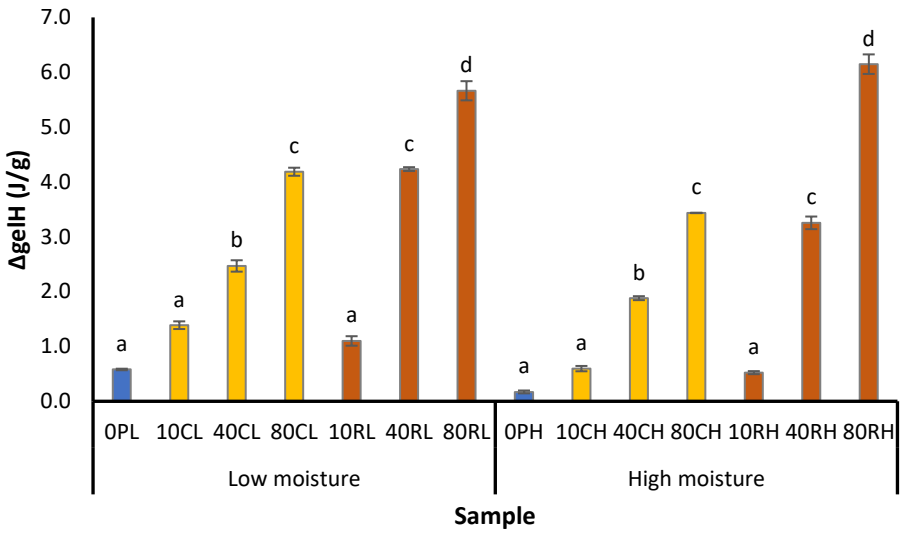

Figure 4.4: Gelatinisation enthalpy of extruded potato starch (P) pellets and chickpea (C) or red lentil (R) flour incorporated extrudates (10, 40 & 80%) at low and high formulation moisture. Analysis was carried out in triplicate. Significant difference is indicated by different letters ($P < 0.05$), determined by ANOVA.

DSC data showed that extrudates incorporating the highest amounts of pulse flour resulted in the least gelatinised starch during extrusion. In comparison to raw potato starch which had an enthalpy value of 15.09 J/g, chickpea flour and red lentil flour had gelatinisation enthalpy values of 4.7 J/g and 8.2 J/g respectively. The corresponding processed samples produced a lower post extrusion enthalpy, the highest being 80% red lentil flour incorporated extrudates at 6.1 J/g. This finding correlates with expected results, as gelatinisation has yet to take place in raw materials, and indicates that partial starch gelatinisation occurred in all of the extruded samples as a result of the extrusion process.

Upon extrusion, potato starch underwent the most amount of gelatinisation, resulting in the lowest post extrusion enthalpy results (0PL & 0PH, Figure 4.4). Chickpea or red lentil flour incorporated at 10% behaved similarly to control potato starch. There was no statistically significant difference observed between the control and 10% pulse incorporation samples observed at high or low formulation moisture levels.

At 40% chickpea flour incorporation, low and high moisture extrudates were found to have undergone significantly less gelatinisation than either potato starch only control extrudates or 10% pulse incorporated samples. 40% red lentil flour incorporated extrudates exhibited gelatinisation at a lower extent to 40% chickpea (Figure 4.4).

80% chickpea flour incorporation resulted in extrudate gelatinisation enthalpies (4.2 J/g at low moisture; 3.4 J/g at high moisture) (Figure 4.4) slightly lower than raw chickpea flour (4.8 J/g) indicating that little gelatinisation had taken place

during extrusion. A similar trend is displayed in 80% red lentil flour (8.2 J/g raw vs 5.7 J/g low moisture; 6.1 J/g high moisture extrudates), indicating that slightly more gelatinisation occurred with the red lentil flours than with the chickpea flours, but that there was still a significant fraction of ungelatinised starch.

For all the extrudate samples measured (0, 10, 40 and 80% pulse flour incorporation), there was no significant differences observed in degree of gelatinisation depending on formulation water content. Although all of the measured enthalpies were slightly lower for the high formulation water compared to the low formulation water, the differences were too small to achieve statistical significance, indicating that, somewhat surprisingly, the impact of formulation water on gelatinisation was limited with the levels tested. The small difference may reflect differences in residence time along the barrel of the extruder, where the different dough consistencies led to the low formulation water dough having a longer dwell time in the extruder than the high formulation water dough, and therefore longer time for gelatinisation despite the lower water content. An alternative hypothesis would be that the gelatinisation in an extruder is as a result of a combination of temperature and shear, and while high temperature gelatinisation is highly dependent on water content, shear/pressure related gelatinisation is much less dependent on moisture content.

In many starch based processed foods, a portion of residual starch remains not fully gelatinised, usually as a consequence of limited water or insufficient heating (Chung et al., 2006). These conditions are true to the extrusion process. These results demonstrate that the optimisation process described in Chapter 3 to produce partially gelatinised doughs based on dough composition and

formulation water were successful, as the conditions selected led to partial starch gelatinisation within the extruded pellets.

Table 4.4: Gelatinisation temperature of expanded snacks

Sample	Low Moisture			High Moisture		
	T_o (°C)	T_p (°C)	T_c (°C)	T_o (°C)	T_p (°C)	T_c (°C)
0% Pulse	59.6±1.1	69.8±0.9	79.4±1.6	67.9±0.0	71.9±0.0	78.3±0.8
10% Chickpea	63.1±0.4	74.9±0.7	84.0±1.1	69.2±0.3	76.0±0.0	85.1±1.6
40% Chickpea	67.9±0.7	75.2±0.8	86.6±1.5	71.8±0.0	76.7±0.0	88.2±1.2
80% Chickpea	69.4±0.7	75.1±0.7	86.3±1.5	72.8±0.7	78.1±0.8	88.9±1.2
10% Red Lentil	62.6±0.9	74.8±0.8	85.7±1.6	68.7±0.7	77.2±0.8	86.7±1.3
40% Red Lentil	65.5±0.1	74.4±0.0	88.6±1.5	70.3±0.1	76.3±0.0	89.4±1.2
80% Red Lentil	67.8±0.8	76.6±0.8	90.8.1.1	70.4±0.2	77.1±0.1	89.8±1.1

Analysis was carried out in triplicate. Values represent mean ± standard deviation.

The gelatinisation temperature of expanded snacks was found to increase with increasing pulse incorporation (Table 4.4). The gelatinisation peak temperature of the expanded snacks was very similar to the gelatinisation peak temperature of the equivalent extrudate product (i.e. with the same pulse incorporation and formulation water), which the exception of 10% low moisture expanded samples which had peak gelatinisation temperatures ~3°C higher than the equivalent extrudate. Similarly to the gelatinisation behaviour of extrudates, high moisture expanded snacks gelatinised at a higher temperature in comparison to low moisture formulations. Expanded samples with 10% and 40% pulse incorporation and high formulation moisture had very similar gelatinisation peak temperatures to the equivalent extruded samples.

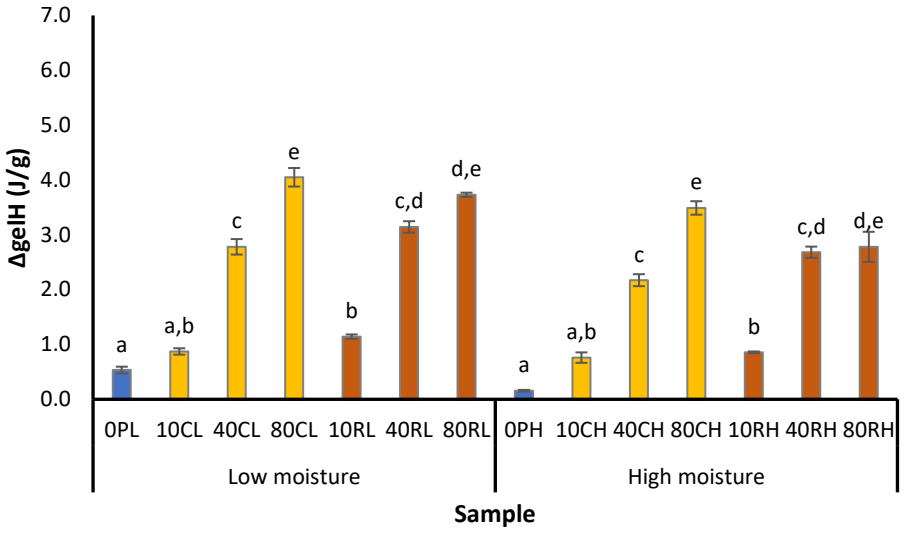

Figure 4.5: Gelatinisation enthalpy of expanded potato starch (P) products and chickpea (C) or red lentil (R) flour incorporated expanded snacks (10, 40 & 80%) at low and high formulation moisture. Analysis was carried out in triplicate. Significant difference is indicated by different letters ($P < 0.05$), determined by ANOVA.

Table 4.5: Difference in gelatinisation enthalpy between extrudates and expanded products

Sample	Low moisture	High moisture
0% Pulse	0.0±0.08	0.0±0.04
10% Chickpea	0.5±0.13	-0.2±0.14
40% Chickpea	-0.3±0.24	-0.3±0.14
80% Chickpea	0.1±0.24	-0.1±0.13
10% Red Lentil	-0.0±0.13	-0.3±0.04
40% Red Lentil	1.1±0.14	0.6±0.22
80% Red Lentil	1.9±0.21	3.4±0.45

Analysis was carried out in triplicate. Values denote means ± standard deviation.

Post expansion DSC enthalpy data measures the remaining ungelatinised starch following the extrusion and expansion processes. Similarly to post extrusion DSC data, potato starch only controls exhibited the most amount of gelatinisation compared to raw (Figure 4.5) consistent with the data presented in Figure 4.8 showing that the majority of the starch in the potato only control was gelatinised during the extrusion process. The measured enthalpy of the 10% chickpea incorporated snacks at low and high moisture were not statistically significantly different to the controls. The 10% red lentil expanded products were significantly less gelatinised than the potato only controls, but were not significantly different to the 10% chickpea expanded products. Expanded 40% pulse samples were significantly less gelatinised than both 10% pulse and potato only controls. At 80% incorporation, expanded chickpea samples were significantly less gelatinised than the 40% chickpea samples, however there was no significant difference observed between the 40% and 80% red lentil expanded samples.

Similar gelatinisation enthalpy values have previously been obtained for expanded potato based snacks (0.2 - 0.9 J/g) (van der Sman et al., 2018) to the values obtained in the present study for the potato only controls with low and high formulation moisture content (0.5 & 0.2 J/g respectively) (Figure 4.5).

The difference in starch gelatinisation enthalpy values between extrudate and expanded final products indicates how much the frying process gelatinised native starch granules beyond the gelatinisation which occurred during extrusion. It has previously been hypothesised that ungelatinised starch granules remaining following extrusion act as key nucleation sites for expansion (Chapter 1.5.2) (Moraru & Kokini, 2003; van der Sman et al., 2018). The largest

differences in enthalpy change between extruded and expanded products were observed for the 40% and 80% red lentil flour incorporated snacks, indicating that a significant amount of gelatinisation of starch granules occurred during expansion of these products (Table 4.5). This was particularly evident in the high moisture formulation at 80% red lentil incorporation.

In comparison to potato starch extrudates (0.6 J/g low moisture; 0.2 J/g high moisture), very little difference in enthalpy was observed in potato starch only expanded snacks (0.5 J/g low moisture; 0.2 J/g high moisture) (Table 4.5). This indicates that a majority of the starch crystallite melting had already occurred during the extrusion process. This result does not align with previous specific volume data (Section 3.3.3.2), which shows high expansion in potato starch control expanded products. 10% chickpea ore red lentil incorporated expanded snacks behave comparably to potato starch controls at both high and low formulation moisture. These results suggest that in these products significant gelatinisation of starch during expansion was not associated with either good expansion or large reductions in specific volume. This suggests that either the hypothesis that ungelatinised starch granules are required for acting as nucleation points during expansion is incorrect for these samples, or that the number of ungelatinised granules required for expansion is so low that their gelatinisation cannot be measured by DSC.

4.3.4. Starch retrogradation of processed materials

The retrogradation behaviour of extrudates and expanded snacks containing 0, 10, 40 & 80% chickpea or red lentil flour incorporated at low or high moisture formulations was determined using DSC, focussing on the endothermic peak occurring at 50-55°C. During storage starches can undergo retrogradation (reformation of crystalline structures), both amylose and amylopectin, with differing kinetics and melting points. The melting point of retrograded amylose is between 50-60°C, therefore the thermal transitions observed in this temperature range were assumed to arise from formation of retrograded amylose in samples during the extended storage which is routine for extruded pelleted snacks. This was to analyse the behaviour of starch-based snacks upon storage.

Table 4.6: Retrogradation temperature of extrudates

Sample	Low Moisture			High Moisture		
	T_o (°C)	T_p (°C)	T_c (°C)	T_o (°C)	T_p (°C)	T_c (°C)
0% Pulse	40.5±1.4	51.5±0.7	60.0±0.6	36.5±1.1	51.9±0.8	64.9±1.1
10% Chickpea	39.2±1.5	51.5±0.8	60.5±0.3	36.7±0.8	52.2±0.8	64.2±1.3
40% Chickpea	n.d.	n.d.	n.d.	41.7±1.9	53.0±0.9	61.4±0.8
80% Chickpea	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10% Red Lentil	40.1±1.6	51.6±0.8	60.5±1.1	37.3±0.6	52.1±0.7	64.0±0.7
40% Red Lentil	n.d.	n.d.	n.d.	43.3±0.3	53.5±0.7	62.1±1.3
80% Red Lentil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Analysis was carried out in triplicate. Values denote means ± standard deviation. n.d. indicates values were not determined as peaks were too small to quantify or absent.

In extrudate samples, only those with a low incorporation of pulse flour exhibited an endothermic retrogradation peak. High moisture extrudates showed broader retrogradation peak in comparison to corresponding low moisture extrudates (resulting in a wider gap between onset and conclusion temperatures (Table 4.6). The peak temperature for the retrograded amylose peak was observed to be slightly elevated with increasing levels of pulse incorporation. Significantly greater amounts of retrogradation (indicated by high enthalpy values, (Figure 4.6) occurred in samples with higher formulation moisture content. Water has a crucial role in starch retrogradation (Wang, 2013) due to its role as a plasticiser increasing the chain mobility of the starch and allowing the starch chains to reassociate, and water also acts as water of crystallisation within the B-type crystalline pattern formed during retrogradation. (Morris, 1990) looked at retrogradation on stored starch gels and found that an endothermic transition occurred at 63°C in gels which had been stored for 7 days. This is higher than results reported here. In contrast, Koev et al., (2020) observed lower melting temperatures for retrograded amylopectin in starch gels, of ~45°C. The results reported here are intermediate to the studies of Koev and Morris. The differences are likely to reflect differences in moisture content and storage conditions, as well as differences in starch source (for example the Koev paper explored a range of maize starches, in contrast to the potato and legume starches studied here). The moisture content in the present study (<10% in the final product) is also much lower than many literature studies which promote retrogradation by producing gels with moisture contents between 50-90%.

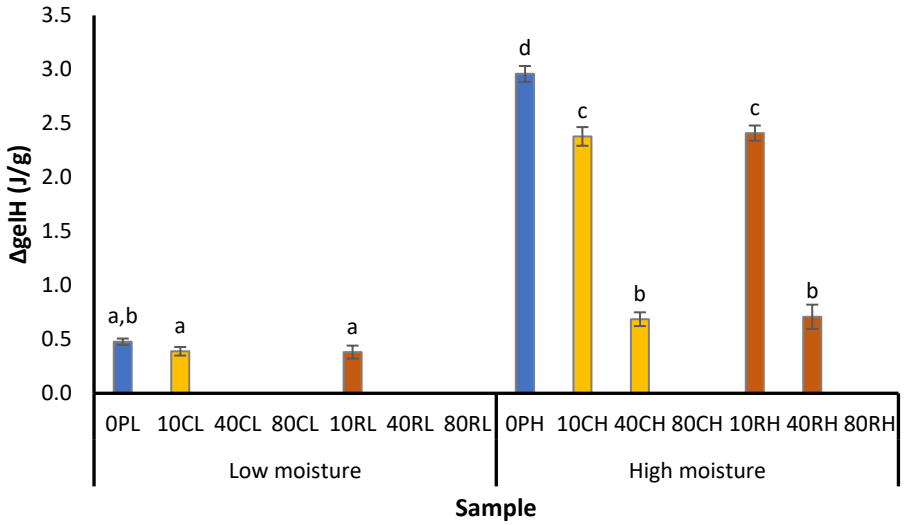

Figure 4.6: Retrogradation enthalpy of extruded potato starch (P) pellets and chickpea (C) or red lentil (R) flour incorporated extrudates (10, 40 & 80%) at low and high formulation moisture. Analysis was carried out in triplicate. Significant difference is indicated by different letters ($P < 0.05$), determined by ANOVA.

Retrograded starch was found to be mostly cooked out, although this was less likely in high moisture extrudates.

As well as lower pulse incorporated extrudates having a lower melting temperature of retrogradation, 10% chickpea or red lentil incorporated snacks also had higher retrogradation enthalpy in comparison to those incorporated at 40%. There was no significant difference in retrogradation enthalpy between chickpea and red lentil flour incorporation at either low or high moisture formulation extrudates.

Pulse starches have higher tendency towards retrogradation (Hoover & Sosulski, 1991). Higher retrogradation due to higher amylose content (Hoover et al., 2010). This tendency was not displayed in extruded samples. Amylose content affects retrogradation, with amylose retrograding much faster than amylopectin. Despite the high amylose content in the legume starches, very little evidence was observed of retrogradation of amylose in these samples. This may be due to the low amount of total gelatinisation observed in pulse incorporated samples (Figure 4.4) resulting in less amorphous starch available for retrogradation.

Table 4.7: Retrogradation temperature of expanded snacks

Retrogradation Temperature of Expanded Snacks						
Sample	Low Moisture			High Moisture		
	T_o (°C)	T_p (°C)	T_c (°C)	T_o (°C)	T_p (°C)	T_c (°C)
0% Pulse	n.d.	n.d.	n.d.	45.3±1.4	54.3±0.5	60.6±1.1
10% Chickpea	n.d.-	n.d.	n.d.	42.3±0.3	52.8±0.1	60.8±0.6
40% Chickpea	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
80% Chickpea	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10% Red Lentil	n.d.	n.d.	n.d.	46.8±2.0	55.0±0.7	64.9±0.3
40% Red Lentil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
80% Red Lentil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Analysis was carried out in triplicate. Values denote means ± standard deviation. n.d. indicates values were not determined as peaks were too small to quantify or absent.

When analysing the retrograded starch remaining post expansion, it was found that all ordered structure melted out in low moisture expanded samples in comparison to extrudates, which had slight endothermic peaks in 10% pulse incorporated extrudates and potato starch only controls.

At high formulation moisture, retrogradation peaks remained in potato starch controls and 10% pulse incorporated expanded snacks, however at 40% chickpea or red lentil incorporation, an endothermic retrogradation peak was no longer visible.

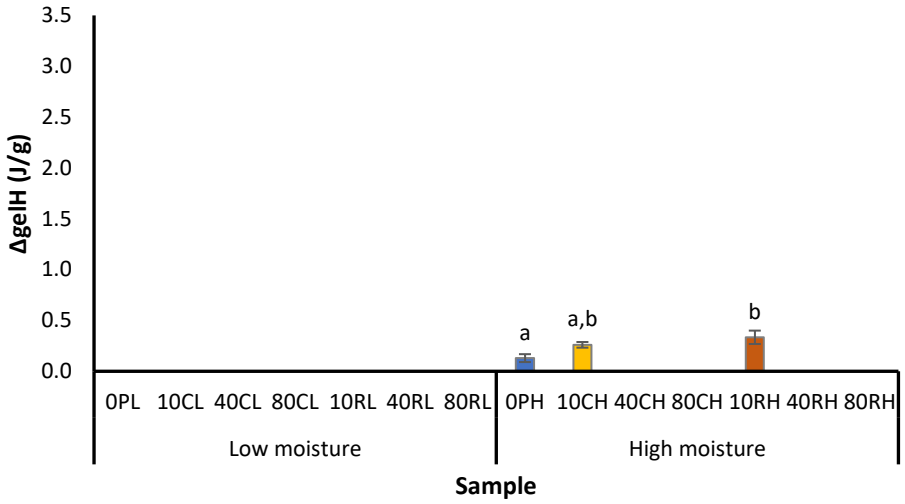

Figure 4.7: Retrogradation enthalpy of expanded potato starch (P) products and chickpea (C) or red lentil (R) flour incorporated expanded snacks (10, 40 & 80%) at low and high formulation moisture. Analysis was carried out in triplicate. Significant difference is indicated by different letters ($P < 0.05$), determined by ANOVA.

Table 4.8: Difference in retrogradation enthalpy between extrudates and expanded products

Sample	Low moisture	High moisture
0% Pulse	n.d.	2.8±0.11
10% Chickpea	n.d.	2.1±0.11
40% Chickpea	n.d.	n.d.
80% Chickpea	n.d.	n.d.
10% Red Lentil	n.d.	2.1±0.14
40% Red Lentil	n.d.	n.d.
80% Red Lentil	n.d.	n.d.

Analysis was carried out in triplicate. Values denote means ± standard deviation. n.d. indicates values were not determined as peaks were too small to quantify or absent.

Very little retrogradation remained in expanded snacks (0.1 J/g in potato starch; 0.3 J/g in both pulses at 10% incorporation).

4.3.5. Starch crystallinity of raw materials and extrudates

X-ray diffraction was carried out in order to determine the starch crystallinity of raw potato starch, pulse flours and processed extrudates.

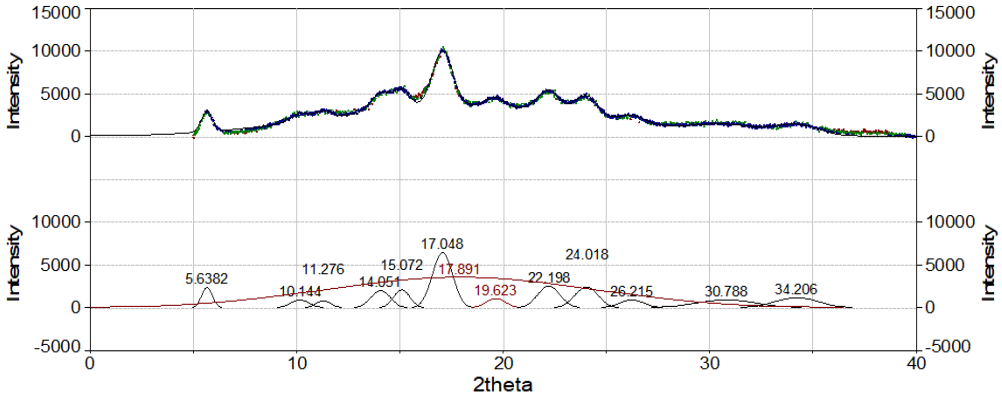

Figure 4.8: Typical representative peak fitting for X-ray diffraction results obtained for raw potato starch. The red peak at 17.891° represents the amorphous content of the starch. The sum of the areas of every other peak corresponds to the crystalline content. The red peak at 19.623° represents the contribution from V-type crystallinity.

Peaks were fitted according to the method described by (Lopez-Rubio et al., 2008). An amorphous peak was fitted using a Lorentz peak shape and crystalline peaks were fitted using Gaussian peak shapes (Figure 4.2 shows an example) according to the position of crystalline peaks referencing previous data (Lopez-Rubio et al., 2008). A good quality of fit was achieved for all of the samples achieving an R^2 value of at least 0.87 for all of the fits. A mixed Lorentzian and Gaussian fitting approach was taken, as this was found to be the most accurate form of fitting the data. A 2-phase model was also tested, in which a simpler form of peak fitting is conducted whereby a single amorphous halo is fitted below the data and the crystallinity is calculated from the ratio of the area above and below the amorphous halo (Nara et al., 1978; Sterlin, 1960), however this approach was found to give inconsistent results and was not as reproducible as the peak-fitting approach. An analytical approach was also tested whereby wholly amorphous starch samples were prepared and analysed to subtract from the diffraction patterns of the samples, however it was found that completely amorphous legume starches could not be prepared.

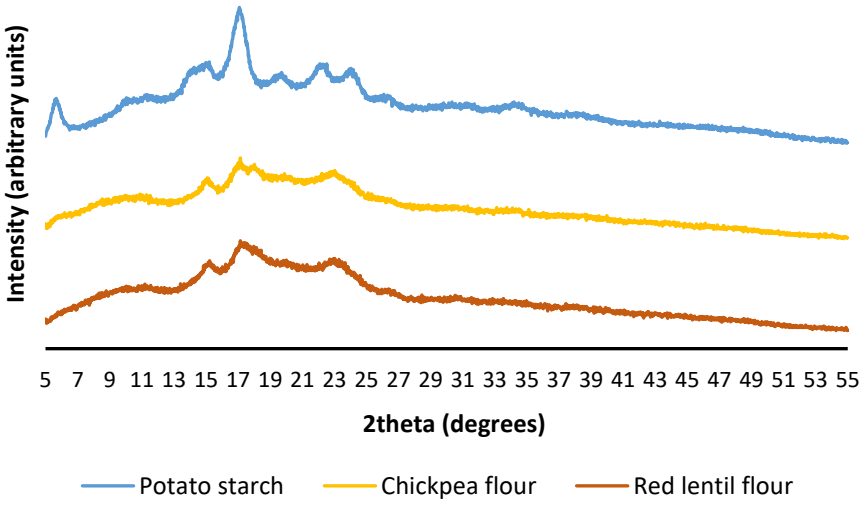

Figure 4.9: Wide angle X-ray diffraction patterns for raw potato starch, raw chickpea flour and raw red lentil flour. Diffractograms have been offset for visual clarity.

The raw materials potato starch, chickpea flour and red lentil flour were analysed using powder x-ray diffraction so that crystallinity could be determined for comparison to pulse enriched extrudates (Figure 4.3). It was observed that peaks were sharper in the raw potato starch sample, indicating that it was more crystalline in comparison to pulse flours. A sharp peak was seen at 17 degrees 2theta in potato starch, which is typically observed only in B-type crystalline starches and indicates that in common with what is widely observed for potato starch, this sample had a predominantly B-type crystalline form (Lopez-Rubio et al., 2008; Pérez et al., 2009; Waigh et al., n.d.). A small peak at 18 degrees 2theta can be detected in both chickpea and red lentil flour which is not seen in potato starch. This diffraction pattern is typically observed in legume starches and is typical of C-type starch crystallinity (Bogracheva et al., 1998). C-type starch is characterised by a combination of A- and B-type crystallinity within a single granule (Hoover & Ratnayake, 2002). A peak at 19.6 degrees 2theta is detected in all samples, most distinctly in potato starch sample. This indicates the presence of v-type amylose lipid complex (Tan et al., 2007).

Crystallinity in starches is associated with the double helical organisation of mainly amylopectin within the starch granules. The crystal structure of B-type starch is hexagonal in arrangement: left-handed double helices are packed in a parallel fashion combined with structured water (Imberty & Perez, 1988). (Bajaj et al., 2018) found that starches containing a higher percentage of amylose correlated negatively with crystallinity, whereas those containing a high percentage of long chain amylopectin branches displayed greater crystallinity. Greater proportion of long chain amylopectin leads to the formation of more perfect crystallites within the granules (Witt et al., 2012). This correlates with the

results of the present study where low amylose potato starch exhibits higher crystallinity than higher amylose containing chickpea and red lentil flour.

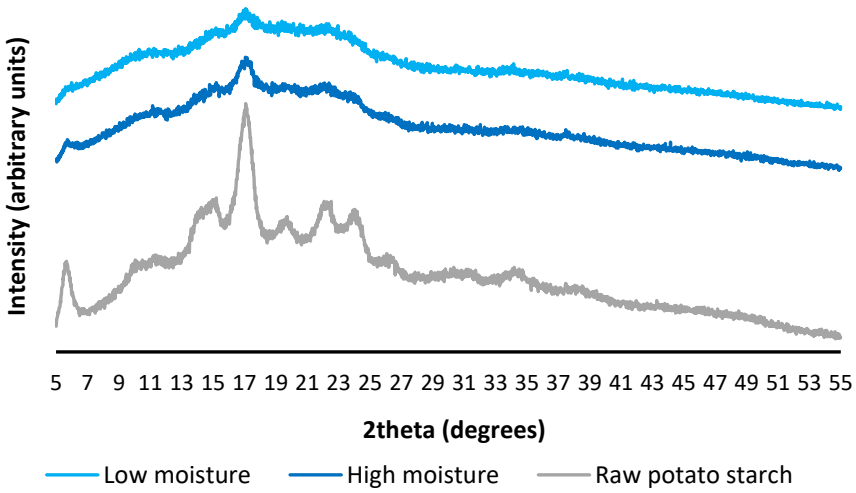

Figure 4.10: X-ray diffraction patterns of raw potato starch and potato starch extrudates at low and high moisture formulation conditions. Diffractograms have been offset for visual clarity.

The crystallinity of potato starch control extrudates was examined at low and high formulation moisture in comparison to raw potato starch in order to investigate the effect that moisture content during extrusion processing had on crystallinity. It was found that mechanical processing through extrusion resulted in a loss in crystallinity compared to the native potato starch, as signified by the broader, less intense peaks shown in processed samples. The quantified percentage crystallinity was reduced from 46.3% in the native potato starch to 16.3% in the low moisture extrudate and 21.1% in the high moisture extrudate. The reduction in crystallinity is likely to be due to the gelatinisation of starch during the extrusion process, however the starch is not completely gelatinised during extrusion. The peak at 17 degrees 2theta is still present in both the low and high moisture extrudates, but is much less pronounced than in the native potato. The peak is larger in the high moisture sample, consistent with the quantification indicating that more crystallinity is present in this sample. The higher crystallinity observed in the high moisture sample may be due to higher levels of retrogradation during storage for this sample, which was observed by DSC (see section 4.3.4).

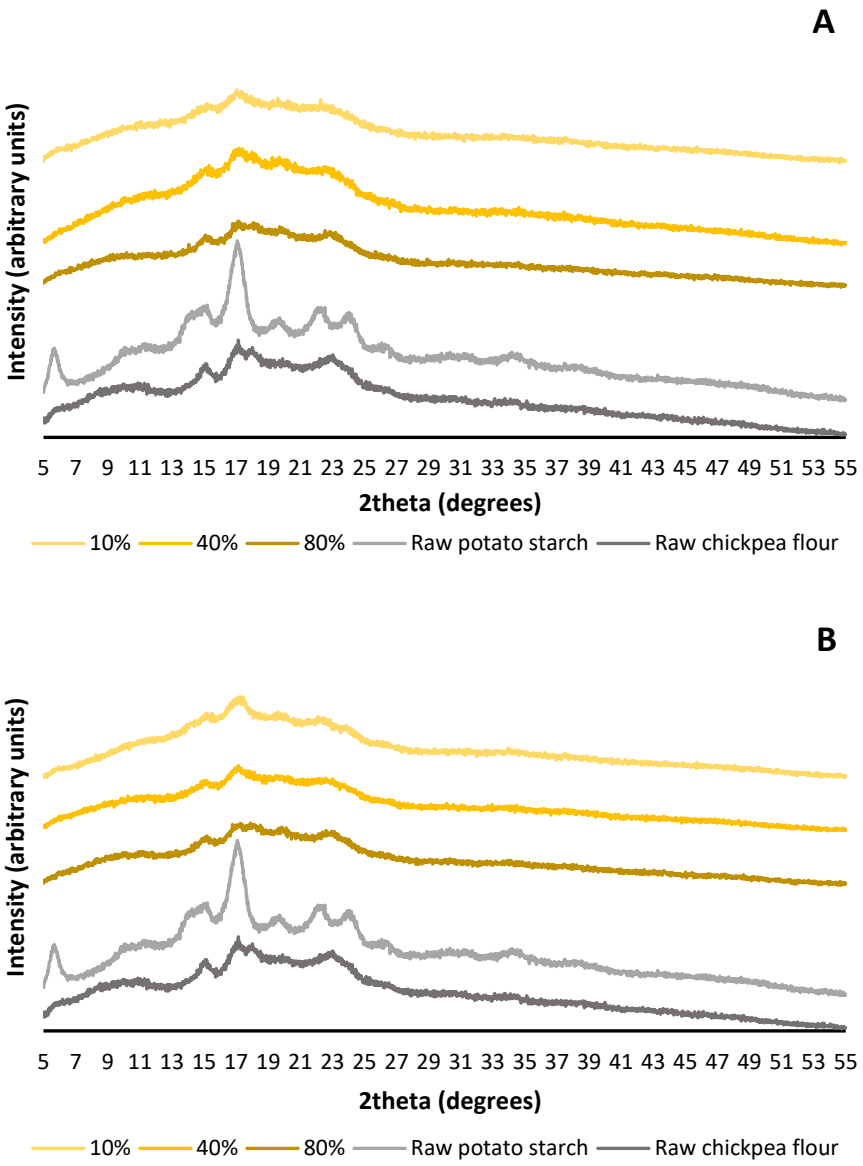

Figure 4.11: X-ray diffraction patterns of raw potato starch, raw chickpea flour and chickpea flour extrudates (10, 40 & 80% incorporation) at low (A) and high (B) moisture formulation conditions. Diffractograms have been offset for visual clarity.

The crystallinity of chickpea flour incorporated extrudates (10, 40, 80% incorporation at low and high formulation moisture) was analysed in comparison to raw potato starch and raw chickpea flour (Figure 4.5). This was carried out in order to determine the effect of processing and chickpea incorporation on starch crystallinity.

For all of the chickpea containing extrudates, a reduction in starch crystallinity was observed relative to the raw materials. Very little difference was observed in the crystalline patterns between the high and low moisture chickpea extrudates, which was reflected in the calculated crystallinity values which were very similar between the two moisture treatments (Table 4.9). Incorporating more chickpea flour resulted in a change in shape of the crystalline peaks, from a diffraction pattern which resembled B-type potato starch in the 10% chickpea extrudates to a diffraction pattern resembling C-type starch in the 80% chickpea extrudates. This was as a result of the B-type potato starch being replaced by C-type chickpea starch in the formulation. The crystallinity quantified in the samples (Table 4.9) was similar across all of the chickpea containing extrudates. All the chickpea containing extrudates at low moisture were more crystalline than the potato only control, but this was not true of the high moisture samples. This may be because in the low moisture samples the crystallinity is dominated by ungelatinised starch, whereas in the high moisture samples there is a significant contribution from retrograded starch, which was highest in the low moisture potato extrudate (Figure 4.6)

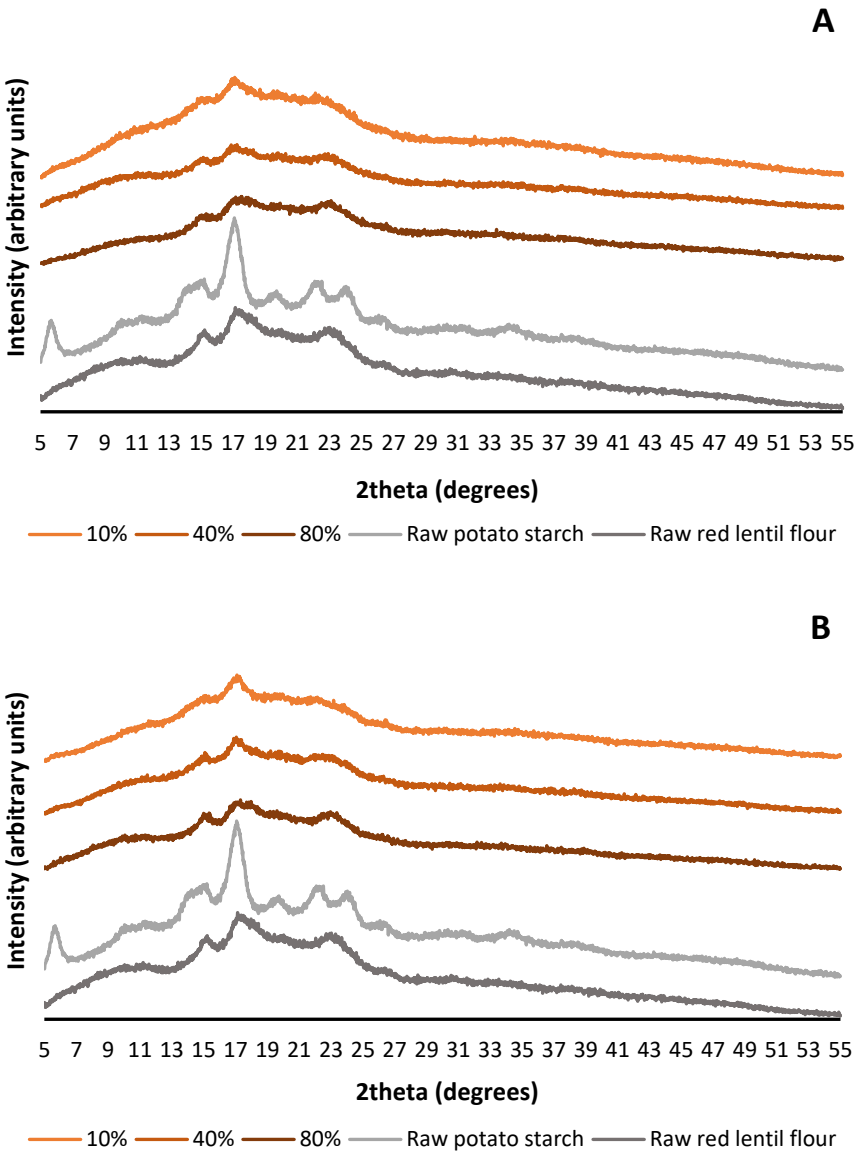

Figure 4.12: X-ray diffraction patterns of raw potato starch, raw red lentil flour and red lentil flour extrudates (10, 40 & 80% incorporation) at low (A) and high (B) moisture formulation conditions. Diffractograms have been offset for visual clarity.

In general, very similar trends were observed between the different samples for red lentil as was observed for the equivalent chickpea samples. There was a large drop in crystallinity in all samples in comparison to the raw materials as a result of gelatinisation during extrusion. The main difference observed with the red lentil samples was that the 80% high moisture extrudate had particularly high crystallinity. This was consistent with the DSC results which showed that in this sample there was very limited gelatinisation during extrusion due to the high legume and moisture content conditions (section 4.3.4).

Starch crystallinity was compared to the combined enthalpy of gelatinisation and retrogradation in raw materials and extrudates, where a good correlation (R^2 = 0.901) was observed (Figure 4.13).

Table 4.9: Starch crystallinity percentage and combined gelatinisation and retrogradation enthalpy of raw materials and extrudates containing 0%, 10%, 40% & 80% chickpea or red lentil flour incorporated at low or high formulation moisture.

Sample		% Starch Crystallinity	Total enthalpy (J/g)
Raw	Potato Starch	46.30	15.08
	Chickpea Flour	22.00	5.24
	Red Lentil Flour	29.30	8.18
Extrudate	Potato Starch Low Moisture	16.30	1.06
	Potato Starch High Moisture	21.10	3.13
	10% Chickpea Low Moisture	20.00	1.78
	10% Chickpea High Moisture	23.90	2.97
	40% Chickpea Low Moisture	18.30	2.47
	40% Chickpea High Moisture	18.10	2.57
	80% Chickpea Low Moisture	22.90	4.19
	80% Chickpea High Moisture	19.10	3.43
	10% Red Lentil Low Moisture	18.55	1.48
	10% Red Lentil High Moisture	22.37	2.93
	40% Red Lentil Low Moisture	22.47	4.23
	40% Red Lentil High Moisture	20.42	3.96
	80% Red Lentil Low Moisture	20.86	5.66
	80% Red Lentil High Moisture	24.50	6.15

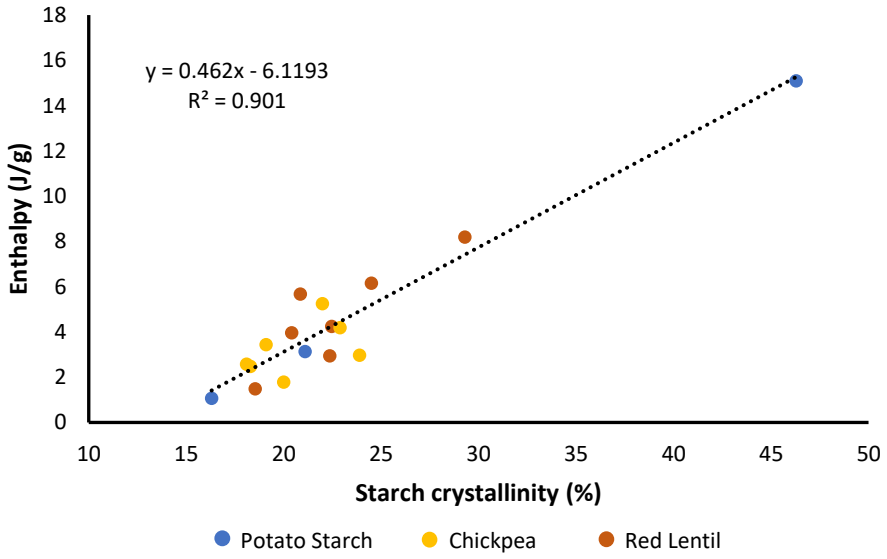

Figure 4.13: Correlation of starch crystallinity and total enthalpy (gelatinisation + retrogradation) in potato starch controls, chickpea (10%, 40, & 80%) and red lentil (10% 40% & 80%) incorporated extrudates at low and high moisture formulations. P-value (Pearson correlation) is < 0.00001; the result is significant at $p < 0.05$.

4.3.6. Microscopy

Microscopy was carried out on extrudates containing 0%, 10%, 40% & 80% chickpea or red lentil flour incorporated at low or high formulation moisture. Changes to the starch granule structure and matrix were examined through iodine staining and viewing through cross polars. Toluidine blue was used to investigate protein and cell wall composition.

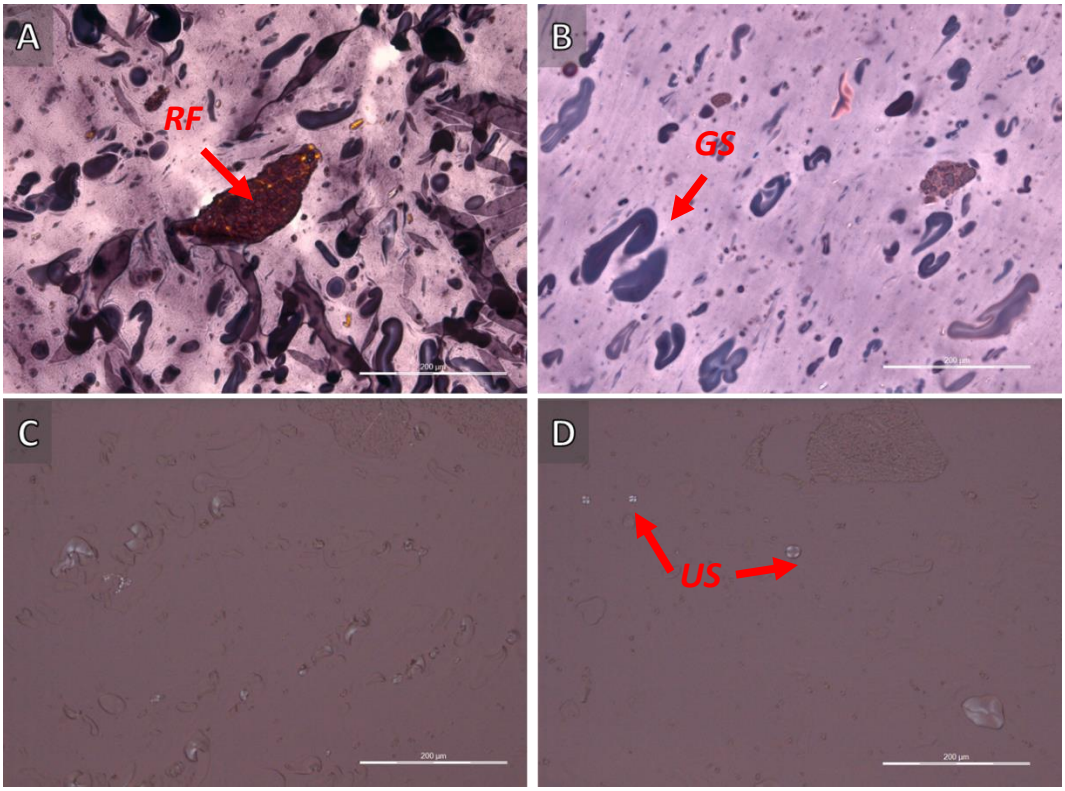

Figure 4.14 Potato starch control extrudates. A) low moisture formulation; iodine stain, red arrow indicates location of rice flour particle B) high moisture formulation; iodine stain C) low moisture formulation viewed between crossed polarisers D) high moisture formulation viewed between crossed polarisers. All images 10x magnification, scalebar 200µm. Illustrating RF: rice flour, GS: gelatinised starch and US: ungelatinised starch.

Native raw potato starch granules are characterised by an oval shape with smooth surface. In the presence of iodine, starch is stained to a deep blue/purple colour. Iodine binding with a 20x greater affinity to amylose producing a deep blue colour; binding to amylopectin is weaker and results in a purple/reddish hue.

When potato starch is extruded gelatinisation occurs, causing a collapse in starch structure and a loss in the smooth surface and oval shape. The potato starch control samples which had been stained with iodine (Figure 4.14, A & B) showed visible intact, swollen and broken starch granules suspended within a clear matrix made up of gelatinised starch. Some small ungelatinised starch granules are also visible within a fragment of rice flour tissue (stained red/orange, indicated with red arrow in Figure 4.14 A). Due to the small starch granule size these particles may be attributable to the small amount of rice flour present in the base formulation. The combination of remaining within an intact cellular structure and the inverse relationship between starch granule size and gelatinisation may account for the lack of gelatinisation of the rice starch. A blue background is observed from leached potato starch polymers forming a continuous matrix, which stains a light blue colour with iodine due to the high amylose content of the leached material, as observed by (Han et al., 2019).

The use of polarised light is effective at showing crystallinity within ungelatinised starch granules. Similar to other optically anisotropic materials, native starch granules exhibit birefringence when illuminated under crossed polarised light, present as a distinctive maltese cross. When starch granules undergo gelatinisation, the semi-crystalline structure is compromised. Lack of crystalline order results in loss of birefringence. Upon viewing potato starch extrudate

sections through crossed polarised light (Figure 4.14, C & D), the majority of the field of view is dark, indicating a lack of crystallinity and a high amount of gelatinised starch. A few granules are illuminated as signified by the presence of maltese crosses.

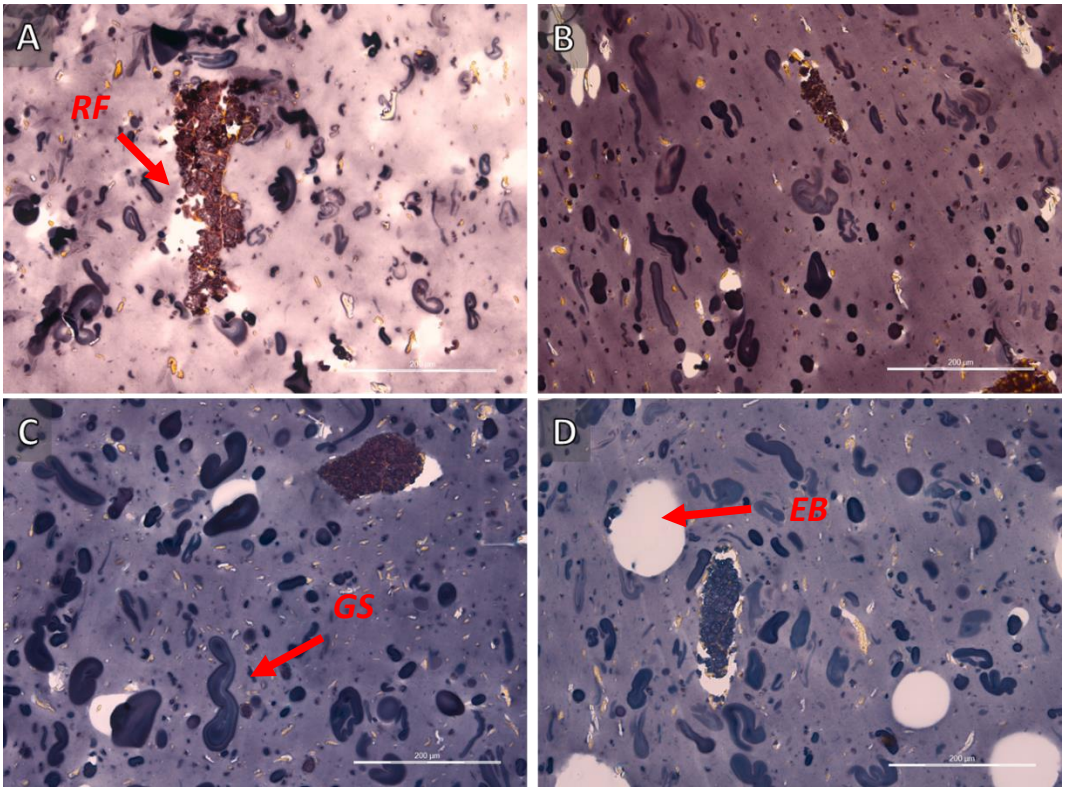

Figure 4.15: 10% pulse incorporated extrudates stained with iodine. A) chickpea enriched low moisture formulation B) chickpea enriched high moisture formulation C) red lentil enriched low moisture formulation D) red lentil enriched high moisture formulation. Brightfield microscopy all images 10x magnification, scalebar 200 µm. Illustrating RF: rice flour, GS: gelatinised starch and EB: expansion bubble.

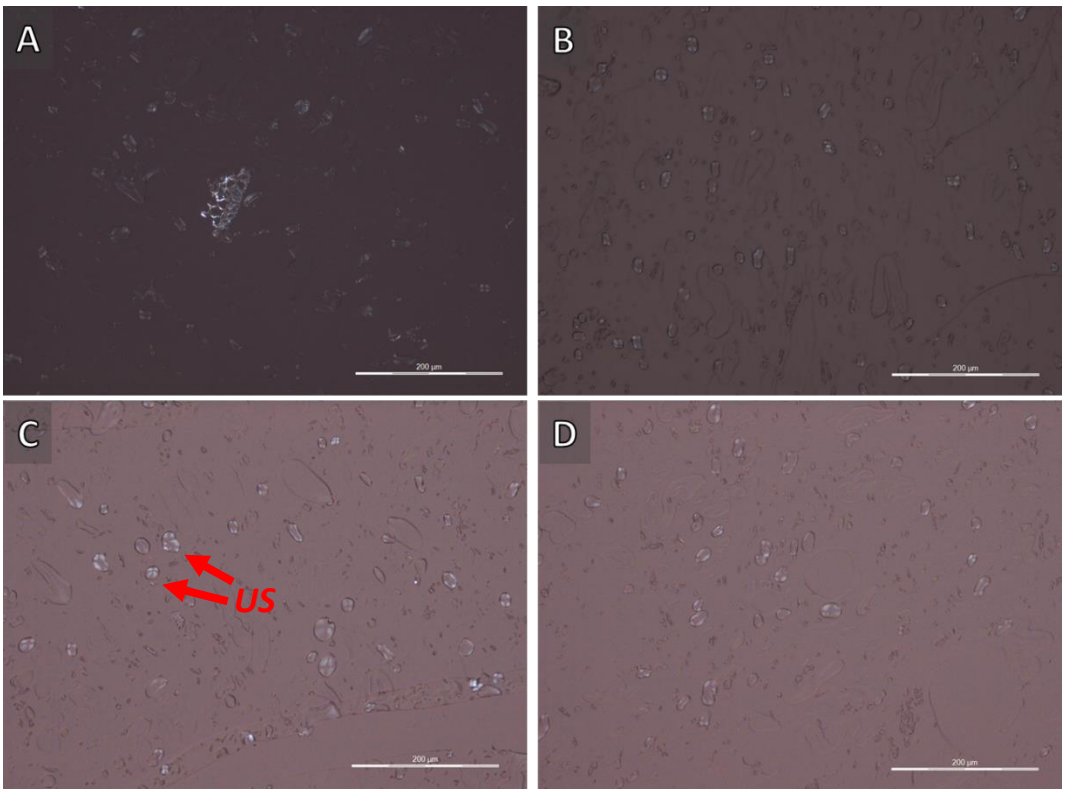

Figure 4.16: 10% pulse incorporated extrudates viewed between crossed polarisers. A) chickpea enriched low moisture formulation B) chickpea enriched high moisture formulation C) red lentil enriched low moisture formulation D) red lentil enriched high moisture formulation. Polarised light microscopy all images 10x magnification, scalebar 200 µm. Illustrating US: ungelatinised starch.

Incorporation of 10% pulse flour resulted in more visible intact starch granules, as shown by iodine staining (Figure 4.15). Incorporating red lentil flour gives rise to a bluer hue than chickpea containing samples. This indicates the presence of more leached amylose in red lentil containing products. In comparison to potato starch control extrudate sections, samples containing 10% chickpea or red lentil had more visible birefringence (Figure 4.16). This indicates that less gelatinisation occurred, which corresponds to previous DSC data in which slightly higher gelatinisation enthalpies were observed for these samples. There was little visible difference between low and high moisture formulations in low pulse incorporated samples.

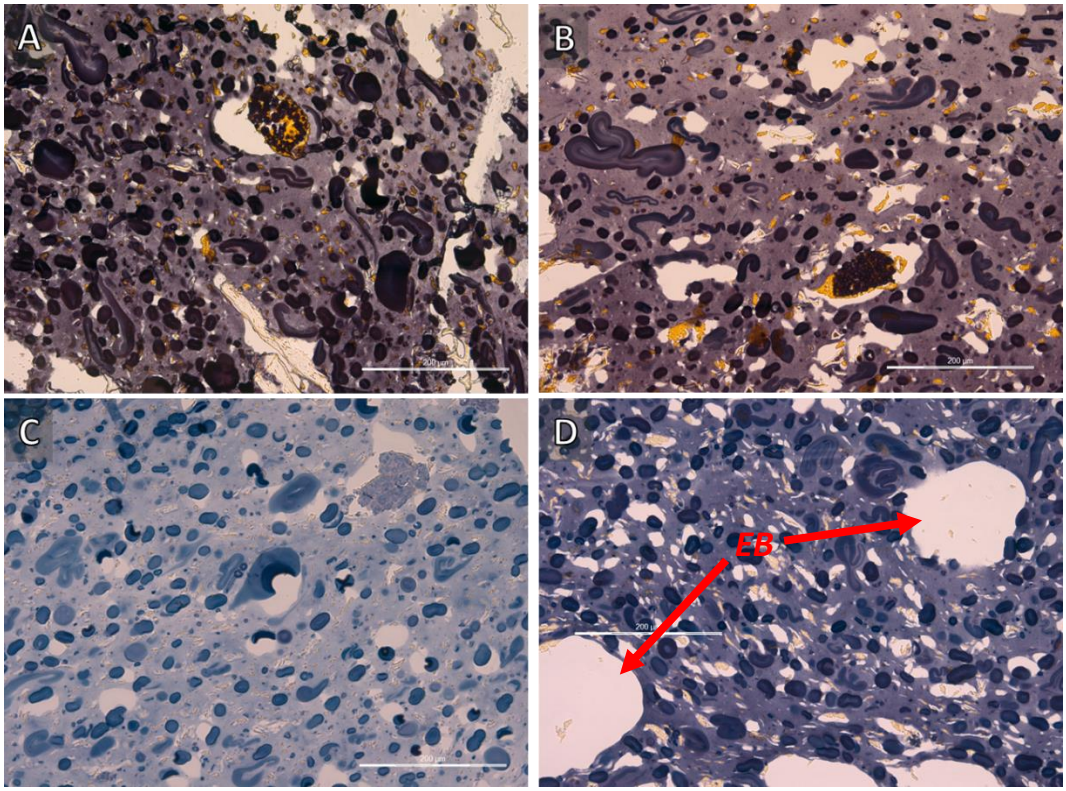

Figure 4.17: 40% pulse incorporated extrudates stained with iodine. A) chickpea enriched low moisture formulation B) chickpea enriched high moisture formulation C) red lentil enriched low moisture formulation D) red lentil enriched high moisture formulation. Brightfield microscopy all images 10x magnification, scalebar 200 µm. Illustrating EB: expansion bubble.

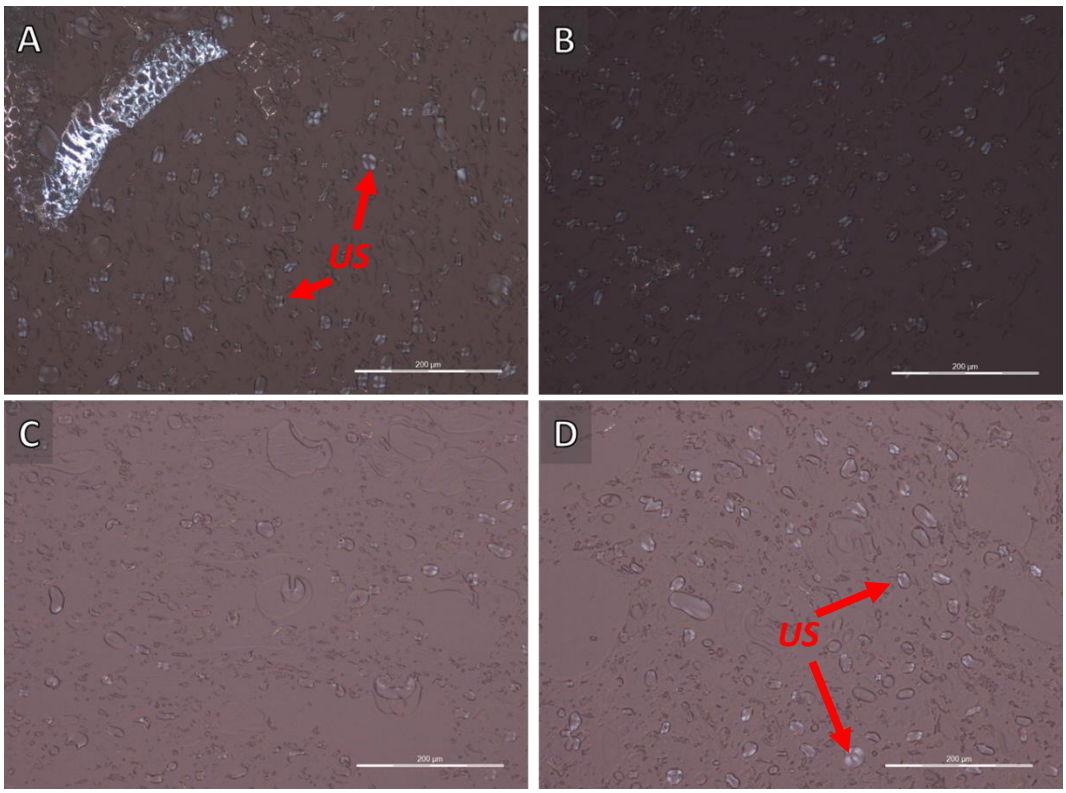

Figure 4.18 40% pulse incorporated extrudates viewed between crossed polarisers. A) chickpea enriched low moisture formulation B) chickpea enriched high moisture formulation C) red lentil enriched low moisture formulation D) red lentil enriched high moisture formulation. Polarised light microscopy all images 10x magnification, scalebar 200 µm. Illustrating US: ungelatinised starch.

Increasing the concentration of chickpea or red lentil flour incorporated into the formulation to 40% resulted in a noticeable reduction in the gelatinised starch matrix (Figure 4.17). Instead, a higher density of ungelatinised starch granules and fragments of non-starch components are visible. This is particularly evident in chickpea containing samples.

This is also shown when viewed under polarised light, with an increase in maltese crosses in comparison to 10% pulse incorporated extrudates and potato starch controls. A high number of ungelatinised starch granules are observed in these 40% samples compared to the 10 and 0% samples, indicated by the high density of maltese crosses (Figure 4.18).

A pulse incorporation concentration of 40% also results in the presence of more pores in the matrix structure. Bigger pores were visible in the high moisture formulation samples. This may be due to slight expansion that occurs during the extrusion process – more water content gives rise to more availability for steam, therefore increase in the number and size of pores. An alternative hypothesis could be that the higher water content results in greater syneresis, as indicated by the higher amount of retrogradation observed in Figure 4.5 for the high formulation moisture samples, which resulted in pores opening during storage. This could be tested by carrying out microscopy analysis of samples immediately following extrusion.

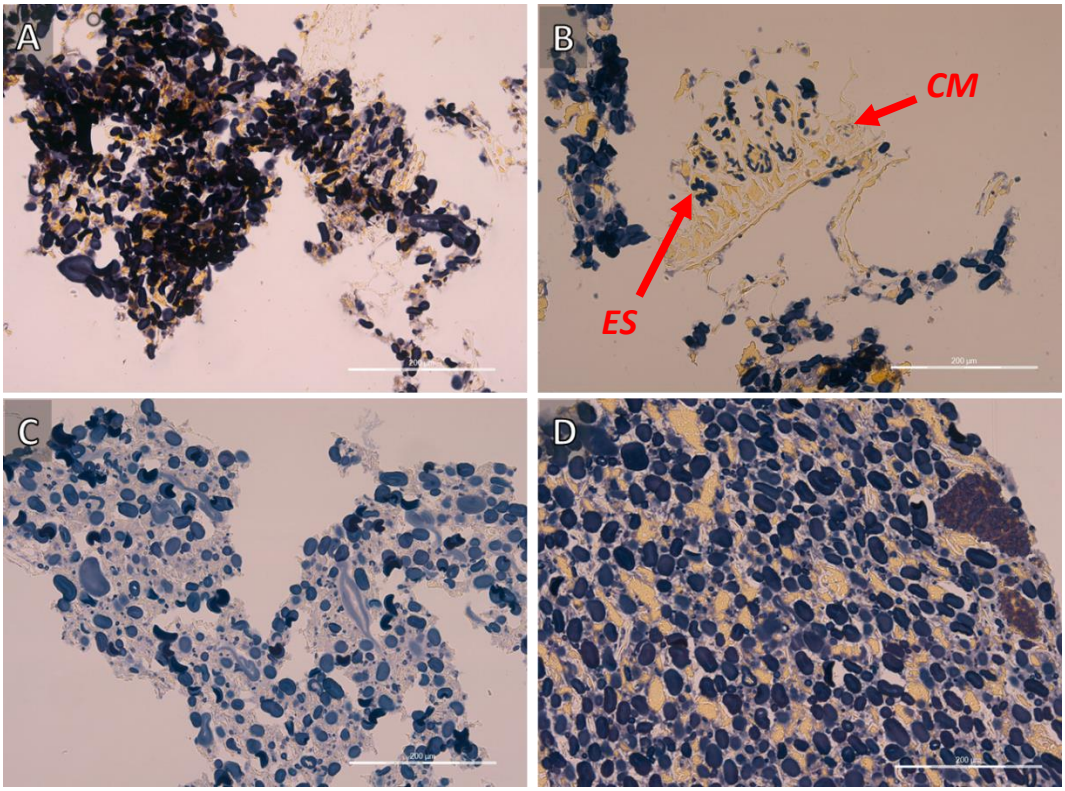

Figure 4.19: 80% pulse incorporated extrudates stained with iodine. A) chickpea enriched low moisture formulation B) chickpea enriched high moisture formulation C) red lentil enriched low moisture formulation D) red lentil enriched high moisture formulation. Brightfield microscopy all images 10x magnification, scalebar 200 µm. Illustrating ES: encapsulated starch and CM: cellular material.

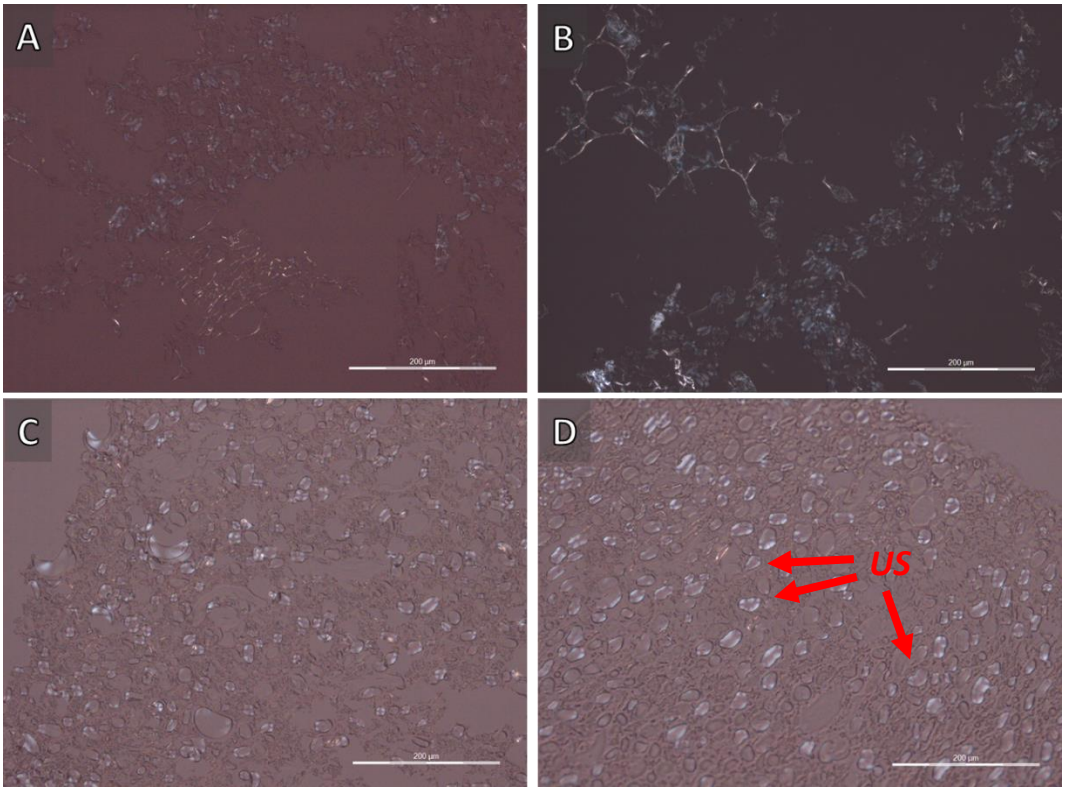

Figure 4.20: 80% pulse incorporated extrudates viewed between crossed polarisers. A) chickpea enriched low moisture formulation B) chickpea enriched high moisture formulation C) red lentil enriched low moisture formulation D) red lentil enriched high moisture formulation. Polarised light microscopy all images 10x magnification, scalebar 200 µm. Illustrating US: ungelatinised starch.

The 80% pulse enriched extrudates showed again an increase in ungelatinised starch granules and tissue fragments. There was a general lack of evidence of a gelatinised starch matrix (Figure 4.19).

The increase in pulse incorporation also resulted in difficulty in sectioning the samples, in particular the chickpea samples. This is shown by a visible break down in structure, due to the brittle samples. In the chickpea 80% samples, particularly clearly in the high formulation moisture sample, encapsulated starch granules are visible within intact chickpea cellular structures.

Red lentil samples were easier to section due to the greater matrix integrity of these samples. More visible gelatinised starch is observed, particularly in the low formulation moisture samples. At high formulation moisture, less matrix is visible as the section is more taken up by whole starch granules and non-starch components. When samples were viewed under polarised light microscopy (Figure 4.20) large numbers of partially gelatinised or ungelatinised starch granules could be observed, reflecting the limited gelatinisation of these samples. The DSC results suggested that there was less gelatinisation in the red lentil samples at 80% compared to the chickpea samples. In the microscopy images it appears that there may be more birefringence in the red lentil samples, but a direct comparison is difficult due to the challenging nature of the chickpea sectioning resulting in less intact sections.

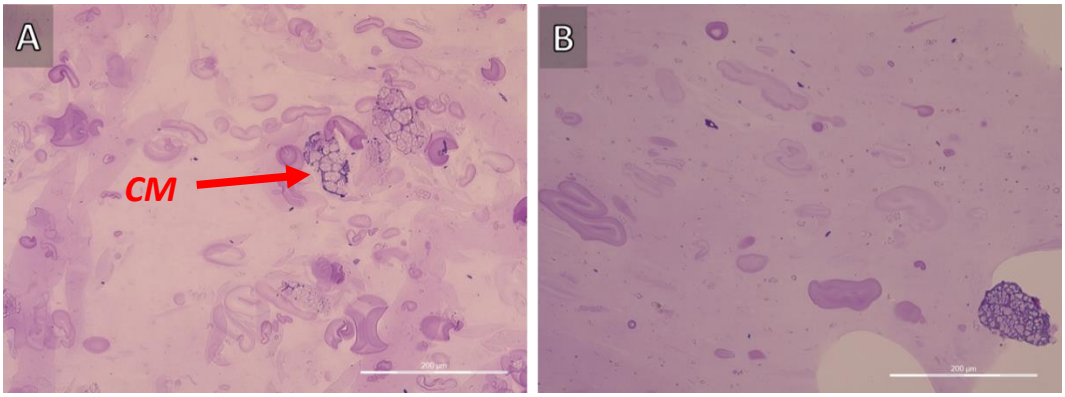

Figure 4.21: Potato starch control extrudates stained with toluidine blue. A) low moisture formulation B) high moisture formulation. Brightfield microscopy 10x magnification, scalebar 200 µm. Illustrating CM: cellular material.

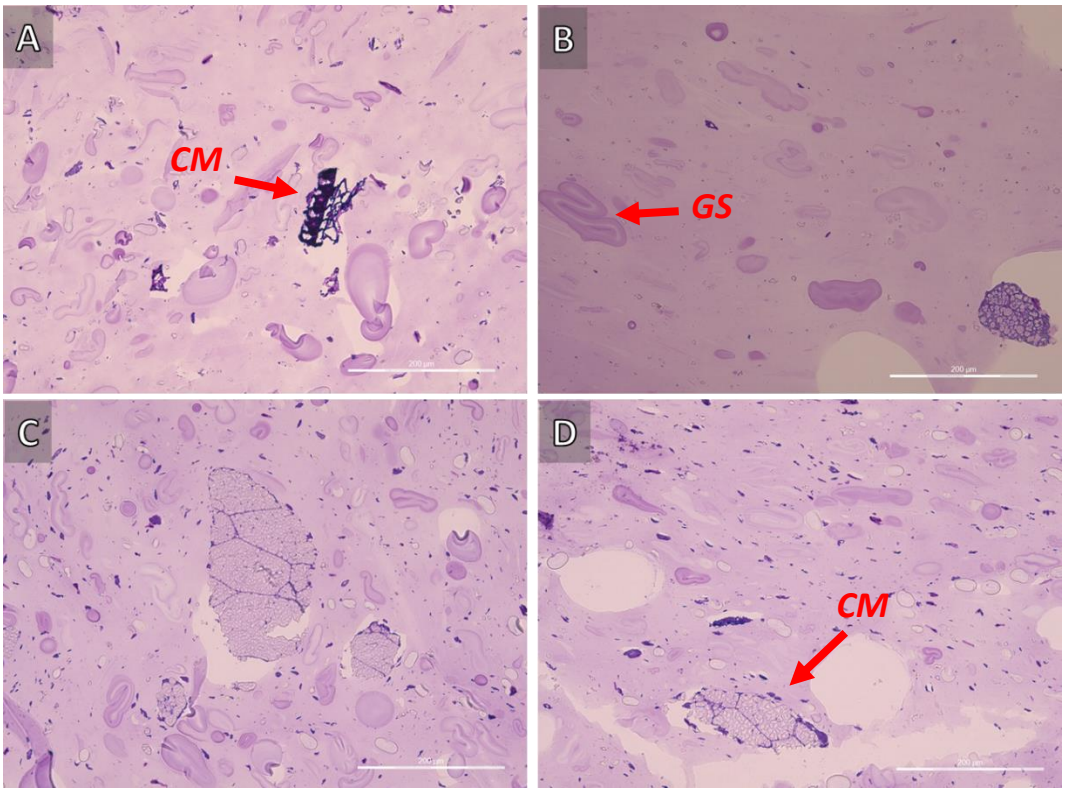

Figure 4.22: 10% pulse incorporated extrudates stained with toluidine blue. A) chickpea enriched low moisture formulation B) chickpea enriched high moisture formulation C) red lentil enriched low moisture formulation D) red lentil enriched high moisture formulation. Brightfield microscopy all images 10x magnification, scalebar 200 µm. Illustrating CM: cellular material and GS: gelatinised starch.

Staining with toluidine blue highlights the presence of protein and cell wall structures. Potato starch only controls shows a small amount of cell wall components, explained by the presence of rice flour within the formulation (Figure 4.21). 10% pulse incorporation has more non-starch components visible, and cell wall fragments can be seen in the chickpea sample sections (Figure 4.22). In red lentil samples, small particles of non-starch are observed to be uniformly mixed within the matrix. A further increase in cell wall and protein is shown when 40% pulse is incorporated (Figure 4.23). Larger structures are clumped together in chickpea containing samples, whereas red lentil samples have a more uniform spread, mixed into the gelatinised starch matrix.

At 80% incorporation, for the chickpea samples whole intact cellular structures can be observed within the extrudates (Figure 4.24). For the 80% red lentil samples large amounts of non-starch cell wall and protein material can be observed, but it is more uniformly spread throughout the matrix of the extrudate. In the red lentil sample at high formulation moisture (Figure 4.24 D) larger clumps of protein and cell wall were observed than in the equivalent red lentil sample at low formulation moisture (Figure 4.24 C). This may be due to the difference in shear experienced by the samples during extrusion where the high formulation moisture sample was more lubricated and therefore underwent lower shear, resulting in large clumps of protein and cell wall material.

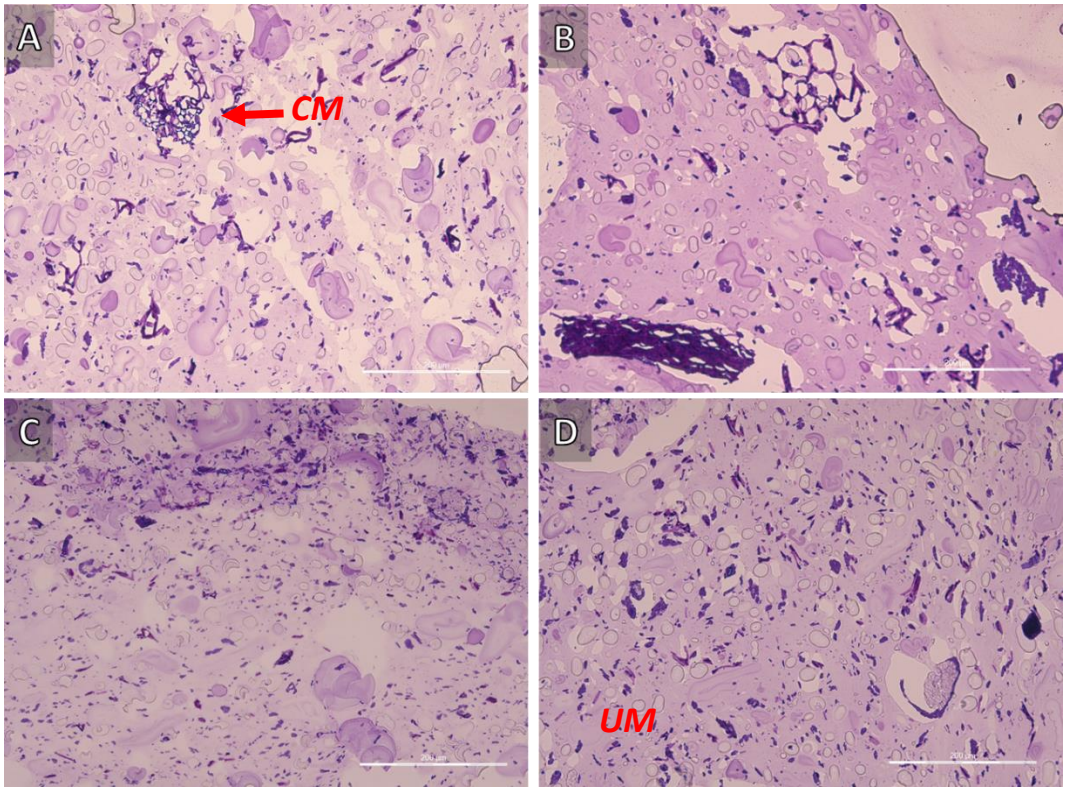

Figure 4.23: 40% pulse incorporated extrudates stained with toluidine blue. A) chickpea enriched low moisture formulation B) chickpea enriched high moisture formulation C) red lentil enriched low moisture formulation D) red lentil enriched high moisture formulation. Brightfield microscopy all images 10x magnification, scalebar 200 µm. Illustrating CM: cellular material and UM: uniform matrix.

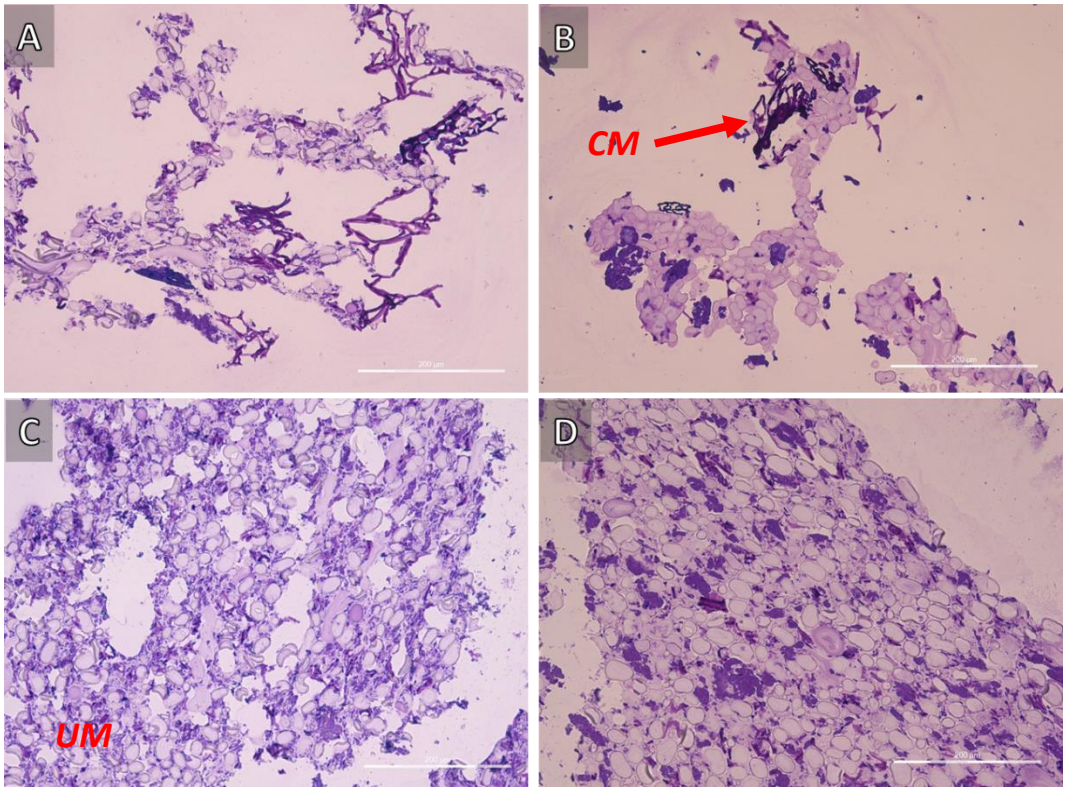

Figure 4.24: 80% pulse incorporated extrudates stained with toluidine blue. A) chickpea enriched low moisture formulation B) chickpea enriched high moisture formulation C) red lentil enriched low moisture formulation D) red lentil enriched high moisture formulation. Brightfield microscopy all images 10x magnification, scalebar 200 µm. Illustrating CM: cellular material and UM: uniform matrix.

4.4. Summary and conclusions

In this chapter, a series of procedures were carried out in order to investigate the impact of pulse flour incorporation and formulation moisture on gelatinisation and crystallinity of third generation extruded and expanded samples.

Gelatinisation behaviour is a significant factor in starch containing food products. In order to assess the effect that incorporating pulse flour or different moisture levels had on different stages of snack processing, the gelatinisation temperature and enthalpy of raw potato starch, chickpea flour and red lentil, extrudates and expanded snacks was determined using DSC in excess moisture conditions. It was found that pulse incorporation decreased the amount of gelatinisation occurring during processing, an effect observed in both extrudates and expanded snacks. In contrast, formulation moisture had no significant effect on gelatinisation. It is proposed that this is due to differences in residence time within the barrel during the extrusion process.

Limited further gelatinisation occurred during the expansion process, when expanded samples were compared to the extruded samples. This was true for all low moisture samples and 10% pulse incorporated samples. This indicates that a low amount of gelatinisation is sufficient for expansion of extrudates at low pulse incorporation levels. At higher pulse incorporation levels, more gelatinisation was observed during the expansion process.

Retrogradation is an indicator of starch behaviour upon storage, and can be determined by DSC. High formulation moisture samples were found to undergo more retrogradation, particularly at low pulse incorporation levels. This is due to

the higher amounts of water allowing for more starch chain mobility which leads to higher levels of retrogradation.

Starch crystallinity of raw materials and extrudates was determined using powder x-ray diffraction. High formulation moisture samples had higher amounts of starch crystallinity. This may be due to the increased retrogradation previously observed by DSC. Water content was not a direct factor in the XRD measurements as all the samples had moisture contents normalised immediately prior to analysis, so differences in XRD patterns reflect differences in starch structure, rather than intrinsic moisture content of the samples.

The amount of enthalpy of gelatinisation and retrogradation positively correlated with the percentage of starch crystallinity detected by XRD (Figure 4.13), showing that for extruded mixtures of starches this relationship is true, as it has been demonstrated in the literature for purified starches (Warren et al., 2016). It was also observed that samples in which a high amount of retrogradation occurred such as 10% high moisture extrudate samples resulted in higher crystallinity. At 40% pulse incorporation there was a minimal amount of retrogradation at high formulation moisture, and there was also a lack of gelatinisation occurring in 40% low moisture samples which would increase starch crystallinity. This therefore led to a similar crystallinity overall at 40% pulse incorporation for the low and high moisture samples, where in the low moisture samples crystallinity mainly arose from ungelatinised starch, whereas in the high moisture samples the crystallinity was mainly due to retrogradation.

There were differences observed in gelatinisation at 40% between chickpea and red lentil (Table 4.5) where red lentil had undergone less gelatinisation which

was reflected in the higher starch crystallinity percentage observed in red lentil extruded samples. This difference in crystallinity may reflect that the red lentil flour had a higher gelatinisation temperature than the chickpea flour (Table 4.2). Indeed, given the barrel temperature of the extruder set at 80°C, at this temperature chickpea flour gelatinisation is almost complete (conclusion temperature of 83.3°C), while red lentil flour would only be partially gelatinised at this temperature (conclusion temperature 96.4°C). While other factors (shear, moisture content) will also be important in the extrusion system, this difference in gelatinisation temperature range may explain the differences observed in crystallinity between the red lentil and chickpea samples which impact their functionality.

In conclusion, in this chapter it has been demonstrated that under 3rd generation extrusion conditions, pulse flour does not fully gelatinise, unlike potato starch. This is due to a combination of the higher gelatinisation temperature of the pulse flour and the different water holding capacities of the pulse flours. The incomplete gelatinisation of the pulse flour impacts on the functionality of the extruded product as it reduces the formation of a continuous starchy matrix as observed by light microscopy. Depending on the moisture content of the extrudates it was also found that retrogradation occurring during storage could contribute to the crystallinity of the extruded products, although retrograded starch was almost completely melted during expansion.

5. Starch digestion kinetics of extruded and expanded prototypes

5.1. Introduction

The main research aim of the project was to assess the starch digestibility of third generation extruded snacks, and to investigate how incorporation of chickpea or red lentil flour processed under different moisture regimes affected digestibility. Starch bioavailability of starches found in pulse flours such as chickpea and red lentil flour is low in comparison to cereal or tuber sources, due to the intrinsic structural differences in pulse starches, high amylose content, and high quantity of soluble dietary fiber components (Hoover & Sosulski, 1991; Hoover & Zhou, 2003). Processing through cooking or mechanical means increases bioavailability and bioaccessibility of pulse starches, similarly to other starches.

Gelatinisation effects starch digestibility as the granules swell and have an increased amorphous structure allowing for greater accessibility of amylolytic enzymes. Conversely, high starch crystallinity influences starch digestibility through reduced enzyme bioaccessibility (Baldwin et al., 2015; Tester et al., 2004; Warren et al., 2011). Pulse starches have a number of intrinsic structural features which make them more resistant to digestion. They have a higher gelatinisation temperature meaning that the starch crystalline structure is more likely to be retained during hydrothermal processing (Chapter 4). This is in part due to their higher amylose content relative to other starches which resists enzyme breakdown through maintaining granular integrity (Jane, 2006). Amylopectin in pulses contains a smaller proportion of DP 6-12 short branch chains, and a larger proportion of DP13-24 branch chains which therefore results in a more ordered crystalline structure which may also be resistant to

hydrothermal treatment, maintaining crystalline structure during cooking and reducing starch digestibility (Li et al., 2019).

Further to intrinsic starch structure, where the cellular structure is maintained the starch digestibility in pulses can also be limited by digestive enzyme accessibility. The rigid cell wall structure and the dense pulse protein matrix can block accessibility of the starch for digestive enzymes (Edwards, Ryden, et al., 2021; Hafiz et al., 2022; Xiong et al., 2018). In Chapter 4 it was observed that for the chickpea samples there were still intact cellular structures, while for the red lentil samples the cell walls were completely disintegrated.

Second generation extrusion under high temperature conditions has been shown to dramatically increase the digestibility of legume starches through a near complete disruption of the granular crystalline structure of the starch, with a dramatically increased ileal digestion in an animal model of extruded legume starches compared to the raw ingredients (Sun et al., 2006). This has also been suggested to be due to the inactivation of endogenous proteinaceous α-amylase inhibitors by the extrusion process, which can further enhance starch digestion (Alonso et al., 2000b). However, starch digestion of legumes subjected to third generation extrusion processing has not been investigated to date.

An *in vitro* starch digestibility assay utilizing α-amylase as the sole starch hydrolysing enzyme was used in order to assess starch digestibility of raw potato starch, chickpea flour, red lentil flour and snacks at different stages of processing, produced under different formulation moisture regimes and with a range of different levels of pulse flour incorporation. This will reveal the impact

of pulse incorporation on starch digestibility in pulse flour based snacks, with potential implications for the glycaemic index of these snack products.

5.2. Materials & Methods

5.2.1. Materials

All raw ingredients and chemical reagents used in this chapter are described in Chapter 2.1.

5.2.2. Starch digestibility assay

Starch digestion kinetics analysis was carried out on the raw materials, extrudates and expanded products using the protocols described in Chapter 2.3 and 2.4. The only change made to this protocol was for the raw ingredients, where 150 minutes of digestion was found to not be sufficient to allow the digestion to plateau, therefore the digestion period was extended to 330 minutes.

5.2.3. Powder X-Ray diffraction analysis

XRD analysis was conducted to determine the degree of crystallinity in the raw materials and the residual crystallinity following processing in the extruded and expanded products. XRD analysis was conducted as described in Chapter 2.12.

5.2.4. Differential Scanning Calorimetry

DSC analysis was carried out to determine thermal transitions present in the raw materials, extrudates and expanded products under heating in excess moisture conditions. DSC analysis was carried out using an MCDSC Instrument (TA Instruments) as described in Chapter 2.11.2.

5.2.5. Water holding capacity

The water holding capacity of the expanded snack products was used to determine if water holding/binding had an impact on the starch digestion kinetic

assay due to the high water holding capacity of the expanded snacks. The water holding capacity was determined as described in Chapter 2.6.

5.2.6. Specific volume

Specific volume was carried out to determine the change in expansion according to different formulations. Specific volume analysis was carried out as described in Chapter 2.8.

5.3. Results

5.3.1. Starch digestibility of raw potato starch, chickpea, and red lentil flour

Starch digestibility kinetics of raw materials potato starch, chickpea flour and red lentil flour were assessed using an *in vitro* starch digestibility assay based on amylase as the sole enzyme. The final timepoint was selected to be taken at an extended time of 330 minutes due to the low digestibility of raw starches based on preliminary experiments to determine the digestion rate of the raw materials. These samples were analysed in order to provide a baseline for further processed samples.

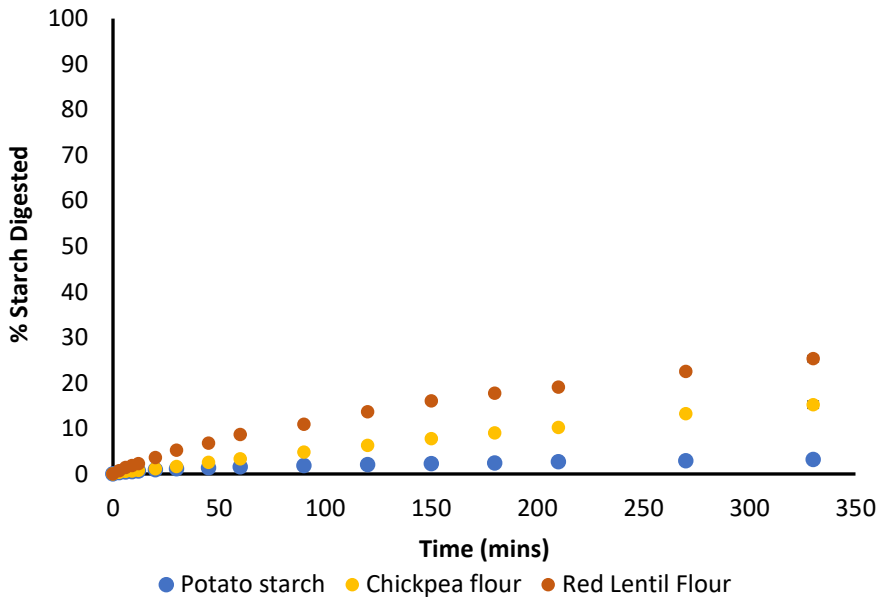

Figure 5.1: In vitro starch digestibility of raw materials up to 330 minutes. Analysis was carried out in triplicate.

In vitro starch digestibility of all three raw materials was shown to be low, with none of the samples reaching beyond 30% starch digestion in the time course of the experiment (Figure 5.1). Raw potato starch digested the least, with 3% digested at the 330 minute timepoint. Chickpea flour was slightly more digestible, reaching 15% starch digested at the end of the assay. Of the three raw materials, red lentil flour was found to be the most digestible by amylase, and reached 25% at the 330 minute timepoint. (Ma et al., 2017) Due to the low digestion rates of the raw materials under the selected digestion assay conditions, LOS plot analysis could not be used to accurately determine kinetic parameters. The low digestibility of the starches in these raw materials is comparable to that observed in comparable studies, and reflects the limited digestion of native starch granules (Bhattarai et al., 2017; Dhital et al., 2017; Edwards, Veerabahu, et al., 2021).

5.3.2. Starch digestibility of extrudates

Following analysis of raw materials, starch digestibility of extrudates was assessed. This was to understand the impact that the extrusion process had on starch digestibility.

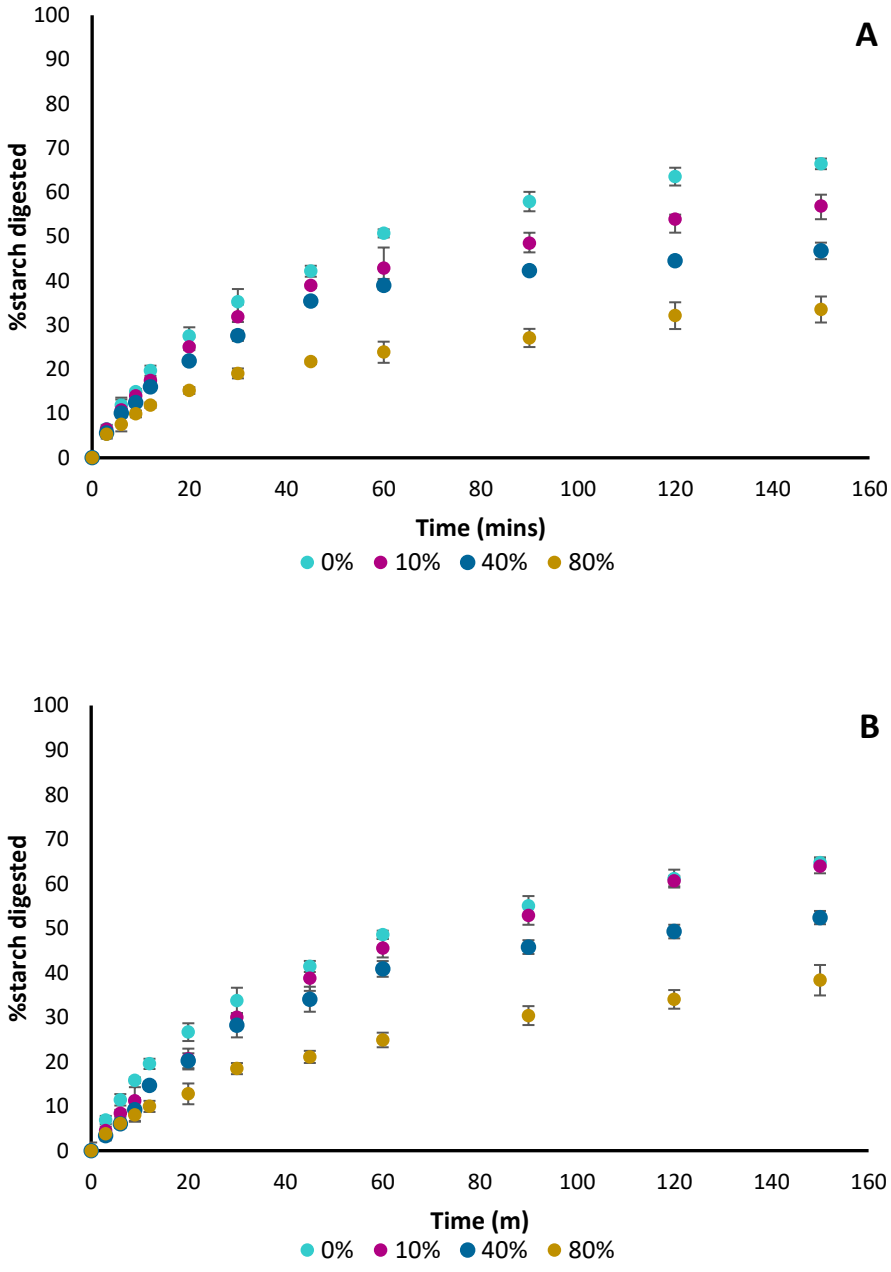

Figure 5.2: Starch digestibility of 3rd generation extrudates composed of potato starch and enriched with 10%, 40 and 80% chickpea flour A) low formulation moisture B) high formulation moisture. Analysis was carried out in triplicate.

Starch digestibility of third generation extrudates was measured for 150 minutes (Figure 5.2 & Figure 5.3). This final timepoint was determined as beyond 150 minutes starch digestibility has plateaued. All of the extrudate samples followed a similar pattern where starch was digested rapidly in the beginning (0 – 20 minutes) then slowed down until finally plateauing, as would be anticipated for data obeying *pseudo*-first order kinetics. Control samples containing no incorporated pulse flour were found to digest the most rapidly, reaching 66% starch digested in low formulation moisture samples and 65% starch digested in high formulation moisture samples

The chickpea incorporated low formulation moisture extrudates (Figure 5.2 A) had a lower digestibility than the potato starch only control. Digestibility decreased with increasing concentration of chickpea flour incorporation. 10% chickpea incorporation resulted in a 10% decrease (~57% starch digested at 150 minutes) in digestibility at 150 minutes in comparison to the potato starch only control. 40% chickpea flour incorporation resulted in a further 10% decrease (~47% starch digested at 150 minutes) in comparison to 10% incorporation. 80% chickpea flour incorporation resulted in the lowest amount of starch digestibility with ~34% digested at 150 minutes.

Chickpea flour containing extrudates which had been processed at a high formulation moisture also resulted in a decrease in starch digestibility (Figure 5.2 B). Unlike the low formulation moisture extrudates, there was a very small decrease in digestibility between 10% chickpea flour incorporated high formulation moisture extrudates and potato starch control high formulation moisture extrudates, with a <1% difference. An increase in chickpea flour incorporation to 40% resulted in a similar decrease to low formulation moisture

extrudate samples, a decrease of 12% starch digestibility at the 150 minute timepoint was observed. 80% chickpea flour incorporation again resulted in the lowest degree of starch digestion, 38% was digested at 150 minutes.

In comparison to low formulation moisture extrudates, high formulation moisture samples which had chickpea flour incorporated had slightly higher starch digestibility.

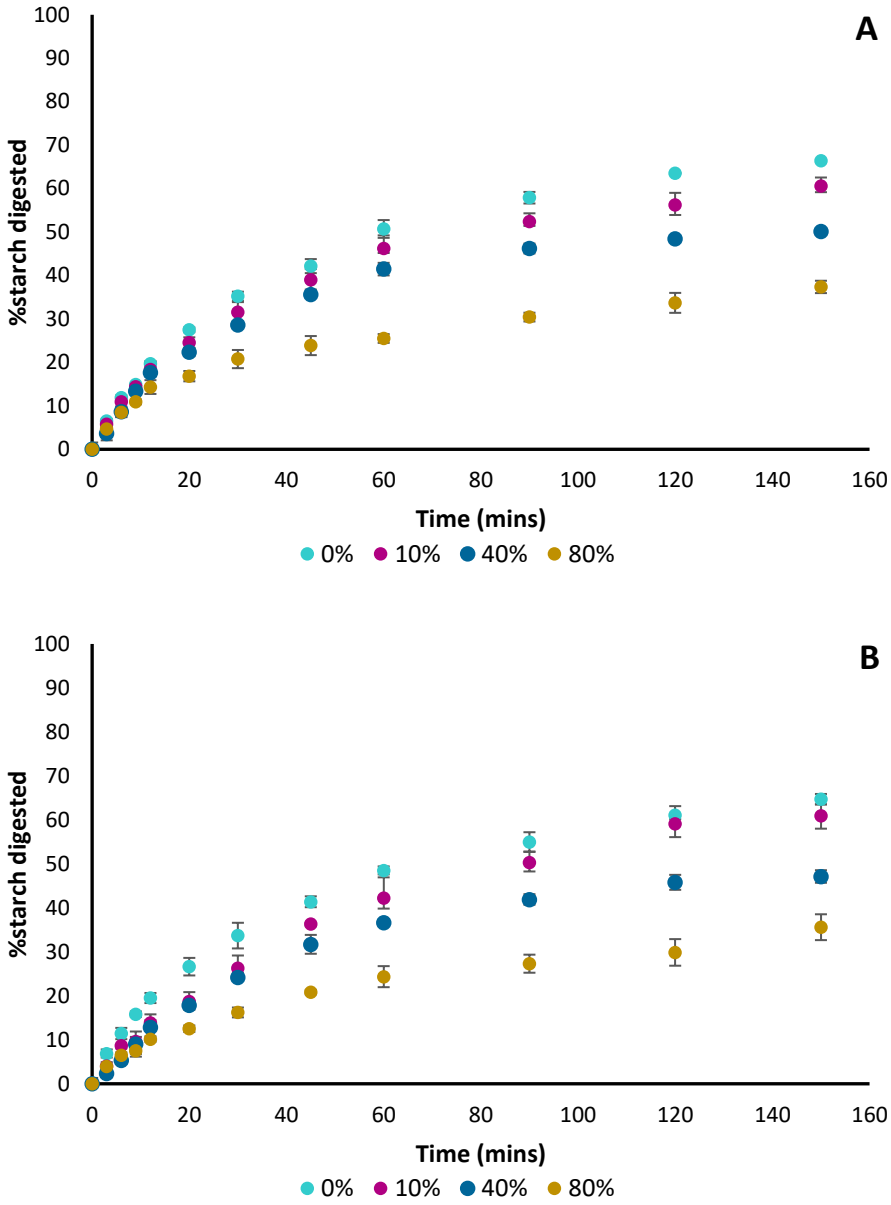

Figure 5.3: Starch digestibility of 3rd generation extrudates composed of potato starch and enriched with 10%, 40 and 80% red lentil flour A) low formulation moisture B) high formulation moisture. Analysis was carried out in triplicate.

Extrudate samples with 10, 40 & 80% red lentil flour incorporated (Figure 5.3) followed a similar trend to chickpea flour (Figure 5.2) incorporated samples. The potato starch control sample digested the most easily in comparison to red lentil enriched extrudates at both low and high formulation moistures. At both low and high formulation moisture, there was a decrease in starch digestibility as the percentage of red lentil incorporation increased.

A 10% red lentil flour incorporation was found to decrease digestibility by ~5% in comparison to potato starch only controls in both low and high formulation moistures. Comparably to chickpea enriched extrudates, 40% red lentil enriched low formulation moisture extrudates also decreased starch digestibility by a further 10% in comparison to 10% red lentil incorporated extrudates. At the 150 minute timepoint 50% of starch had digested. In high formulation moisture 40% red lentil enriched extrudates, 47% starch was digested at the 150 minute timepoint, a 13% decrease in digestibility in comparison to 10% red lentil incorporation at high formulation moisture. The lowest percentage of starch digestibility was observed in the 80% red lentil incorporated extrudates in both low and high formulation moistures. ~37% was digested in the low formulation moisture extrudates and ~35% was digested in the high formulation moisture extrudates. In comparison to low formulation moisture extrudates, a lower percentage of digestibility was observed in high formulation moisture extrudates.

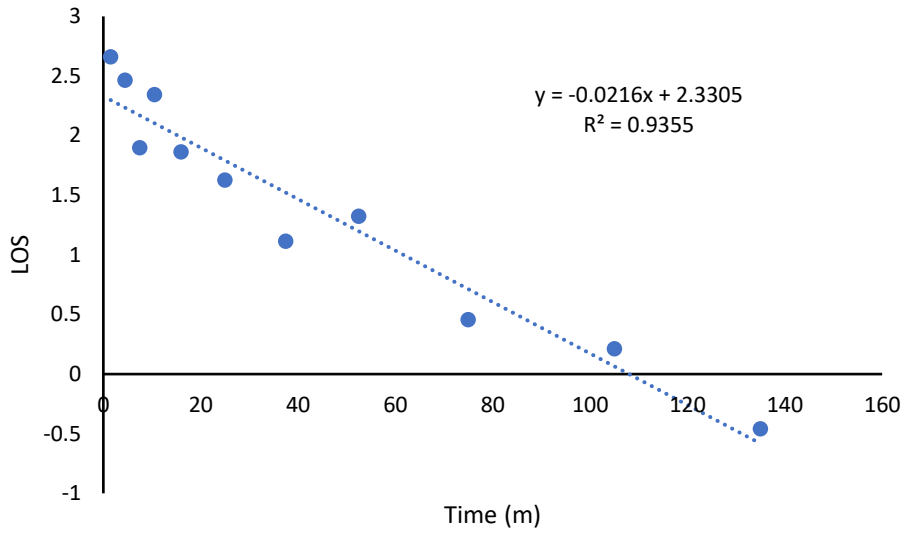

Figure 5.4: Representative LOS plot of starch digestibility of potato starch extrudate formulated at low moisture.

LOS plots were constructed in order to analyse the starch digestibility kinetics of extrudate starch digestion (Figure 5.4). The data do not show any breaks or discontinuities, and a single linear correlation could be fitted to the data demonstrating that the starch was digested at a single rate, and there was no need to fit multiple rate constants. This trend was observed for all extrudate and expanded samples analysed.

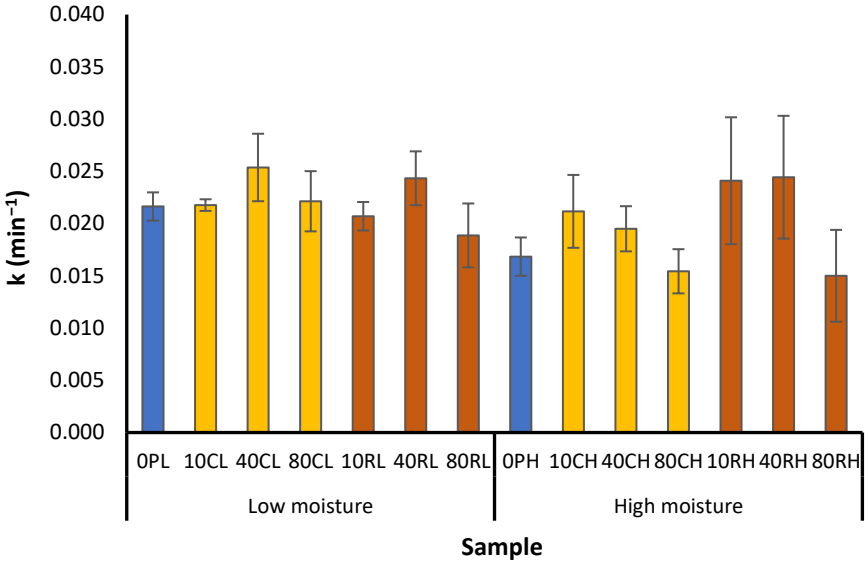

Figure 5.5: Calculated rate of digestion of 3rd generation extrudates composed of potato starch (P) and enriched with 10%, 40 and 80% chickpea (C) or red lentil (R) flour, at low and high formulation moisture levels. Analysis was carried out in triplicate. No significant difference in rate was observed between samples, determined by one way ANOVA.

The rate of starch digestion was calculated using LOS plots for each of the extrudates (Figure 5.5). There was no significant difference observed in digestion rate constant between any of the samples analysed, although there was a slight trend towards lower digestion rate constants at higher levels of pulse incorporation.

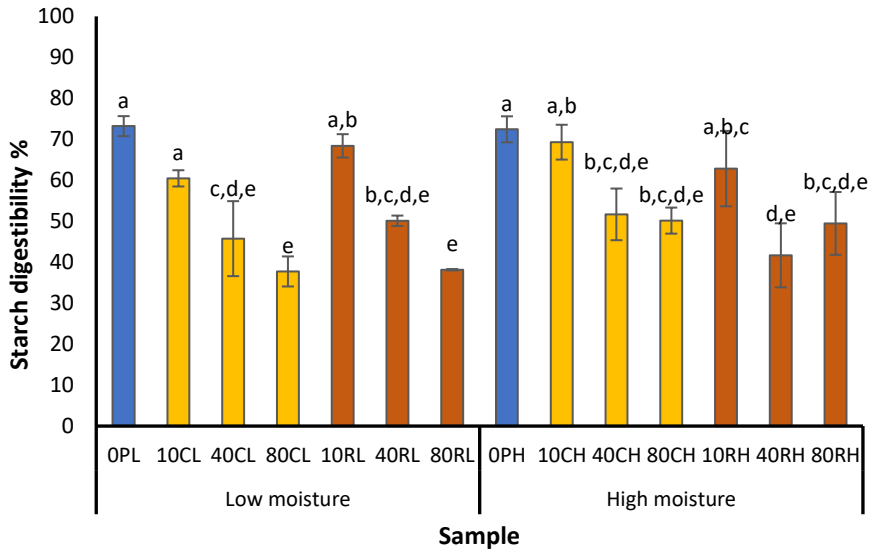

Figure 5.6: Calculated starch digestibility end point of 3rd generation extrudates composed of potato starch (P) and enriched with 10%, 40 and 80% chickpea (C) or red lentil (R) flour, at low and high formulation moisture levels. Analysis was carried out in triplicate. Different letters denote significance ($P < 0.05$) determined by one way ANOVA.

The C_∞ was determined for all extrudate samples. In comparison to the potato only control sample, the 10% chickpea and red lentil samples did not show a statistically significant reduction in C_∞ values. The 40% and 80% pulse incorporation samples had significantly lower C_∞ values than the potato only control sample at high and low moisture, demonstrating that these samples were digested to a significantly lower extent. At low formulation moisture, the 80% pulse incorporated samples were also significantly less digestible than the 10% pulse incorporated samples, for both red lentil and chickpea. Although a similar trend was observed in the high formulation moisture samples, the difference between 10% and 80% pulse incorporation was not statistically significant.

The low formulation moisture chickpea incorporated samples exhibited slightly lower C_∞ values in comparison to equivalent red lentil incorporated samples at

10% and 40%, although this was not statistically significant. This was not observed in the high moisture samples, where no clear trend was observed between the red lentil and chickpea samples.

5.3.3. Starch digestibility of expanded snacks

The starch digestibility of extrudates which had been expanded in an air fryer was assessed using the same starch digestibility assay. It was important to analyse the digestibility of expanded snacks as they are the final product which will be consumed and their structure will be altered by the expansion process which may alter their digestibility relative to extruded products.

Due to the large difference in water holding capacity of expanded snacks in comparison to extrudate samples, *in vitro* starch digestibility assays in which the volume of PBS was adjusted for the water holding capacity of each individual sample were carried out (Figure 5.7) as it was hypothesised that water bound to the expanded snack product would not be available for the digestion reaction.

This resulted in a drop in digestibility of the expanded snacks relative to extrudates, which was unexpected for expanded snacks which would be expected to digest at the same rate or faster than the extrudates. The *in vitro* starch digestibility assay was repeated with a standard volume of PBS (Figures 5.8 & 5.9). This resulted in an increase in the percentage of starch digested, with data more aligned with the extrudate data. From the results of the moisture corrected analysis, it was determined that adjusting the volume of PBS used during the starch digestibility assay resulted in less accurate data, as the water that was bound to the expanded snack was still available to take part in the digestion reaction and to act as a diluent for the generated maltose.

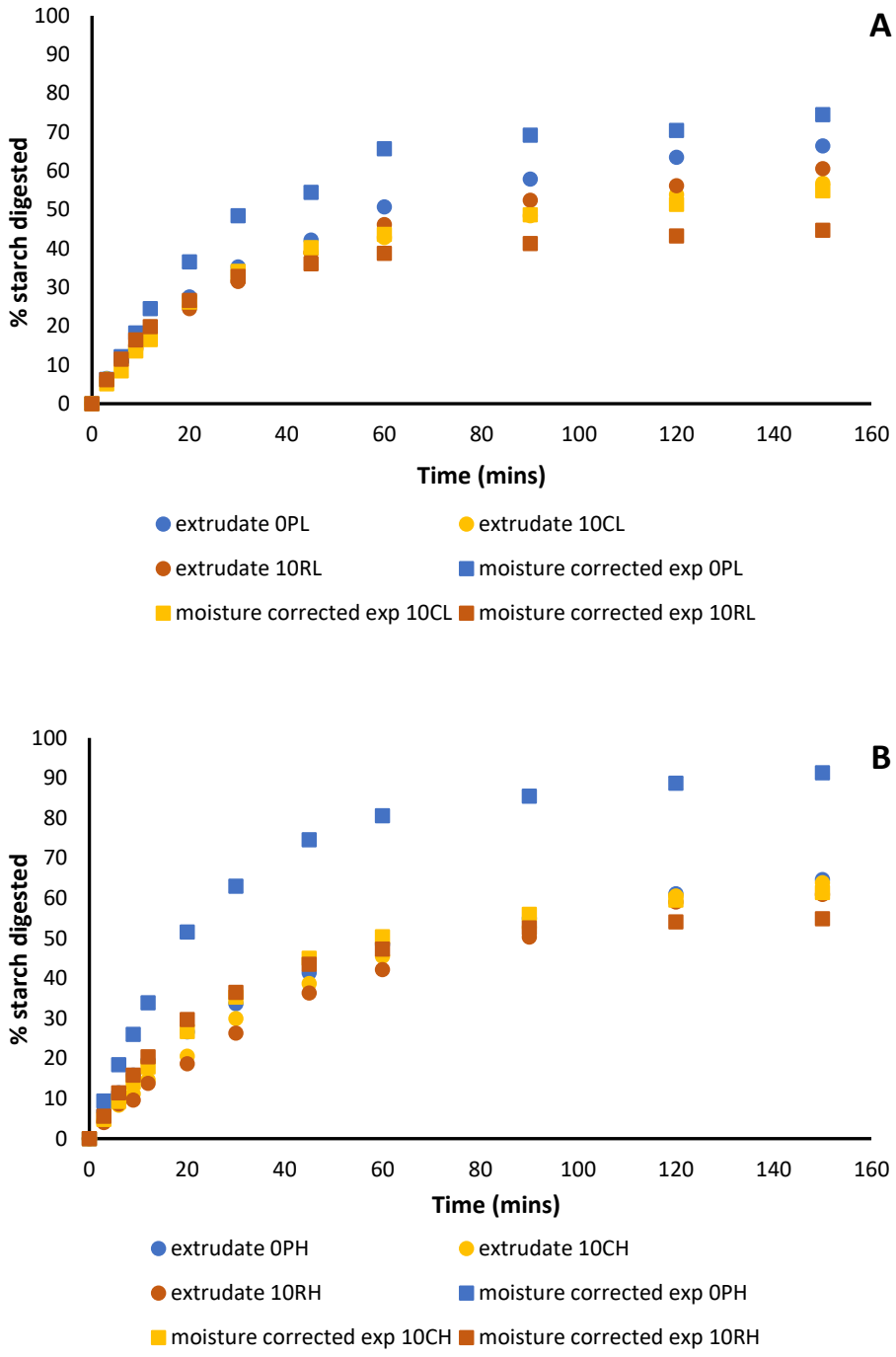

Figure 5.7: Starch digestibility of extrudates composed of potato starch (P) and enriched with 10% chickpea (C) or red lentil (R) flour and expanded snacks digested in vitro *with PBS added according to water absorption capacity. A) low formulation moisture B) high formulation moisture. Analysis was carried out in triplicate.*

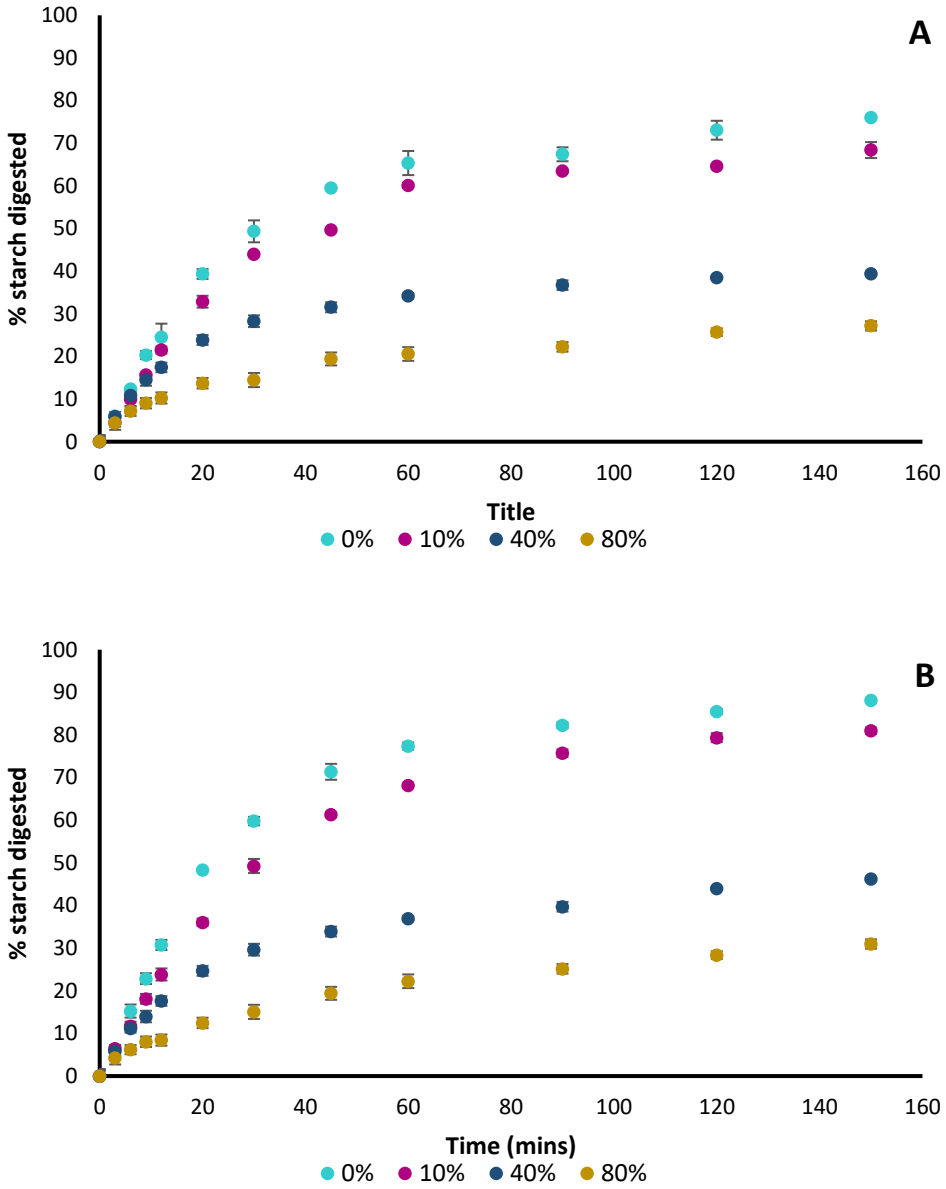

Figure 5.8: Starch digestibility of expanded snacks composed of potato starch and enriched with 10%, 40% and 80% chickpea flour A) low formulation moisture B) high formulation moisture. Analysis was carried out in triplicate.

The starch digestibility of legume enriched snacks expanded at 210°C using an air fryer was assessed (Figure 5.8 & Figure 5.9). This was carried out in order to understand how the expansion of the structure in legume flour incorporated snacks affected starch digestibility.

Potato starch controls exhibited higher starch digestibility in expanded snacks in comparison to extrudates at both low and high formulation moisture. The low moisture potato starch control had a starch digestibility of 76% at the 150 minute timepoint, whereas the high moisture potato starch control reached 88% by the 150 minute timepoint, which was the highest digestibility observed for any tested product in this study.

The starch digestibility of expanded chickpea snacks was found to decrease with increasing incorporation of chickpea flour. Similar to the extrudate samples, where a 10% incorporation of chickpea at low formulation moisture samples resulted in ~10% decrease in starch digestibility relative to the potato only control, a 10% chickpea flour low formulation moisture expanded snacks resulted in ~10% starch digestibility decrease relative to the potato only control. After 150 min the 10% expanded chickpea snack was digested to 68% (Figure 5.8 A) which was higher than the 57% starch digestibility observed in the equivalent chickpea extrudate sample. Starch digestibility decreased again with 40% chickpea flour incorporation at low formulation moisture, reaching ~39% at the 150 minute timepoint. Analogously to previous samples, the 80% chickpea flour enriched sample achieved the lowest amount of starch digestibility, with ~27% starch digested at the 150 minute timepoint. Both 40% and 80% chickpea flour incorporated low formulation expanded samples were less digestible than equivalent extrudate samples.

The high formulation moisture potato starch control was highly digestible (88% starch digested at 150 min), and there was only a small reduction in digestibility when 10% chickpea flour was incorporated at high formulation moisture. At 150 minutes, 81% starch digested was recorded in the 10% chickpea flour high formulation moisture samples, a drop of less than 10% in comparison to the potato starch only high formulation moisture control. This value was ~6% higher than the equivalent low formulation moisture chickpea incorporated sample. An incorporation level of 40% chickpea flour at high formulation moisture in the expanded sample reduced the starch digestibility once again in comparison to the 10% chickpea incorporated high formulation moisture sample to ~46 % at the 150 minute timepoint. 80% exhibited the lowest level of starch digestibility in the high formulation moisture expanded samples, with a value of ~31%.

In comparison to the low formulation moisture expanded snacks enriched with chickpea flour, the high formulation moisture equivalents achieved higher digestibility in all samples.

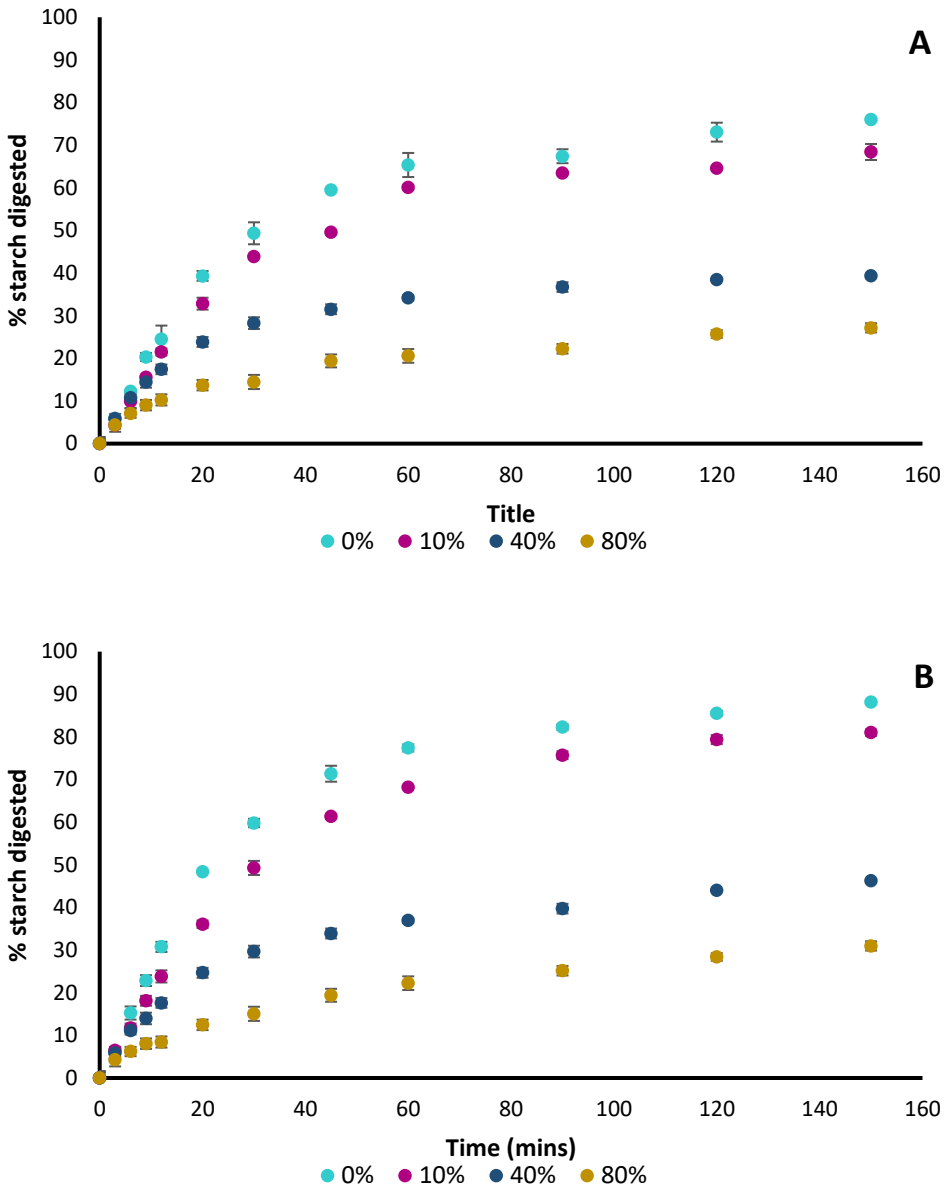

Figure 5.9: Starch digestibility of expanded snacks composed of potato starch and enriched with 10%, 40% and 80% red lentil flour A) low formulation moisture B) high formulation moisture. Analysis was carried out in triplicate.

This analysis was also carried out in red lentil samples made up at low and high formulation moisture and expanded through air frying. There was a much greater difference in digestibility of red lentil flour incorporated expanded snacks in comparison to chickpea incorporated expanded snacks. At low formulation moisture, a 10% incorporation of red lentil flour resulted in ~68% digestibility at the 150 minute timepoint. 40% red lentil flour incorporation resulted in 40% starch digested by the 150 minute timepoint. This value is similar to the equivalent chickpea enriched sample (39%), but 10% lower than the equivalent red lentil enriched extrudate result. At 80% red lentil flour incorporation, ~35% starch was digested in low formulation moisture expanded snacks. This is 8% higher digestibility than the equivalent chickpea expanded sample, and ~2% lower than the equivalent red lentil incorporated extrudate sample.

In high formulation moisture red lentil incorporated snacks, an increasing incorporation level red lentil flour resulted in a decrease in starch digestibility. In comparison to the low formulation moisture red lentil incorporated expanded snacks, the equivalent high formulation moisture snack achieved a higher starch digestibility percentage.

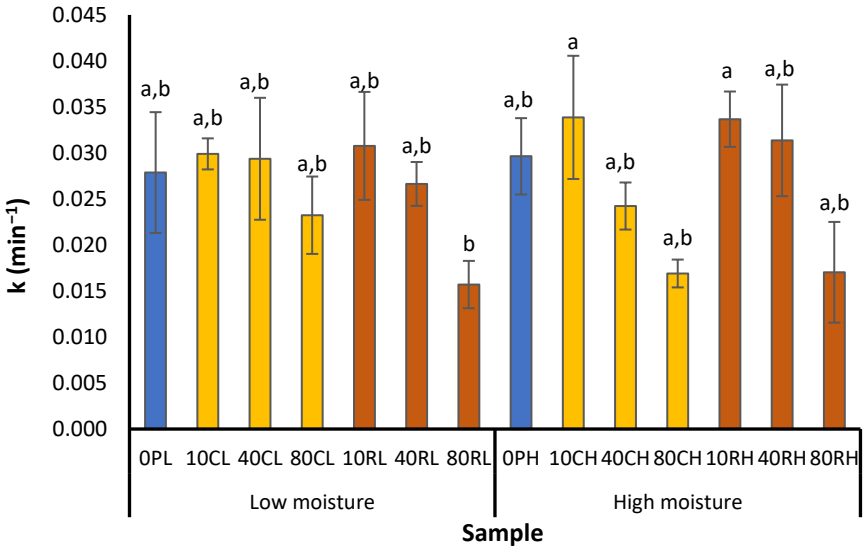

Figure 5.10: Calculated rate of digestion of 3rd generation expanded snacks composed of potato starch (P) and enriched with 10%, 40% and 80% chickpea (C) or red lentil (R) flour, at low and high formulation moisture levels. Analysis was carried out in triplicate. Different letters denote significance ($P < 0.05$) determined by one way ANOVA.

The rate of starch digestion was calculated using LOS plots for expanded snacks containing different levels of chickpea or red lentil flour, and potato starch based non-pulse flour containing controls formulated at low and high moisture (Figure 5.9). This was carried out in order to understand the effect of formulation and processing on the starch digestibility rate.

An incorporation of chickpea or red lentil flour was found to have a non-significant effect on starch digestibility rate at all pulse incorporation levels and formulation moistures tested. The reduction in digestion rate of the 80% chickpea flour samples was not statistically significant. It was hypothesised that high formulation moisture expanded snacks would have a higher rate of starch digestion as the higher formulation moisture would allow for more complete gelatinisation during extrusion. However, the data presented in Chapter 4 from DSC and XRD analysis demonstrated that this was not the case, and this was reflected in the results presented in this chapter where no significant differences were observed in starch digestion rate between high and low formulation moisture for the expanded snacks.

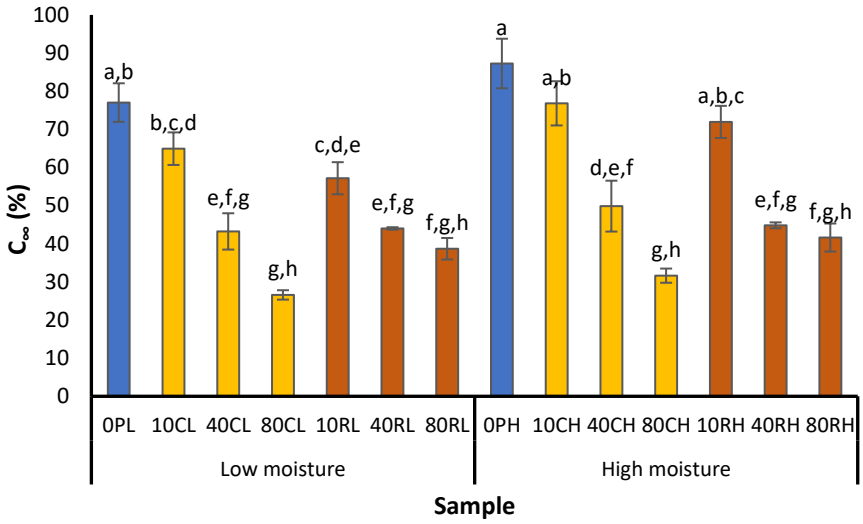

Figure 5.11: C∞ (%) of 3rd generation expanded snacks composed of potato starch (P) and enriched with 10%, 40% and 80% chickpea (C) or red lentil (R) flour, at low and high formulation moisture levels. Analysis was carried out in triplicate. Different letters denote significance (P < 0.05) determined by one way ANOVA.

C_∞ was calculated for expanded snacks enriched with chickpea or red lentil flour and expanded snacks made up of potato starch in order to understand the effect of formulation on starch digestibility (Figure 5.11).

As previously observed in the raw digestion data (Figure 5.8 and 5.9), an increase in the concentration of pulse incorporation resulted in a decrease in C_∞ value. The potato starch only controls had the highest C_∞ values. With the exception of the 10% chickpea sample at low and high formulation moisture, and the 10% red lentil at high formulation moisture all of the C_∞ values for the extruded legume flour enriched samples were significantly lower than their respective control samples. In the expanded samples, increasing the chickpea incorporation level had a much larger effect on digestibility than increasing the red lentil incorporation. This may reflect the more similar gelatinisation enthalpy observed between the expanded red lentil samples at different pulse incorporation levels (Figure 4.5) compared to the chickpea expanded samples, which had significant increases in gelatinisation enthalpy with increasing pulse incorporation.

In comparison to low formulation moisture expanded samples, those formulated at high moisture were slightly more digestible, with a higher C_∞, although this trend was not significant.

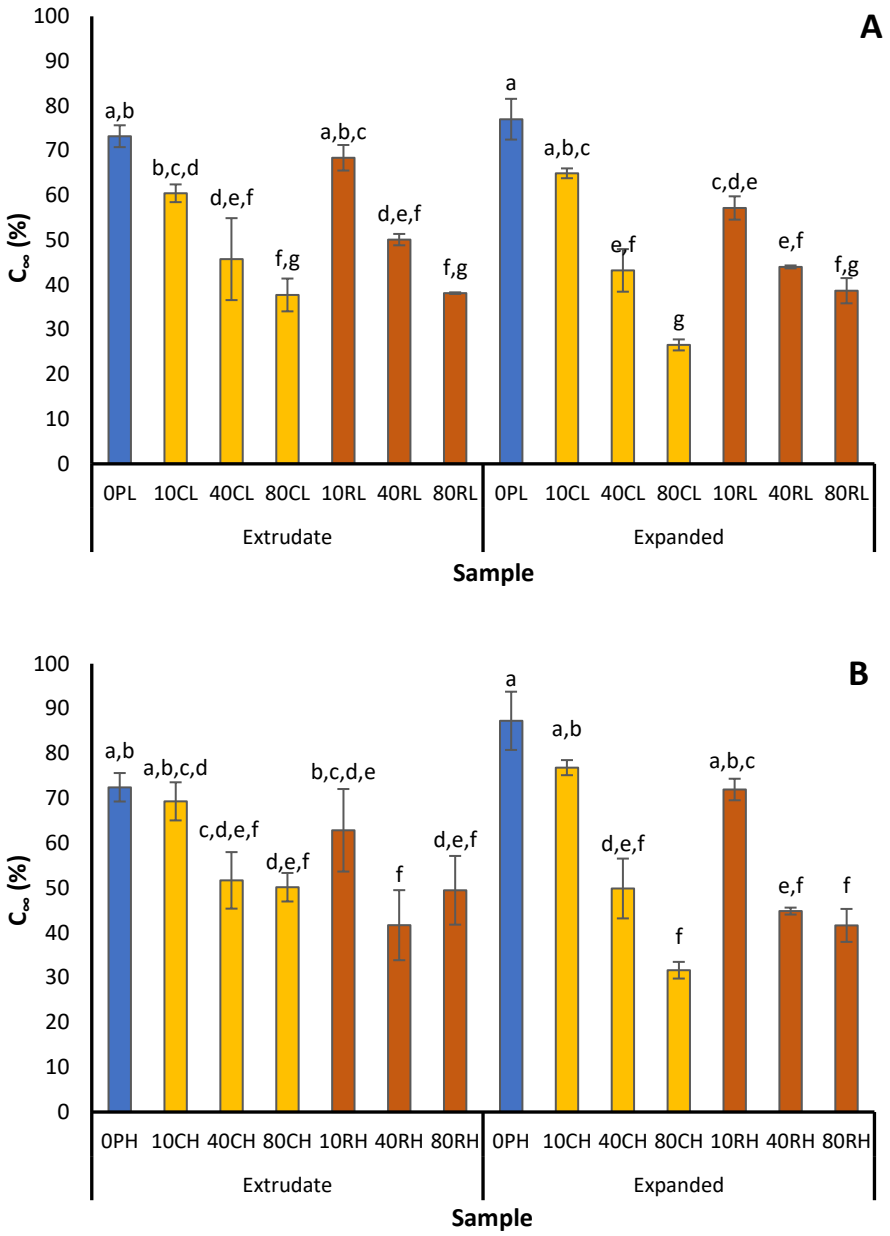

Figure 5.12: C∞ percentage of 3rd generation extrudates and expanded snacks composed of potato starch (P) and enriched with 10%, 40% and 80% chickpea (C) or red lentil (R) flour A) low formulation moisture B) high formulation moisture. Analysis was carried out in triplicate. Different letters denote significance ($P < 0.05$) determined by one way ANOVA.

C_∞ was compared between extrudate and expanded samples in order to determine effect of expansion on starch digestibility. It was hypothesised that the expansion process would increase the starch digestibility. However, it was observed in all cases that there were no significant differences in starch digestibility (C_∞) between the extruded and equivalent expanded samples, at either formulation moisture level.

5.3.4. Starch digestibility and starch gelatinisation enthalpy

The C_∞ of extrudate samples incorporated with chickpea or red lentil flour at 0%, 10%, 40% & 80% and formulated with low and high formulation moisture were correlated with the corresponding starch gelatinisation enthalpy in excess moisture (Figure 5.13). This was carried out in order to investigate the association between the gelatinisation enthalpy and starch gelatinisation of extrudates.

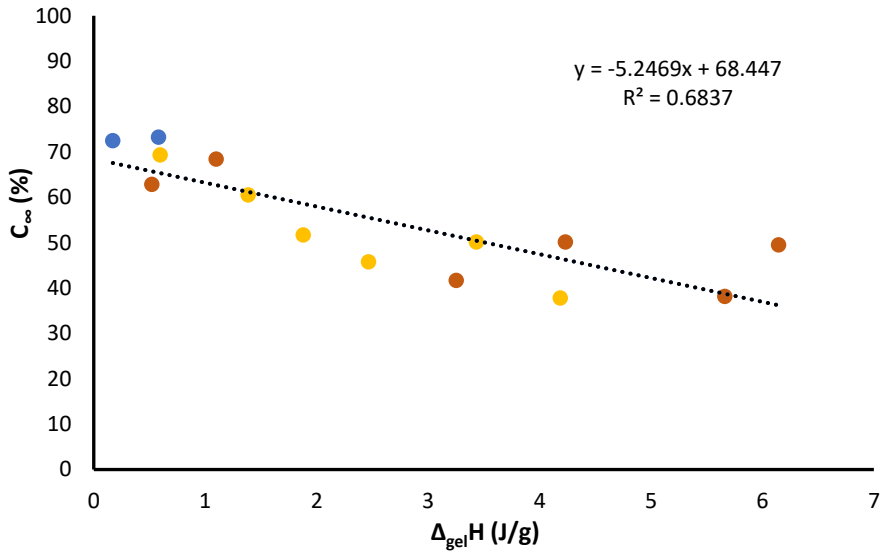

Figure 5.13: Correlation of C_∞ percentage and gelatinisation enthalpy of low and high formulation moisture extrudates made up of potato starch only (blue) and samples incorporating chickpea flour (yellow) and red lentil flour (orange)). P-value (Pearson correlation) is 0.000265; the result is significant at $p < 0.05$.

A significant correlation was observed between the gelatinisation enthalpy of the extrudates and the starch digestibility (C_∞). This indicates that the samples that achieved more gelatinisation during the extrusion process resulted in higher digestibility.

The correlation between C_∞ and starch gelatinisation was also analysed for expanded samples made up of potato starch only and chickpea and red lentil flour incorporated samples (Figure 5.14).

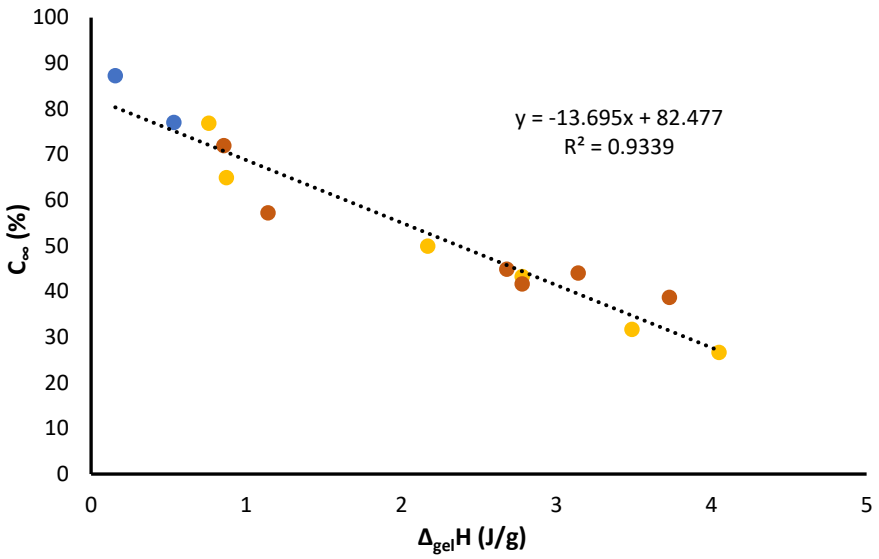

Figure 5.14: Correlation of C_∞ percentage and gelatinisation enthalpy of low and high formulation moisture expanded snacks made up of potato starch only (blue) and samples incorporating chickpea flour (yellow) and red lentil flour (orange). P-value (Pearson correlation) is < 0.00001; the result is significant at $p < 0.05$.

This once again resulted in a significant correlation, with a higher P-value in comparison to the extrudate samples. This suggests that further gelatinisation through the expansion process enhanced capacity for digestion and strengthened the link between starch gelatinisation enthalpy and starch digestibility.

5.3.5. Starch digestibility and starch crystallinity

Starch digestibility (C_∞) was correlated with XRD data of extrudate samples incorporated with chickpea or red lentil flour at 0%, 10%, 40% & 80% and formulated with low and high formulation moisture in order to understand how starch crystallinity effects the digestibility of starch.

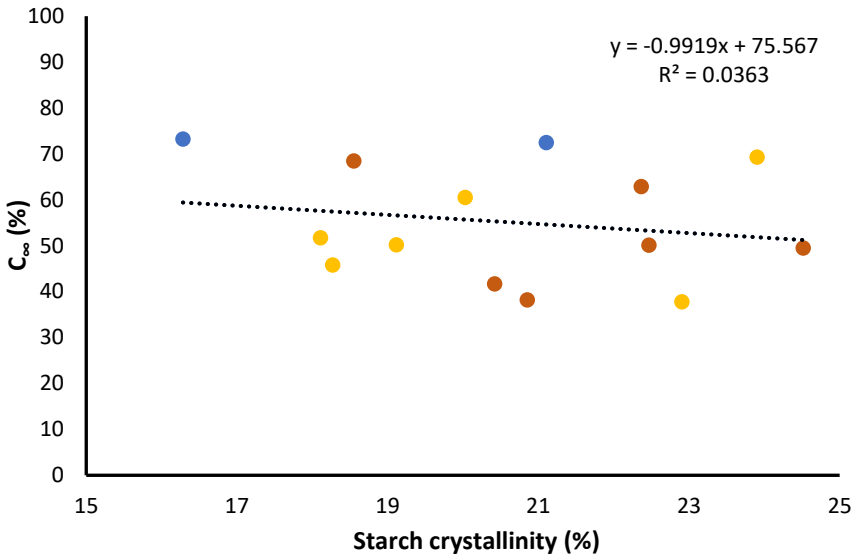

Figure 5.15: Correlation of C_∞ percentage and starch crystallinity percentage of potato starch control extrudates (blue) and extrudates incorporating chickpea flour (yellow) and red lentil flour (orange) at low and high formulation moistures. P-value (Pearson correlation) is 0.51; the result is not significant at $p < 0.05$.

Although it was hypothesised that higher crystallinity would impact digestibility negatively, there is no significant correlation between the starch crystallinity percentage and digestibility of starch in extrudate samples. This indicates that starch crystallinity is not the driving force behind starch digestion. Instead, it is hypothesised that the presence ungelatinised starch is the main limiting factor behind the reduction in starch digestibility. This is hypothesised to be due to the lower concentration of native starch granules facilitating more starch digestibility; as enzyme accessibility is increased when more gelatinised starch

matrix is present (Baldwin et al., 2015). Highly crystalline starch can also act as an amylase inhibitor, reducing the digestion rate of more digestible starch fractions (Patel et al., 2017).

5.3.6. Starch digestibility and specific volume

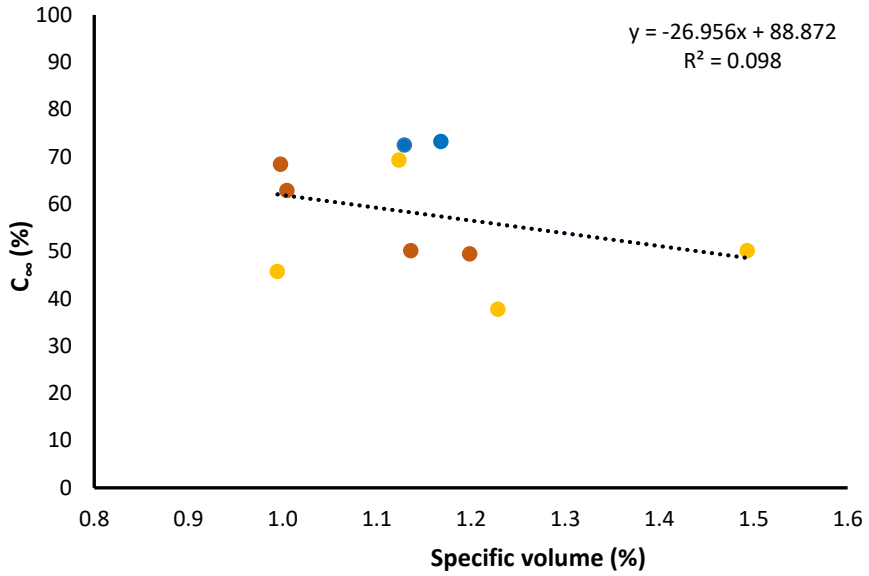

Figure 5.16: Correlation of C∞ percentage and specific volume of low and high formulation moisture extrudates made up of potato starch only (blue) and samples incorporating chickpea flour (yellow) and red lentil flour (orange). P-value (Pearson correlation) is 0.379; the result is not significant at p < 0.05.

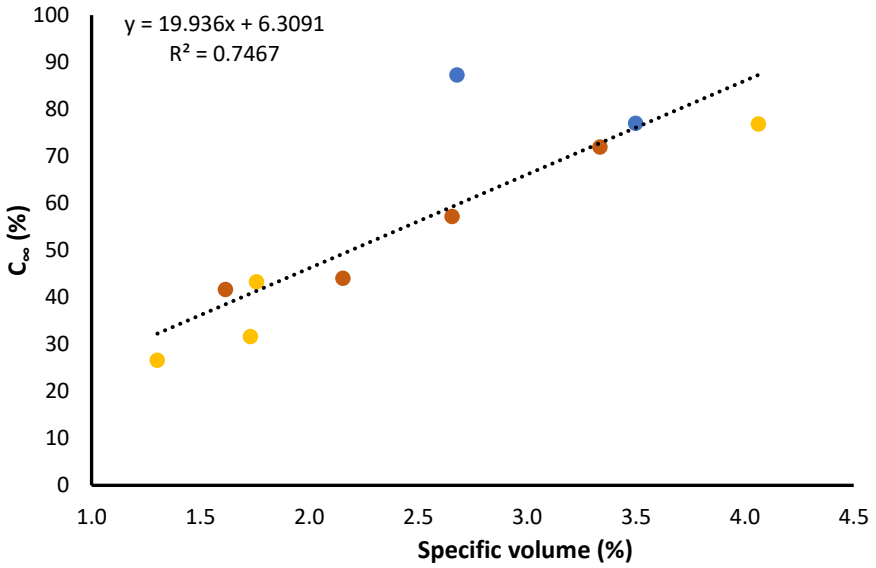

Figure 5.17: Correlation of C∞ percentage and Specific volume of low and high formulation moisture snacks expanded at 210°C in an air fryer, made up of potato starch only (blue) and samples incorporating chickpea flour (yellow) and red lentil flour (orange). P-Value (Pearson correlation) is 0.0013; the result is significant at p < 0.05.

The starch digestibility (C_∞) was correlated with the specific volume of extrudates and expanded snacks. This was carried out in order to further understand how the expansion of the snacks affected the starch digestibility, and to explore if bulk structural properties as well as starch molecular structural factors had an impact on starch digestibility.

When starch digestibility of extrudates containing potato starch only, chickpea flour enriched or red lentil flour enriched were correlated with the corresponding specific volume of extrudates, there was no significant result, with a P-value of 0.379. When the same analysis was carried out in expanded snacks, a significant correlation (P-value of 0.0013) was observed. This indicates that snack expansion has an effect on the starch digestibility, but only in the expanded snacks. This may be because the more open structure of the expanded snacks increases enzyme bioaccessibility, whereas the denser glass like structure of the extrudates did not contribute to their digestibility.

5.4. Summary and conclusions

The aim of this chapter was to understand the starch digestibility of third generation extruded snacks incorporating chickpea or red lentil flour at different enrichment levels and formulation moisture and the factors that affect the digestibility of these products.

5.4.1. Processing and starch digestibility

Starch digestibility is known to be low in native starch sources. In order to assess how the food processing through extrusion and air fryer expansion affects starch digestibility, the initial assessment was carried out on the raw materials. All native samples achieved minimal starch digestion (<30% starch digested) despite the extended timescale of the assay (330 minutes as opposed to 150 minutes) for these samples. This level of digestion is similar to literature values for the digestion of these starch types (Bhattarai et al., 2017; Dhital et al., 2017; Edwards, Veerabahu, et al., 2021).

When samples were processed using extrusion, an increase in starch digestibility was observed, with all samples achieving >30% starch digested. The most digested in extrudate samples was 66%. Expansion of the extruded half products using air frying was shown to increase starch digestibility further. The highest percentage of starch digested was 88%. This demonstrates that despite the relatively low temperatures and shears involved in third generation extrusion processing the processing conditions involved are able to significantly increase the digestibility of starch in legumes, as has been previously demonstrated for other extrusion technologies (Sun et al., 2006).

5.4.2. Product formulation and starch digestibility

In order to determine the effect of pulse flour incorporation on starch digestibility of third generation extruded snacks, samples of extrudates and expanded snacks incorporating different levels (0%, 10%, 40%, and 80%) of either chickpea or red lentil flour were produced.

Control samples containing no pulse flour were found to be the most digestible by α-amylase. When chickpea or red lentil flour was incorporated, the starch digestibility decreased with increasing incorporation percentage. This was true for both extrudate and expanded samples. 80% achieved the least amount of starch digestibility, with the lowest overall starch digestibility observed in the 80% chickpea flour incorporated low formulation moisture sample by the 150 minute timepoint. This was true for both extrudates and expanded snacks. The rate of starch digestion in extrudate samples did not change significantly according to the incorporation level of chickpea or red lentil flour. There was also no significant difference in the rate of expanded samples with incorporation of pulse flour, although there was a non-significant trend of lower starch digestibility rate with increasing pulse percentage in pulse containing samples in both low and high formulation moisture samples. This demonstrates that for these products the main factor limiting the starch digestion was incorporation of pulse, rather than processing conditions such as expansion or formulation moisture. This is in contrast to pulse based snacks produced with alternative extrusion technologies in which the high temperatures and shears result products with starch digestion rates comparable to controls. It is interesting to note, however, that despite differences between the red lentil and chickpea samples in a range of properties, including gelatinisation properties and the

amount of starch encapsulated in intact cells, there was no significant differences observed between equivalent chickpea and red lentil products in starch digestion rate or extent. This implies that the impact of pulse flour on the overall processing properties of the extrudates is more important than individual differences between pulse flours.

5.4.3. Formulation moisture content and starch digestibility

A third factor that may affect the starch digestibility of pulse flour incorporated third generation extruded snacks is the amount of water within the formulation. This is due to the difference in water holding behaviour between pulses and potato starch.

In extruded half products, the rate and extent of starch digestibility was not significantly different between samples low and high formulation moisture samples. The same was observed for equivalent pulse flour incorporation level in expanded snacks. The C_∞ of potato starch and pulse flour incorporated extrudates was not significantly different depending upon the formulation moisture content. Upon expansion, the C_∞ was significantly higher in only the potato starch control and 10% red lentil flour incorporated samples at high formulation moisture. In all other samples, no significant difference was observed between a low and high formulation moisture.

Formulation moisture made less of a difference to starch digestion rate and extent than originally hypothesised. The DSC and XRD data confirm that the formulation moisture during extrusion had a limited impact on starch molecular structure. This may be due to competing factors contributing to starch gelation during extrusion. While the higher formulation moisture samples had more

water available for gelation of starch, much of the water may be bound by the legume fibre components and will not be available for starch gelation. Also, the high formulation moisture legume samples had batters which were much wetter and runnier than the low formulation moisture batters. This resulted in the high formulation moisture batters having a shorter time-of-flight in the extruder, potentially exposing the product to high temperatures for a shorter period of time, limiting gelatinisation in the high formulation water samples.

5.4.4. Starch digestibility and gelatinisation/crystallinity/specific volume

The extent of starch digestion (C_∞) was corelated against a range of factors. The strongest correlation for both extruded and expanded samples was with the starch gelatinisation enthalpy, while the correlation between crystallinity measured by XRD and C_∞ was not statistically significant. This is potentially surprising as the gelatinisation enthalpy of starch and the crystallinity measured by XRD are closely related measurements, which both reflect the degree of ordered structure within the starch (Bogracheva et al., 2001; Warren et al., 2016), and effect which has been demonstrated for legume starches (Bogracheva et al., 1999). However, in these samples the crystallinity measured by XRD contains two components, the crystallinity of starches which have escaped gelatinisation during processing, and crystallinity from starch which has retrograded during storage. The DSC is able to differentiate these two forms of crystallinity due to their different melting temperatures (Karlsson & Eliasson, 2003). The relationship between C_∞ and gelatinisation enthalpy is only significant when the retrograded starch enthalpy is discounted. Therefore, we can conclude from this data that the ungelatinised starch is the main factor which is limiting starch digestion extent in these samples, as demonstrated by

the limited digestibility of the native flours, while the retrograded starch does not significantly limit starch digestion in these products.

5.4.5. Conclusions

In conclusion, while third generation extrusion processing can increase the starch digestibility of legume starches, the incomplete cooking of the starches during this form of extrusion does place some limits on starch digestibility. Even following expansion, the higher pulse snacks were significantly less digestible than the potato controls. A combination of DSC and XRD revealed that this was primarily due to the ungelatinised starch present in these samples, rather than retrogradation that occurred during storage. A further factor in the expanded samples, but not the extruded pellets, was the role of the specific volume, where in expanded products the reduced specific volume contributed to increased digestibility. Therefore, in third generation legume enriched snacks, a combination of ungelatinised starch granules and reduced expansion combined to lead to lower starch digestibility than equivalent potato starch only controls.

6. Discussion and future work

6.1. Discussion

6.1.1. Formulation moisture impacts the final product less than hypothesised

In this project, a sample set of third generation extruded snacks incorporating chickpea and red lentil flour at 0%, 10%, 40%, and 80% with low (25%) and high (35%) formulation moisture were produced using a pilot scale single screw extruder. Final expanded products were optimised for expansion capacity and Browning index. It was hypothesised that the incorporation of chickpea or red lentil flour would decrease the expansion capacity, due to the low gelatinisation of these materials resulting in a lack of sufficient gelatinised starch matrix for expansion. Upon expansion by air frying, the specific volume was found to decrease with increasing pulse incorporation, demonstrating that increased pulse incorporation produced a less highly expanded snack. High formulation moisture was hypothesised to increase the expansion capacity. Specific volume data showed that this was in fact not the case.

(Norton et al., 2011) found that a high moisture atmosphere reduced the expansion capacity in half products which were expanded using hot air expansion, and suggested that the moisture content of starch extrudates needs to be carefully controlled in order to achieve optimum expansion. In my snacks, despite the difference in visual appearance of pre extrusion dough, the degree of gelatinisation was similar for equivalent low and high moisture formulation samples. This may account for the lack of significant difference in expansion of low and high formulation moisture expanded snacks.

In terms of starch digestibility, it was originally hypothesised that a high formulation moisture would increase starch digestibility. This was found to be true for only two of the expanded sample, 0% pulse flour and 10% red lentil flour which had a significantly higher C_∞ in comparison to the equivalent low formulation moisture expanded samples. When referring back to the gelatinisation that occurred during processing, it was observed that a higher amount of gelatinisation had occurred in the high formulation moisture 0% and 10% red lentil expanded samples in comparison to the equivalent low formulation moisture expanded samples, however this difference was not significant.

6.1.2. The differences between ungelatinised starch and retrograded starch in third generation snacks and the impact on digestibility

The starch digestibility of raw potato starch, chickpea flour and red lentil flour was compared to the starch digestibility of processed extrudates incorporated with 0%, 10%, 40% & 80% chickpea or red lentil flour with low or high formulation moisture as well as their equivalent expanded final products. It was hypothesised that processing through extrusion and expansion would change the starch structure and result in increased starch digestibility. As hypothesised, the native potato starch and pulse flours exhibited the lowest extent of starch digestion. When processed using extrusion, the starch digestibility was higher in comparison to the raw materials. This is in agreement with results produced by (Yağcı et al., 2020) where the in vitro starch digestibility of chickpea flour increased upon first generation extrusion. Expansion of the half products in the current study did not further increase digestibility however, despite the vast

difference in product structure. Therefore a different factor must be driving starch digestibility.

The incorporation of chickpea or red lentil flour was hypothesised to decrease the starch digestibility of processed snacks. C_∞ data showed that increasing the incorporation level of chickpea or red lentil flour did indeed reduce the starch digestibility in expanded snacks. As snack microstructure is not the driving force behind reduced starch digestibility, the C_∞ was correlated with starch gelatinisation data of expanded snacks. A strong and significant correlation suggests that a limiting factor in starch digestibility is the non-gelatinised starch content. This may be contributions from both ungelatinised starch granules, and retrograded starch. 0% and 10% pulse incorporated high formulation moisture extrudates achieved the highest levels of retrogradation. This did not impact the digestibility of these samples as C_∞ was still higher than 40% and 80% extruded samples at high formulation moisture. In addition, starch digestibility was correlated with starch crystallinity data. A lack of significant correlation despite the large contribution of crystallinity from retrogradation of high moisture samples indicates that total starch crystallinity does not affect the starch digestibility. This suggests that it is the portion of ungelatinised starch granules alone which influences starch digestibility in third generation extruded snacks. A higher ratio of ungelatinised starch within the product results in a decreased amount of starch matrix, as can be seen in the microscopy images of 80% chickpea or red lentil flour extrudates. This increase in amorphous starch matrix through increased gelatinised starch may facilitate higher starch digestibility. The quantity of ungelatinised starch granules remained similar for extrudates and equivalent expanded snacks, as exhibited by the lack of significant

difference between the gelatinisation enthalpy between the two samples. This indicates that ungelatinised starch granules may play a smaller role in the expansion process than previously hypothesised. Instead expansion is driven by the presence of a gelatinised starch matrix. When correlating C_∞ and specific volume data, a significant correlation was observed. This indicates that a highly expanded snack has high starch digestibility due to the presence of a large portion of gelatinised starch.

6.2. Conclusion

The aim of this study was to understand how pulse ingredients and formulation moisture impacts the starch digestibility of third generation extruded snacks. From this series of investigations, it was found that the starch gelatinisation and starch retrogradation of the product has the highest impact on both starch digestibility and expansion capacity. A main finding of the study was that the starch gelatinisation may be influenced by ingredients such as incorporation of pulse flours, but is less impacted by formulation moisture. Incorporation of pulse flour may be used to reduce starch digestibility and influence expansion. 10% pulse flour incorporation was found to increase specific volume (thereby increasing expansion), whereas higher incorporation percentages decreased specific volume. Formulation moisture was found to have no significant effect on expansion capability.

These findings are relevant to industry when producing expanded snacks incorporating pulse ingredients, as these ingredients are typically used to increase protein content and lower starch digestibility. When aiming to reduce

starch digestibility, it is useful to know the parameters to control gelatinisation during snack processing to affect how well starch digests. Also, an understanding of how we can increase gelatinisation to increase expansion, in order to balance between an industry accepted snack on sensory terms and nutrition.

6.3. Future work

A next step in understanding and optimising the third generation extruded pulse incorporated snacks, and a step that had been planned for this PhD project was to conduct a sensory trial at PepsiCo to analyse the chickpea and red lentil incorporated expanded snacks. Preparations for this trial were made prior to lockdown restrictions being put in place – sensory had been approved by the relevant colleagues, documents were drawn up and weeks were spent producing test samples. Sensory aspects that would have been targeted would be texture, crispiness, and flavour (such as beany flavours). Hardness, crispiness and crunchiness would be focused upon through the use of a texture analyser.

Another continuation of this work that would be of interest is analysis of the snack digestibility using *in vitro* digestibility models such as the INFOGEST model. Use of the Infogest model as a static digestion model has superior capabilities in comparison to the single enzyme starch digestibility model as protein and lipid digestion enzymes are utilised, allowing for a more well-rounded outlook on overall digestibility. It would also be useful to understand the interaction of protein and starch only digestibility. The availability of essential amino acids could in turn be measured and analysed using LC-MS based proteomics. For a further in depth look at *in vitro* digestibility of third generation extruded pellets and snacks, the semi dynamic and dynamic gastric model (DGM) could be utilised to consider the gradual pH shifts, gastric emptying and the peristaltic movements that occur *in vivo* to give more physiologically relevant information.

7. Abbreviations

AM	Amylose
AP	Amylopectin
BI	Browning index
DG	Degree of retrogradation
DSC	Differential scanning calorimetry
D.W.B	Dry weight basis
GI	Glycaemic index
LOS	Log of slope
XRD	X-ray diffraction
WHC	Water holding capacity
WAC	Water absorption capacity
0PL	0% pulse incorporation at low formulation moisture
0PH	0% pulse incorporation at high formulation moisture
10CL	10% chickpea flour incorporation at low formulation moisture
10CH	10% chickpea flour incorporation at high formulation moisture
10RL	10% red lentil flour incorporation at low formulation moisture
10RH	10% red lentil flour incorporation high formulation moisture
40CL	40% chickpea flour incorporation at low formulation moisture
40CH	40% chickpea flour incorporation at high formulation moisture

40RL	40% red lentil flour incorporation low formulation moisture
40RH	40% red lentil flour incorporation high formulation moisture
80CL	80% chickpea flour incorporation at low formulation moisture
80CH	80% chickpea flour incorporation at high formulation moisture
80RL	40% red lentil flour incorporation low formulation moisture
80RH	40% red lentil flour incorporation high formulation moisture

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