Cooking quality and nutritional properties of extruded maize pasta with orange-fleshed sweet potato

By

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DECLARATION

I Rose Otema Baah declare that this dissertation, which I hereby submit for the degree MSc Food Science at the University of Pretoria, has not previously been submitted by me for a degree at this or any other university or institution of higher education.

SIGNATURE: _____

DATE: _____

DEDICATION

I dedicate this MSc research to my family especially my loving parents, Mr. Andrew Baah-Kwako and Mrs. Sarah Baah.

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ABSTRACT

Cooking quality and nutritional properties of extruded maize pasta with orange-fleshed sweet potato

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Vitamin A deficiency is prevalent in sub-Saharan Africa and is considered a serious problem of public health significance. The use of bio-fortified staple foods is an intervention strategy to address vitamin A deficiency and one example is orange-fleshed sweet potato (OFSP) cultivar with increased beta-carotene content. Replacing white-fleshed potato cultivars with orange-fleshed sweet potato can improve the vitamin A status of school-aged children. Taking this information into consideration, the diversification of diet through inclusion of orange-fleshed sweet potato could be a useful approach for alleviating vitamin A deficiency. This presents an opportunity whereby white maize flour can be composited with orange flesh sweet potato flour to produce gluten-free pasta with enhanced β-carotene content. This product will also be suitable for celiac patients and other consumers who want to abstain from gluten due to some health related issues, and contribute to the RDA requirements for vitamin A for various groups. This study therefore investigates the effect of extrusion processing on cooking quality, β-carotene content and antioxidant properties of gluten free maize – orange-fleshed sweet potato pasta.

Maize and orange-fleshed sweet potato composite (100:0, 80:20, 70:30, 50:50 w/w) flours were extruded into pasta using a twin-screw extruder. Raw flours and raw pasta samples were analysed for proximate composition, β-carotene content and *in-vitro* radical scavenging properties. Cooked pasta samples were analysed for cooking quality (cooking time, cooking loss and water absorption capacity), textural properties (firmness, stickiness and fracturability), *in-vitro* starch digestibility and *in-vitro* protein digestibility. Raw and cooked pasta samples were analysed for thermal properties. Gluten free commercial pasta made from corn and rice was used as a reference.

Increase in addition of OFSP flour increased the cooking loss and decreased cooking time and water absorption capacity of pasta. There was an increase in insoluble and soluble dietary fibre as the proportion of OFSP flour in the composite decreased. The fibre content in the OFSP flour

caused a loosening of the compact structure of the pasta disrupting the compact protein-starch matrix resulting in higher cooking loss and sticky pasta. This also led to lower hydration of starch as well as protein hydration resulting in lower water absorption capacity. However, compared to the commercial pasta which was conventionally made, extruded pasta samples showed indistinct starch granules as observed with scanning electron microscopy (SEM) indicating higher amount of disrupted starch thereby leading to faster absorption of water during cooking resulting in lower optimum cooking time.

Extruded pasta samples exhibited lower *in-vitro* protein digestibility as compared to the commercial pasta that was conventionally made. Higher temperature used during extrusion cooking leads to the disruption of protein structures by unfolding, re-organization and polymerization through the formation of disulphide bonds. Disulphide bonds can result in reduction in protein solubility resulting in lower *in-vitro* protein digestibility. Extruded pasta samples showed lower *in-vitro* starch digestibility. Differential scanning calorimetry showed that amylose-lipid complexes were formed which reduced starch digestibility.

Increasing proportion of OFSP in the composites increased β-carotene content and antioxidant properties of both raw flour and pasta samples. After extrusion, β-carotene content of pasta samples decreased but antioxidant properties increased. The high temperature and shear rate conditions used during extrusion processing may have caused losses of β-carotene through cis-trans isomerization, fragmentation and oxidative decomposition. The possible formation of Maillard reaction and caramelization products with reducing properties as a result of extrusion could have contributed to the observed increased antioxidant properties of the pasta samples.

In conclusion, addition of 20 % OFSP to maize flour produces pasta by extrusion that have similar characteristics in terms of its good cooking quality and textural properties to the commercial pasta and 100 % maize pasta as evidenced by low cooking time, less cooking loss, less stickiness and more firmness and required greatest amount of force to break. The cooking quality of extruded pasta seems to be related to the microstructure and dietary fibre seems to play an important role in contributing to negative cooking qualities. The addition of OFSP flour in cereal-based pasta could potentially meet an appreciable amount of the recommended daily amount of vitamin A for various groups and exerting good antioxidant properties.

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CHAPTER 1: INTRODUCTION

Micronutrient deficiency continues to be a major nutritional concern in poor communities, especially in sub-Saharan Africa with children and women being the most vulnerable (Bain *et al.*, 2013). Vitamin A deficiency impairs numerous functions and, as a result, can lead to many health consequences such as, impaired iron mobilization, growth retardation, blindness, reduced immune response, increased susceptibility to infectious disease and increased childhood mortality in mostly developing countries (Sommer and Davidson, 2002; Müller and Krawinkel, 2005). Currently, a number of different successful intervention strategies to address vitamin A deficiency is being promoted (Bengtsson *et al.*, 2010). Some of these interventions are food supplementation, food fortification and bio-fortification of stable crops. The sustainable way to address micronutrient malnutrition in an innovative strategy is bio-fortification, which involves increasing the concentrations of nutrients in crops by the use of agronomic approaches and plant breeding (Bouis *et al.*, 2011). Example of bio-fortified food crops are yellow maize, yellow cassava, golden rice and orange-fleshed sweet potato.

Sweet potato (*Ipomoea batatas*) is among the top seven staple foods in the world and adjudged to be fourth after rice, corn and cassava in terms of important carbohydrate food sources with China being the country with highest production, cultivating over 3.5 million hectares which makes up 43 % of the world total (Omran and Hussien, 2015; Tan, 2015). Orange-fleshed sweet potato can be used as food in developing countries due to its great potential: that is, it can grow and mature within the shortest possible time and also has the ability to grow in different climatic conditions and on less fertile soils as well (Zuraida, 2003; Tan, 2015). According to Laurie *et al.* (2018) Orange-fleshed sweet potato (OFSP) is considered the most successful example of bio-fortified staple crop, and presents a possible option to address vitamin A deficiency. A study by Van Jaarsveld *et al.* (2006) reported the vitamin A status of school-aged children was improved by substituting white-fleshed potato cultivars with orange-fleshed sweet potato. Orange-fleshed sweet potato being rich in β-carotene is also a cheap food source with antioxidant properties having potential physiological attributes like protection against oxidative stress-induced diseases such as cancer and other non-communicable diseases (Vimala *et al.*, 2011).

Pasta is traditionally made from durum wheat and it is mostly preferred due to its convenience, palatability, high shelf stability and its nutritional properties. Currently consumers are shifting

towards the consumption of gluten free pasta due to personal health choice to exclude gluten from their diet and for patients with gluten intolerance and celiac disease. This has led to the development of cereal based gluten free pasta made from rice and maize. However, the gluten free diet may be deficient in certain nutrients such as fibre, B vitamins, iron, and trace minerals (Marti and Pagani, 2013; Theethira and Dennis, 2015). Taking this information into consideration, the diversification of diet through the inclusion of orange-fleshed sweet potato could be useful in alleviating vitamin A deficiency.

Apart from the nutritional aspect of gluten free pasta production, the cooking and textural qualities also remain a major technological challenge. The difficulty in producing gluten free products is associated with the lack of gluten in the food system (Marti and Pagani, 2013). Extrusion processing may help develop a compact structure with gelatinized starch imbedded in protein matrix and aligned in the direction of flow through the extruder barrel which may help create the matrix that imitates the viscoelastic properties of gluten (Wang *et al.*, 1999).

Extrusion processing is a food processing technique that uses high-temperature short time to produce foods including breakfast cereals, snack products and pasta products. Brennan *et al.* (2011) also reported that extrusion processing is preferred by food processors because it produces products with a wide range of distinct textural properties such as crispiness, expansion and desirable mouthfeel as compared to products produced using conventional processing technique. The extrusion processing technique is also versatile, has high productivity, lower operation costs, high-energy efficiency and shorter cooking time.

Extrusion processing is reported to improve the digestibility of proteins through the denaturation of proteins and the reduction of antinutrients (Alonso *et al.*, 2000). Starch digestibility is also increased through the loss of structural integrity of the starch granules as a result of the high shear and temperatures used, increasing their susceptibility towards enzymatic attack (Singh *et al.*, 2010) as compared to conventional cooking. The key parameters in extrusion processing are temperature, pressure, shear rate and residence time (Stojceska, 2013). Raw food materials during extrusion processing are subjected to thermal energy and shear forces causing structural, chemical, and nutritional transformations such as gelatinization and degradation of starch, denaturation of protein, oxidation of lipid, degradation of vitamins, antinutrients and phytochemicals (Ilo and Berghofer, 2003; Singh *et al.*, 2007a) formation of flavors, increase of mineral bioavailability and

solubility of dietary fibre which affects the physical, functional and nutritional properties of the end product (Riaz *et al.*, 2009).

Even though carotenoids are very stable in fresh plant tissue, they can be totally degraded or partially isomerized when they are processed by the action of light, heat and oxygen (Marx *et al.*, 2003). The aim of this work is to produce a maize gluten free pasta composited with orange-fleshed sweet potato flour for enhanced β-carotene content using extrusion processing technology.

CHAPTER 2: LITERATURE REVIEW

2.1 Maize

2.1.1 Production, grain structure and nutritional value of maize

Maize (*Zea mays*) is believed to have originated 7000 years ago from a wild grass in central Mexico and was then transformed into a better source of food by native Americans (Brown and Darrah, 1985). Currently, the United States, Argentina, Brazil, India, Mexico, France, Italy Indonesia and South Africa produce 79 % of the world's maize production (Ranum *et al.*, 2014). About 60–70 % of maize production worldwide is used domestically as livestock feed, and 30–40 % is used for the production of items for human consumption (Gwirtz and Garcia-Casal, 2014).

From a processing perspective, the maize kernel is composed of four primary structures. They are endosperm, germ, pericarp, and tip cap consisting of about 83 %, 11 %, 5 % and 1 % of the grain respectively. Starch is the main component of the endosperm and is surrounded by a protein matrix (Gwirtz and Garcia-Casal, 2014). Maize contains approximately 72 %, 10 %, 4 % starch, protein, fat respectively, and supplies about 365 J/100 g of energy (Nuss and Tanumihardjo, 2010). Sugars range from 1 % - 3 % with sucrose as the main component and, glucose, fructose, maltose, and raffinose in small amounts (Boyer and Shannon, 1987), and 25 % of these free sugars are located in the endosperm with most exclusively located in the germ.

Starch in the maize kernel is located predominantly in the endosperm (Inglett, 1970). In cereals, starch molecules (amylose and amylopectin) are organized into structural organelles called granules which consist of crystalline lamellae and alternating amorphous regions (Zobel, 1988). It exists as A pattern form in X-ray powder diffraction studies (Cheetham and Tao, 1998). White maize is mostly used as food and it provides many of the B vitamins excluding vitamin B12 and is in general, a poor source of vitamin A, iron and calcium. It also provides essential minerals along with fibre if eaten as whole grain (Dale and Nierberger, 1982). The nutritional value of maize is shown in Table 2.1.

Table 2.1 Nutritional value of white maize
(Nuss and Tanumihardjo, 2010)

Nutritional value per 100 g (dry basis)	
Energy	365 kcals
Carbohydrate	74.3 g
Sugar	0.64 g
Protein	9.43 g
Thiamin	0.39 mg
Riboflavin	0.20 mg
Pantothenic acid	0.42 mg
Vitamin B6	0.62 mg
Vitamin A	11 µg
Niacin	3.63 mg
Folate	19 µg
Calcium	7 mg
Phosphorus	210 mg
Potassium	287 mg
Magnesium	127 mg
Sodium	35 g

2.1.2 Maize flour as an ingredient in food products

Milling of maize grains produces maize meal or flour. Maize meal is used as a staple to make stiff porridge in Southern Africa whereas meal flour is mostly used as an ingredient in many food products such as corn bread, corn chips, porridges, extruded snack foods, etc. (Li *et al.*, 2014a). Starch accounts for more than 50 % of cereal grain weight and bring about changes in its structure

during milling which affects the functional properties of the flour, such as swelling properties, solubility, pasting properties and digestibility (Chen *et al.*, 1999).

Maize flour is one of the preferred ingredients used in the production of gluten free pasta. The challenge concerning this ingredient is that, even though they provide mostly energy, the amount of some essential nutrients are inadequate. Therefore, consumption of products made from only maize flour contributes mostly energy and is limited in fibre, protein, vitamins and minerals, thereby increasing the risk of nutritional deficiencies associated with celiac disease (Padalino *et al.*, 2016). Maize meal lacks Vitamin A, but in South Africa, the maize meal sold is fortified with vitamins according to law. Pertaining to regulation the final minimum level of vitamin A in fortified maize meal should not be less than 187.7 µg RE/100 g (Department of health, 2003). Bio-fortified sweet potato as orange-fleshed sweet potato is rich in β-carotene that can be converted to Vitamin A and therefore can be used in fortification of cereal-based foods.

2.2 Sweet potato

2.2.1 Production and nutritional value of sweet potato

Sweet potato (*Ipomoea batatas*) is a perennial tuber which forms part of the family Convolvulaceae (Tan, 2015) and planted by vegetative reproduction using stem cutting or storage roots (Mohanraj and Sivasankar, 2014). The stem is cylindrical, and its leaves are arranged spirally on the stem and also the length of the tuber depends on the cultivar in terms of its growth habit (Mohanraj and Sivasankar, 2014). The colour of the leaves can have a purple pigmentation in part of the leaf blades or can be green and yellowish-green. The edible part of sweet potato is the hardy storage roots (Huaman, 1992).

Sweet potato can withstand a lot of climate conditions with less labour force than many other staple crops (Woolfe, 1992). According to Ciad (1996), a single sweet potato plant may produce 40-50 roots ranging in length from a few to 30 cm, and weighing between 100 and 1000 g. The colour of its flesh ranges from beige to white, pink, red, violet, purple, yellow and orange.

Sweet potato has high nutritional value in terms of dietary fibre, certain minerals and vitamin contents (Tan, 2015). The nutritional value of sweet potato is shown in Table 2.2. Sweet potato contains more than 90 % of nutrients per calorie that most people require. The OFSP roots are

good source of carbohydrates, vitamins and provide 49 % of the recommended daily allowance (RDA) for vitamin C and 100 % of the RDA for vitamin A, and minerals also providing 15 % of the RDA for potassium and 10 % of the RDA for iron (Mohanraj and Sivasankar, 2014). Sravanthi (2012) reported that sweet potato is also a good source of carbohydrate whose energy content comes from starch. The total carbohydrates in sweet potato are 80 % starch and 20 % simple sugars. It has higher amylose content of about 38 % compared to maize, cassava or wheat (Tian *et al.*, 1991). In comparison to simple sugars, amylose slowly raises the blood sugar levels; therefore, it is recommended as a healthy food for diabetic patients. High amylose starches can be resistant to intestinal enzymes and have fibre-like function (Behall and Hallfrisch, 2002). According to Granfeldt *et al.* (1995), increase in amylose consumption was shown to decrease the concentration of glucose or insulin response after a meal.

2.2.2 Orange-fleshed sweet potato

White-fleshed sweet potatoes are the main varieties currently growing in most countries. These varieties lack an essential component of human diets that is, β-carotene, the plant precursor of vitamin A (Low *et al.*, 2017). Sub-Saharan Africa and South Asia record the highest prevalence in vitamin A deficiency which is considered as a serious problem of public health significance (Stevens *et al.*, 2015). A ready and relatively cheap source of beta carotene is orange-fleshed sweet potato (OFSP) (Simonne *et al.*, 1993).

Orange-fleshed sweet potato is rich in β-carotene with values of 100–1600 µg retinol activity equivalents (RAE)/100 g reported in some African varieties (Hagenimana *et al.*, 2001; Van Jaarsveld *et al.*, 2006). According to Tsou and Hong (1992), children less than five years can get the recommended daily amount of vitamin A when they consume orange-fleshed sweet potato roots having about 3 mg/100 g β-carotene on a fresh weight basis. Jalal *et al.* (1998) reported that incorporation of orange-fleshed sweet potato into meals eaten by 3 to 6 years old improved vitamin A status.

On dry matter basis, some orange-fleshed sweet potato cultivars contain 20 to 30 times more β-carotene than Golden Rice (Ye *et al.*, 2000). The sweet potato has immense potential and has a major role to play in human nutrition, food security, and poverty alleviation in developing

countries (Van Jaarsveld *et al.*, 2005). β-carotene exhibits the highest provitamin A activity among all carotenoids, and they exist in trans configuration in plants (Sravanthi, 2012).

Table 2.2: Nutritional value of orange-fleshed sweet potato
(Mohanraj and Sivasankar, 2014)

Nutritional value per 100g	
Energy	360 kJ (86 kcal)
Carbohydrates	20.1 g
Starches	12.7 g
Sugars	4.2 g
Dietary fiber	3.0 g
Fat	0.1 g
Protein	1.6 g
Vitamin A equivalent	709 µg (89 %)
▪ β-carotene	8509 µg (79 %)
▪ Lutein and zeaxanthin	0
Thiamine (vitamin B_1)	0.1 mg (9 %)
Riboflavin (vitamin B_2)	0.1 mg (8 %)
Niacin (vitamin B_3)	0.16 mg (4 %)
Pantothenic acid (vitamin B_5)	0.8 mg (16 %)
Vitamin B_6	0.2 mg (15 %)
Folate (vitamin B_9)	11 µg (3 %)
Vitamin C	2.4 mg (3 %)
Vitamin E	0.26 mg (2 %)
Calcium	30.0 mg (3 %)
Iron	0.6 mg (5 %)
Magnesium	25.0 mg (7 %)
Phosphorus	47.0 mg (7 %)
Potassium	337 mg (7 %)
Sodium	55 mg (4 %)

2.3 Carotenoids

2.3.1 Chemistry and properties of carotenoids

Carotenoids are natural isoprenoid compounds comprising about 700 pigments that give the reddish, yellow and orange colour of fruits, vegetables, flowers and leaves (Tanaka *et al.*, 2012). Carotenoids occur in photosynthetic organelles such as higher mosses, plant, algae and ferns capable of converting sunlight into chemical energy (Fiedor and Burda, 2014) and can also be found in photosynthetic membranes of cyanobacteria and phototropic bacteria (Scheer, 2003). Carotenoids are present in the blood and tissues of humans and animals although these organisms cannot synthesize carotenoids on their own. The only essential function of carotenoids recognized in humans is that of provitamin A carotenoid (β carotene, α carotene and β-cryptoxanthin) to serve as a source of vitamin A (Rock, 1997).

Carotenoids are known for being efficient physical and chemical quenchers of singlet oxygen, and also their potential to scavenge other reactive oxygen species (ROS) (Fiedor *et al.*, 2005; Edge and Truscott, 2010). The carotenoid structure is a symmetrical tetraterpene skeleton formed by tail-to-tail linkage of two C20 units. The carotenoid skeleton is illustrated by the cyclic hydrocarbon lycopene, but in many carotenoids, the end groups are modified into six-membered rings at one or at both ends of the molecule to give mono and dicyclic carotenoids respectively (Young and Britton, 2012). The simple hydrocarbon carotenes (e.g. β-carotene) are the most widely distributed carotenoids. Lutein and zeazanthin are also hydrocarbons which contain two hydroxylated terminal ring systems joined by the chromophore which bears the chain of conjugated double bonds. Oxygen can also be introduced in the form of keto-groups, with or without additional hydroxyl groups (canthaxanthin and astaxanthin) (Walter and Strack, 2011). Chemical structures of some carotenoids are shown in Figure 2.1

Some important functions of carotenoids include: (a) cell differentiation; (b) regulation of the cell cycle and apoptosis; (c) modulation of growth factors (Krinsky, 1993); (d) modulation of intracellular signaling pathways (gap junction communication); (e) stimulation of the immune system (Bertram, 1993) and (f) modulation of various types of receptors or adhesion molecules and many other physiologically significant processes (Palozza *et al.*, 2009).

Carotenoids present in fresh plant tissue are very stable. However, they are susceptible to degradation in the presence of oxygen, heat and light. Depending on the applied temperature and time of processing conditions, carotenoids can be partially isomerized or totally degraded (Marx *et al.*, 2003). Further explanation is discussed later in the text.

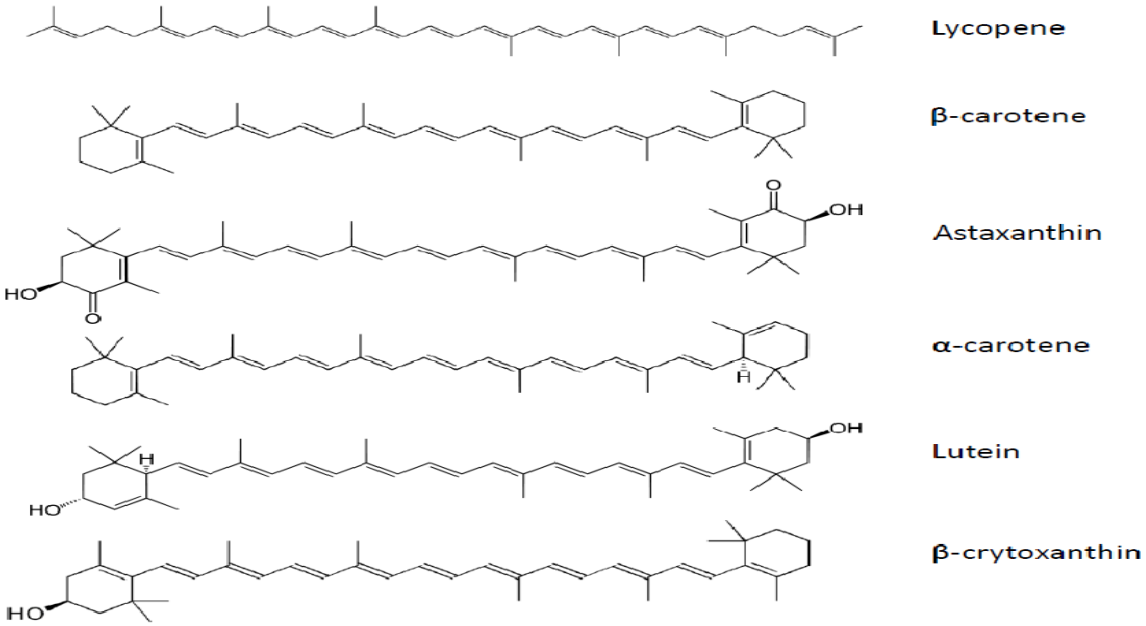

Figure 2.1: Chemical structures of Carotenoids.

2.3.2 β-carotene

β-carotene belongs to the hydrocarbon group of carotenoids and is the most widespread in foods. It is composed of eight isoprenic units with specific end groups or two β-ionone rings (Pénicaud *et al.*, 2011). β-carotene is lipophilic and soluble in organic solvents such as hexane, petroleum ether etc. Its physical, chemical and biological properties are mainly derived from its sequence of conjugated double bonds (Pénicaud *et al.*, 2011). In biological systems, the all-*trans β*-carotene (E-isomer) is the predominant isomer. However, in living organisms and food samples, *cis*-isomers are mostly found (O'neil and Schwartz, 1992; Stahl *et al.*, 1992) among them are 9-*cis*-, 11-*cis*, 13-*cis*-, and 15-*cis*-*β*-carotene (Z-isomers) (Figure 2.2).

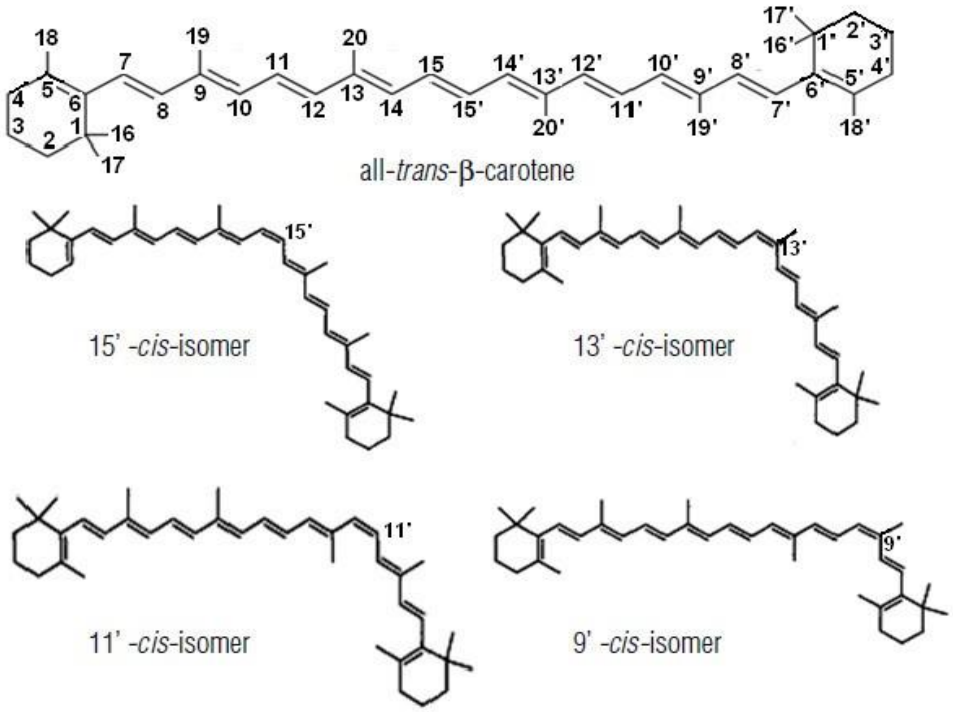

Figure 2.2: Structure of β-carotene and its four cis-isomers

Adapted from (Jing *et al.*, 2012)

β-carotene is valuable due to its nutritional benefit as a precursor of vitamin A and its antioxidant property. It is a vitamin A precursor because of the fact that it is stoichiometrically equivalent to two molecules of retinol (Mohamed *et al.*, 2001; Rodrigez-Amaya, 2001)). The β- carotene dioxygenase enzyme catalyze the breakdown of β-carotene in the walls of the small intestine to form retinal. Retinaldehyde reductase further reduce the retinal aldehyde by the addition of hydrogen atom to form the alcohol, retinol in the intestines (Figure 2.3) (Green and Fascetti, 2016). Vitamin A is essential for many functions in the human body; in particular, it helps in normal growth and development, improves immune function and vision. Vitamin A is present only in animal products (Grune *et al.*, 2010), thus vitamin A requirement from plant foods are met by carotenoids.

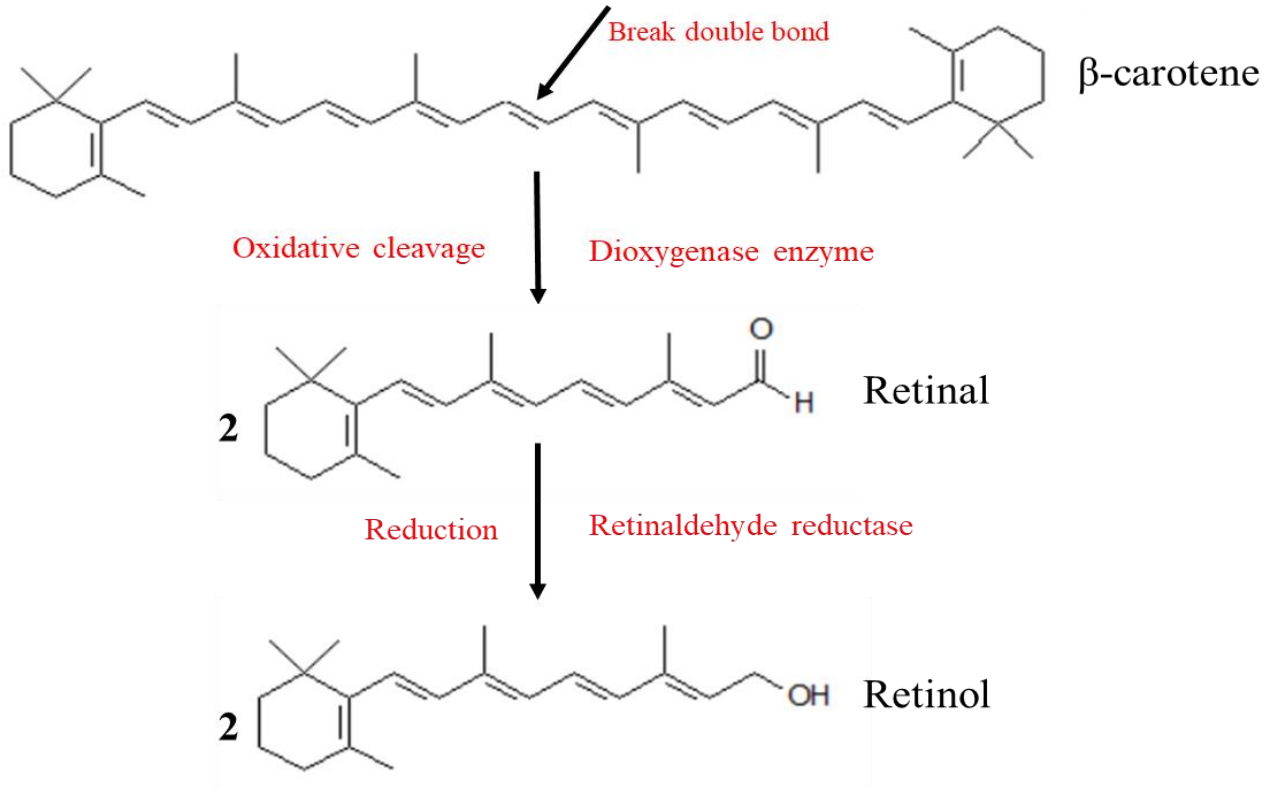

Figure 2.3: Conversion of β-carotene to retinol

Adapted from (Green and Fascetti, 2016)

2.3.3 Antioxidant properties of carotenoids

Carotenoids are known to be strong antioxidants that scavenge singlet oxygen free radicals (Foote and Denny, 1968). The beneficial effects of carotenoids attributed to their antioxidant activity include: prevention of lipid peroxidation, antiaging and anticancer properties, protection against cardiovascular diseases etc. (Alves-Rodrigues and Shao, 2004). According to Shimidzu *et al.* (1996) carotenoids act against free radicals through physical quenching by direct transfer of energy between both molecules and chemical reaction. The energy of singlet molecular oxygen is transferred to the carotenoid molecule to yield ground state oxygen and a triplet excited carotene. Since the carotenoids remain intact during physical quenching of singlet oxygen or excited sensitizers, they can be reused several fold in such quenching cycles. The efficacy of carotenoids for physical quenching is related to the number of conjugated double bonds present in the molecule which determines their lowest triplet energy level. Carotenoids may scavenge radicals in an initial step that involves one or more of the following three possibilities namely, electron transfer, allylic

hydrogen abstraction, and addition as shown in Figure 2.4 (Polyakov *et al.*, 2001; Kiokias and Gordon, 2004).

$$CAR + ROO^{\cdot} \rightarrow CAR^{\cdot+} + ROO^{-} \quad \text{(Electron transfer)}$$

$$CAR + ROO^{\cdot} \rightarrow CAR^{\cdot} + ROOH \quad \text{(hydrogen abstraction)}$$

$$CAR + ROO^{\cdot} \rightarrow ROOCAR^{\cdot} \quad \text{(Addition)}$$

Figure 2.4: Steps in radical scavenging by carotenoids (CAR)

β-Carotene and structurally related carotenoids have triplet energy levels close to that of singlet oxygen enabling energy transfer (Stahl and Sies, 2003). β-carotene also exerts antioxidative functions such as quenching singlet oxygen and also trapping propagation peroxyl radicals (Yamauchi *et al.*, 1998). According to Yamauchi *et al.* (1998), during chlorophyll-sensitized photo-oxidation of methyl linoleate, β-carotene reacts with singlet oxygen leading to the formation of β-carotene 5,6-epoxide and β-carotene 5,8-endo-peroxide. In addition, Krinsky (1979) demonstrated that, β-carotene is capable of inhibiting free radical-induced oxidation in liposomal lipids.

In a carotenoid system, the antioxidant activity depends on the oxygen tension present (Burton and Ingold, 1984). In most tissues under physiological conditions whereby there is low partial pressure of oxygen, β-carotene is found to inhibit the oxidation. In contrast, the initial antioxidant activity of β-carotene is followed by a prooxidant action at high oxygen tension (Palozza, 1998; Stahl and Sies, 2003).

2.4 Gluten and gluten free pasta production

2.4.1 Gluten pasta production

Pasta is a popular food product in many parts of the world due to its convenience, palatability, long shelf life and nutritional properties. Pasta is conventionally made from durum wheat semolina. The use of semolina as an ingredient and a sequence of hydration, mixing, forming and drying steps during processing is used for the production of pasta and plays a significant role in determining

the quality of the final product (De Noni and Pagani, 2010). Protein quantity and quality (gluten) (in terms of its viscoelasticity) are necessary to produce a suitable pasta with an optimal cooking performance (Ames *et al.*, 1999). Gliadin is responsible for the extensibility of the dough, and the elastic property of the dough depends on the glutenin in gluten (Sissons *et al.*, 2007). Gliadin is a monomeric protein that is stacked together with peptide bonds and disulphide bridges. During the manufacturing of pasta, these disulphide bridges break and the gliadin molecule unfolds when it comes into contact with water. The disulphide bridges link with glutenin to form new bridges (Wagner *et al.*, 2011). These new bridges make the gluten network stronger forming large and insoluble aggregates (as shown in Figure 2.5) which is important in the manufacturing of gluten pasta (Haraldsson, 2010).

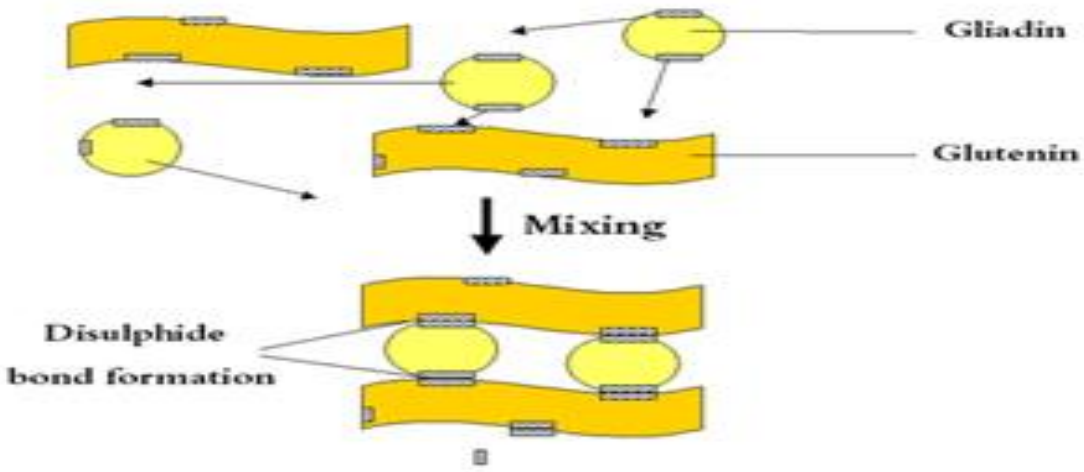

Figure 2.5: Establishment of a gluten network via disulphide bond formation

Adapted from (Haraldsson, 2010)

Durum pasta processing and the conventional way of pasta production consist of three main unit operations as shown in Figure 2.6:

a) Hydrating, mixing and kneading of flour with water to make a dough

b) Extrusion of dough at room temperature or forming sheet of dough to form different shape and size followed by cutting

c) Drying of pasta.

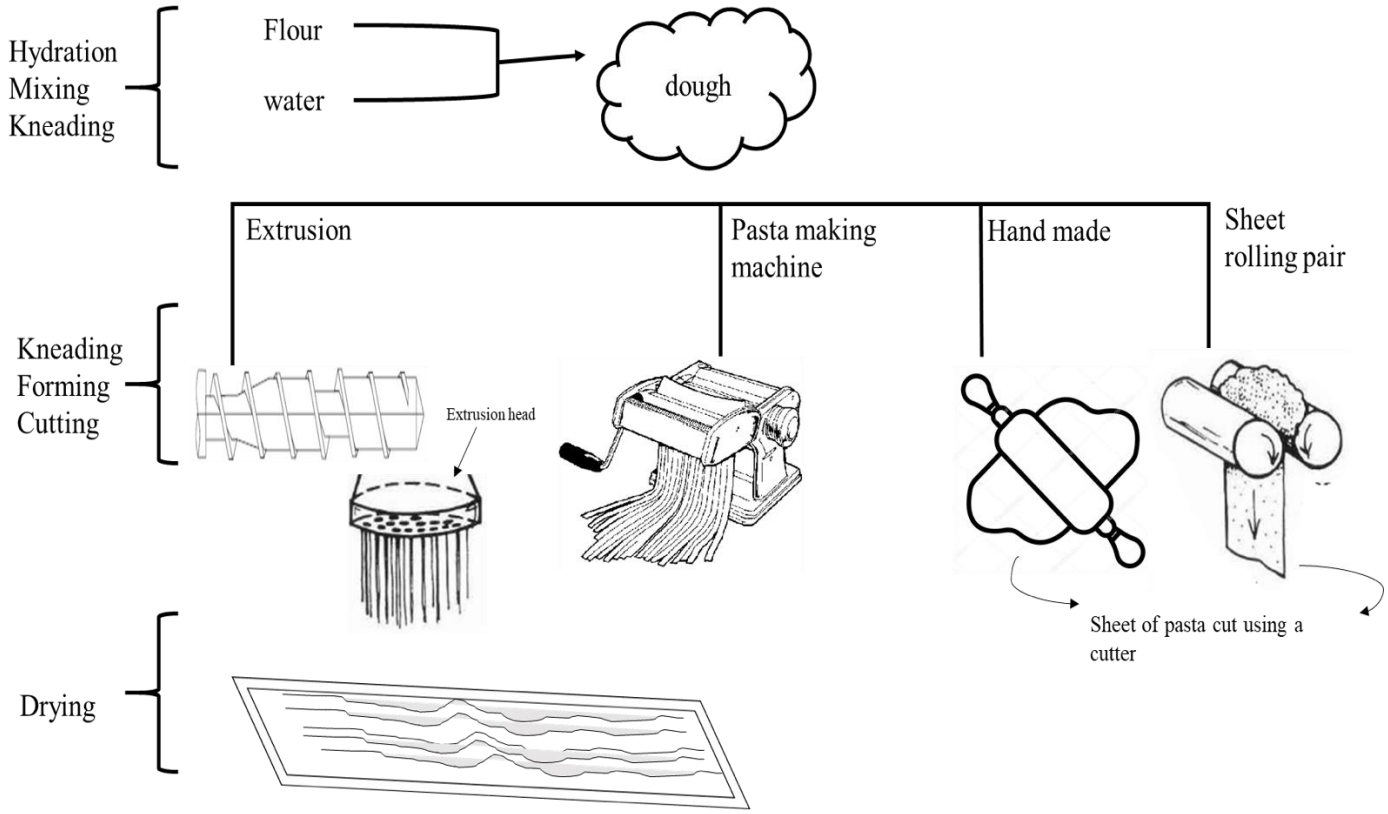

Figure 2.6: Diagram of the steps of production of conventional gluten pasta

Adapted from (Pagani *et al.*, 1989).

The initial step for the conventional production of semolina pasta is the raw material dosing. This is the dough formation step where water is added to the flour mixed and kneaded to form a uniform dough. The mixing-kneading action produces homogeneous hydrated dough where water diffuses evenly into the centre of the granules (Pollini *et al.*, 2012). According to Icard-Vernière and Feillet (1999) the hydration of semolina flour also promotes molecular mobility of its components before dough development, enabling biochemical modifications of proteins and their interactions. Water in a proportion of about 18-25 % of the dry material is added to form a dough containing an average of about 30-32 % moisture. Factors such as the semolina composition (protein, starch and fibre content) are considered in determining the water: semolina ratio.

The amount of water used in pasta processing should be optimized to achieve pasta colour and textural characteristics according to market preferences (Fu, 2008; Hou *et al.*, 2010). Over-hydration (>25 % moisture) results in soft and sticky pasta because it requires more energy to dry leading to pasta stretching and sticking together. Under-hydration (<18 % moisture) leads to the

production of pasta with rough surface and cracks since it requires more energy that is, high pressure and friction to be extruded, resulting in the generation of heat leading to pasta breakage (Manthey and Twombly, 2006).

After hydration, mixing and kneading, semolina dough can be formed into various shapes of pasta by cold extrusion, pasta making machine or hand made. This extrusion process is mostly without heat or with a minimum heat of less than 50 °C. This involves the operation of a screw inside a barrel that transports the pasta dough along the screw concomitant with increasing compression or pressure to stimulate dough development and a die that restricts the flow of the dough and further shapes the product (Sissons, 2004). The lack of excess thermal energy given to the dough could result in no damaged starch granules or protein matrix (Sissons, 2004) or any starch pregelatinisation. On the other hand, some pasta can be processed using the pasta making machine, roll sheeting and handmade sheeting. Pasta making machine, roll sheeting and handmade sheeting process further knead the dough by compressing between a series of rotating cylinders pairs with decreasing roll gaps and forming a sheet without tearing the surface (Petitot *et al.*, 2009; Pollini *et al.*, 2012). The sheet is folded several times favouring the cross-linking of gluten protein (Feillet *et al.*, 1977). The sheet obtained is then cut into strands of the desired width and length.

The drying step is the last and an important step in the production of pasta since it improves the product colour, control of micro-organisms and prolongs the shelf life of the product (Stefanis and Sgrulletta, 1990). The principle involves passing a current of hot or warm air over a fresh pasta product so that the air progressively decreases product moisture.

2.4.2 Gluten free pasta production

In order to cater for consumers that are gluten-intolerant, gluten-free pasta products are being produced and for consumers who for health issues wish to exclude gluten-based products from their diet. Gluten-free pasta is normally produced from refined flour and starch from rice, corn, potato and other tubers. To increase the quantity and quality of products for celiacs, Alvarez-Jubete *et al.* (2010) stated that the diversity of gluten-free raw materials with nutrient dense cereals such as pseudo cereals, amaranth, buckwheat and quinoa is being used recently.

Gluten-free pasta is a most challenging product to formulate and produce since it lacks gluten (Marti and Pagani, 2013). As a result, starch is a key component in gluten free pasta because the reorganization of its macromolecular structure provides good texture and overall quality (Lucisano *et al.*, 2012). Formulating gluten-free pasta requires knowledge of the starch characteristics of the gluten free flour and the appropriate additive such as gums and emulsifiers to be used to produce a cohesive mass in the product (Marti and Pagani, 2013). Bhattacharya *et al.* (1999) reported that to ensure good cooking qualities in terms of lower cooking loss and good texture qualities, starch for gluten-free pasta products should have the tendency to retrograde.

Gluten-free pasta can be processed by using the conventional method (cold extrusion) or high temperature extrusion processing. The conventional method for pasta production involves a discontinuous process mainly based on heat treatment and cooling of flour and subsequently mixing, kneading, cold extrusion and drying as shown in Figure 2.7. The heating involves the pregelatinization of starch and then the cooling for retrogradation. The heat-treatment of flour has been studied to mimic the viscoelastic properties of the gluten by forming three dimensional network which implies the high degree of gelatinization and retrogradation, the latter being responsible for network stabilization (Mariotti *et al.*, 2011). The cooking quality of the pasta therefore depends on the degree of starch gelatinization (Marti and Pagani, 2013) and its tendency to retrograde (Mariotti *et al.*, 2009). After heat treatment, the dough is extruded to shape and form at a minimum temperature usually less than 50 °C. Pasta is further dried to attain a moisture content less than 12 %.

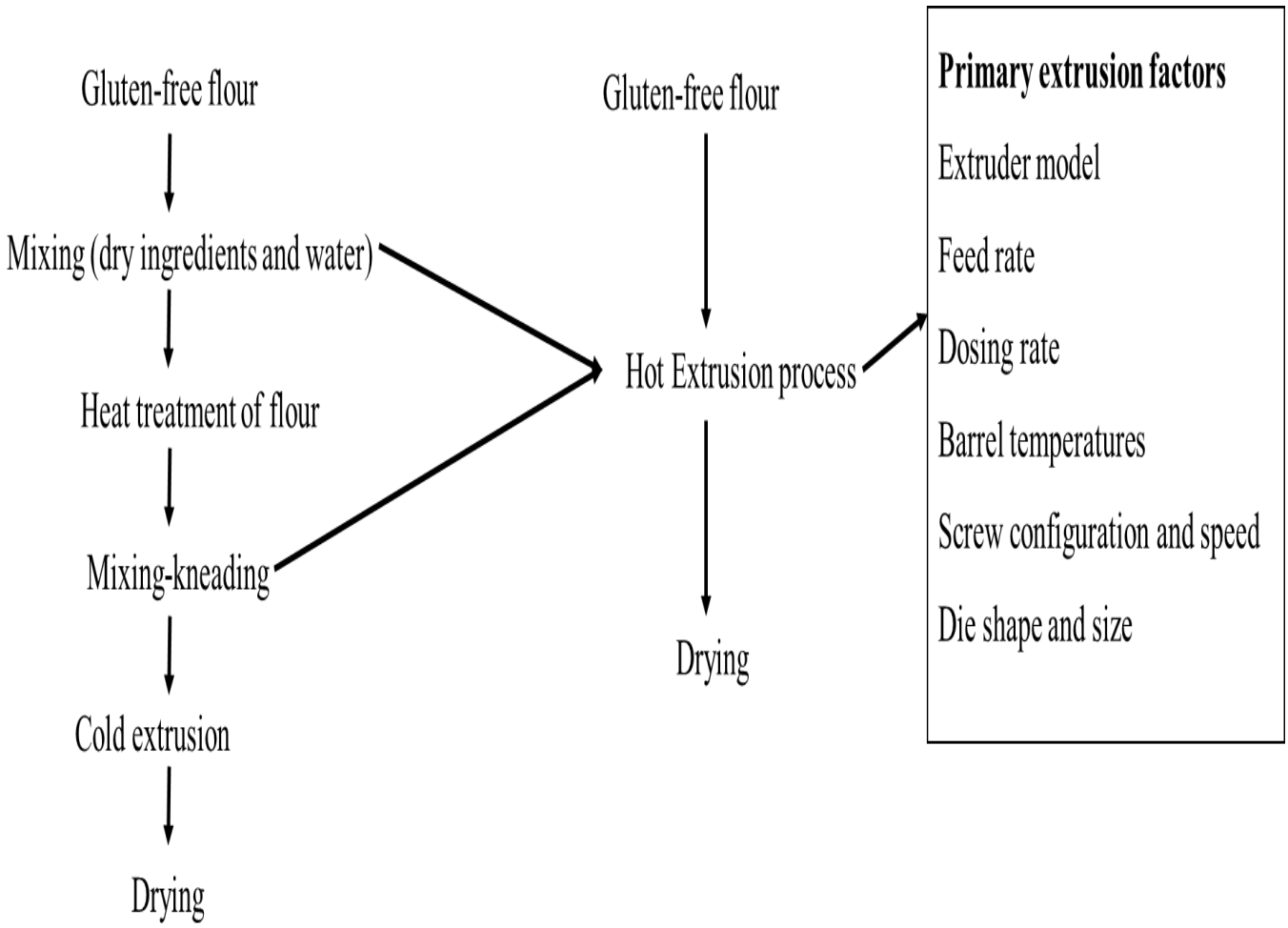

Figure 2.7: Steps in the production of conventional (cold extrusion on the left) and hot extrusion (on the right) gluten-free pasta

Adapted from (Aastha *et al.*, 2017).

Due to the discontinuity of the conventional method, the use of extrusion process, which is a continuous processing method where raw material mixing, heating and kneading happens at the same time in the extruder barrel with a high temperature and short time operation has become a suitable technology for the production of pasta. Many researchers have reported production of pasta with high quality in terms of firmness, flavour and texture after cooking by using extrusion processing as compared to conventional method (Wang *et al.*, 1999; Marti *et al.*, 2010).

2.5 Extrusion technology for pasta manufacturing

2.5.1 Principles of extrusion for pasta manufacturing

Extrusion can be used to manufacture expanded snacks and breakfast cereals. This extrusion is generally known as extrusion cooking and can be used to produce instant products. Extrusion cooking is a high-temperature, short-time process in which starchy and/or proteinaceous food materials are moistened, expanded, plasticised and cooked in a tube by a combination of temperature, pressure, mechanical shear and moisture, resulting in molecular transformation and chemical reactions (Castells *et al.*, 2005; Singh *et al.*, 2007b). Extrusion processing is preferred over other processes because it is a one-step process which can carry out a number of operations in one equipment requiring less labour. The extruder with slight modification can be used to achieve different objectives or for processing many different products (Berk, 2013). Due to its relatively short retention time, it is advantageous for vulnerable food and feed as exposure to high temperatures for only a short time will prevent rapid denaturation effects on vitamins, starches and proteins (Moscicki, 2011).

The energy expenditure used for extrusion cooking is usually lower than other alternative processes because the major part of the energy (heat and mechanical work) is delivered to the product directly and not through an intermediary medium (Guy, 2001; Berk, 2013). Its applications include; nutritious precooked food mixtures for infant feeding, increasing numbers of ready-to-eat cereals; indirect expanded products; co-extruded snacks; salty and sweet snacks; texturised meat-like materials from defatted high-protein flours; an expanding array of dry pet foods and fish foods; croutons for soups and salads and confectionery products (Harper, 1989; Eastman *et al.*, 2001). Some beneficial effects of extrusion cooking include; gelatinization of starch, reduction of lipid oxidation, increased soluble dietary fibre and destruction of antinutritional compounds (Singh *et al.*, 2007b). Bhandari *et al.* (2001) reported that extrusion process denatures undesirable enzymes, improves on the natural flavours and colour of the product.

2.5.2 Steps and parameters used in the production gluten-free pasta by extrusion process

The main role of extrusion is for conveying and shaping fluid forms of processed raw materials, such as pastes and doughs. An extruder can be a single screw extruder or a twin-screw extruder. Twin-screw extruders have greater flexible modular configuration of screw and have greater control of parameters to achieve the desired product as compared to the single screw extruder. The only disadvantages of the twin screw extruder are its high cost and complexity (Berk, 2013). These rotating screws transport and mix the ingredients to a uniform dough-like mass, which passes through a die which is designed in the desired shape (Björck *et al.*, 1984).

In the production of gluten free pasta, the processing units include: conveying, mixing, shearing, melting and plasticization and shaping (Guy, 2001).

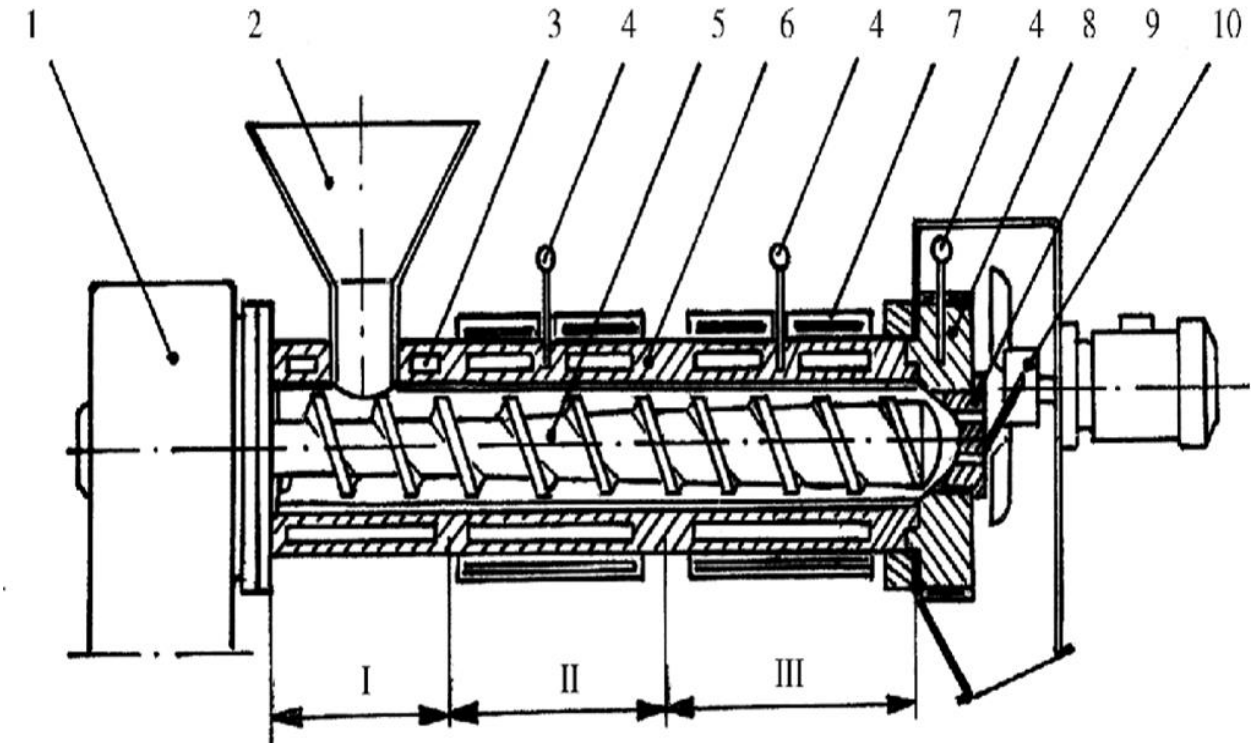

Figure 2.8: A cross-section of a single-screw extrusion cooker

1- engine, 2- feeder, 3- cooling jacket, 4- thermocouple, 5- screw, 6- barrel, 7- heating jackets, 8- head, 9- die, 10-cutter, I- transport section, II- compression section, III- melting and plasticization section (Moscicki *et al.*, 2013).

During the process, the gluten free flour is subjected to intense mechanical treatment extruding in a continuous nature through the action of one or two rotating screws as its main operating body (Moscicki, 2011). The flour in the feeder (2) (Figure 2.8) is fed into the barrel (6). Flour first enters the transport section (I) where it is conveyed by the rotating screw and mixed as the flour is being dosed (5) into the compression section (II). In the compression section, the mixture is compressed and heated with the heating zones (7) at different and high temperatures. The cooling jacket (3) is there to prevent over heating by stabilizing the desired temperature. After the compression section, the mixture is further carried into the melting and plasticization section (III). In this section, the objectives of the extrusion process such as melting, texturization, kneading, chemical reactions etc. occur through shear and mixing. The mixture which has formed into a dough is then shaped and escapes through the die (9) with a design shape. The shaped pasta is further dried. It is noted that the extrusion condition during pasta manufacturing is less severe compared to expanded snacks or breakfast cereal manufacturing. This is shown in Table 2.3. The lower temperature and lower screw speed for gluten free pasta manufacturing ensure better quality in terms of lower degree of solid loss during cooking of the pasta.

2.5.3 Macro structure, molecular structure and cooking qualities of gluten free pasta produced by conventional and extrusion process.

The kind of processing used for the kneading and forming of pasta greatly affects the macro and molecular structure of the pasta affecting its cooking qualities. In conventional processing for gluten free pasta production, the heating treatment of dough promotes a loss of native granular structure of starch and an extensive reticular and fibrillar network after cooling (Resmini and Pagani, 1983). Furthermore, the insufficient mixing and lower temperatures used, do not allow the complete development of gelatinized starch and starch protein network preventing protein coagulation and exhibiting visible starch granules (Resmini and Pagani, 1983). The biopolymer interactions such as starch-starch and starch-protein interactions may not be evenly distributed as a result of insufficient shear rate in mixing.

On the other hand, concerning the starch fraction, the higher temperature and mechanical stress process associated with the screw turning used during modern extrusion promotes a faster rate of starch gelatinization, even distribution and disruption of the starch granules (Karim *et al.*, 2000).

Differential scanning calorimetry measurement reveal a lower gelatinisation enthalpy of extruded pasta compared to non-extruded pasta (Vansteelandt and Delcour, 1998; Zweifel *et al.*, 2000). In extrusion process, the addition of heat and water causes starch gelatinization where, water penetrates into the amorphous region of the starch granule, which swells, and loses its crystallinity (Eliasson, 2004). Due to the higher mechanical shear, there is total degradation of the starch granules (Li *et al.*, 2014b) as shown in Figure 2.9, but the shear should not be too high to cause excessive starch depolymerisation.

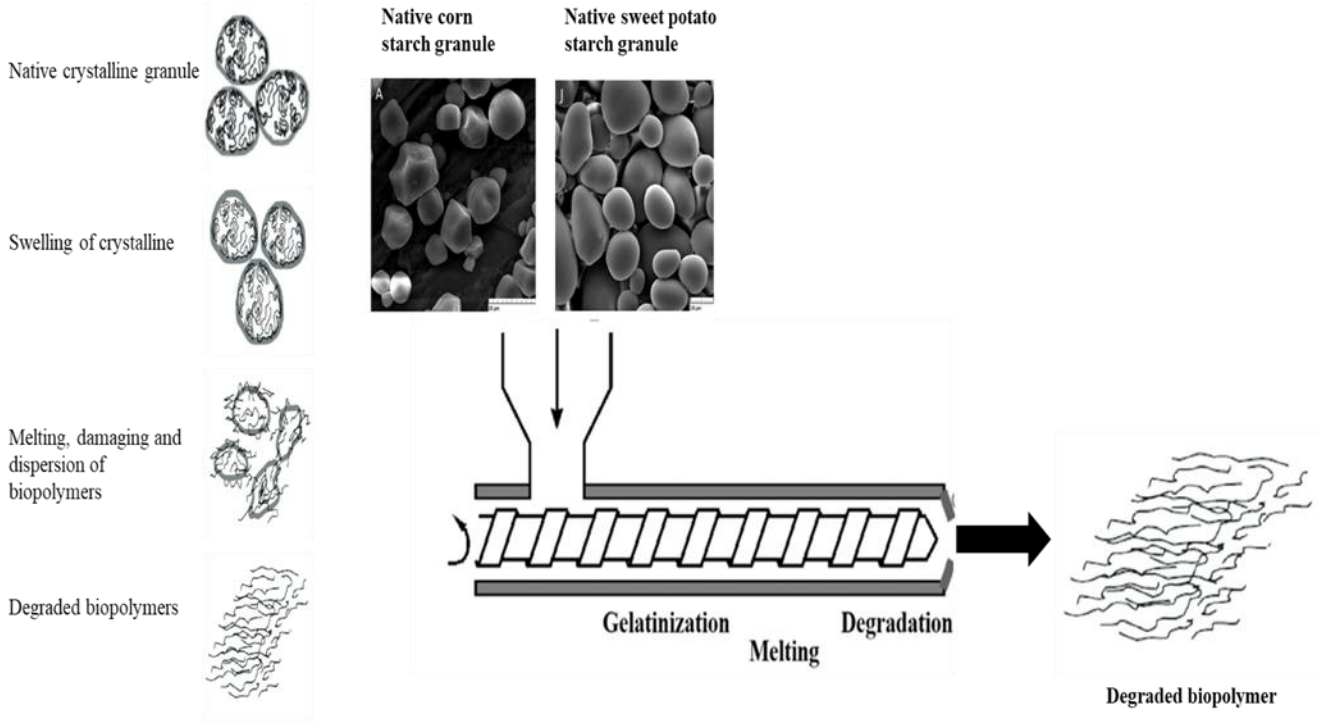

Figure 2.9: Stages of transformation of starch during extrusion cooking

Adapted from (Sujka and Jamroz, 2013; Méndez-Hernández *et al.*, 2018; Ye *et al.*, 2018a).

After heating, retrogradation occurs and starch polymers show a tendency to re-associate in an ordered structure, resulting in a new rearrangement (Atwell, 1988). The internal structure could exhibit relatively few swollen starch granules of irregular size and shape and discontinous protein matrix, aligned along the direction of flow of extruded gluten free pasta (Wang *et al.*, 1999).

2.6 Cooking qualities of pasta manufactured by extrusion

Essentially, cooking quality of pasta depends on the type of raw materials used and their intrinsic characteristics, and also the processing conditions (Cubadda and Carcea, 2003). High-quality pasta is characterized by good cooking resistance, high water absorption capacity, firmness, low stickiness and a limited release of soluble materials into the cooking water (Mariotti *et al.*, 2011). Summary of the effect of extrusion on the cooking qualities of gluten free pasta is shown in Table 2.3.

Comparing the cooking qualities of gluten-free pasta with conventionally made pasta, excessive shear and high temperature and low moisture extrusion may result in lower cooking time, higher cooking loss and lower water absorption capacity of gluten free pasta as compared to conventionally made pasta (Pagani *et al.*, 1989). The high temperature and high shear used during extrusion cooking leads to the depolymerization of starch molecules to form low molecular weight dextrins and the latter can leach out leading to high cooking loss. The higher amount of starch already gelatinized and partially depolymerised starch molecules during extrusion processing at high temperature showed that starch granules are weakened (Wójtowicz and Mościcki, 2009). This results in the absorbed water not held by the pasta strand resulting in lower water absorption capacity (Camire *et al.*, 1990). Thus it is important to have an optimal condition for extrusion process.

Table 2.3: Cooking qualities of extrusion cooked gluten free pasta

Raw materials	**Extrusion conditions**	**Effect on cooking qualities**	**References**
Rice flour	Extruder: single screw Temperature: 120 °C, 50 °C Moisture: 40 g/100 kg Screw speed: 25 rpm	Cooking time: 9 min for extrusion cooked pasta, 11 min for conventional pasta Cooking loss: 9.8 % for extrusion cooked pasta, 10.3 % for conventional pasta WAC: 77.6 % for extrusion cooked pasta, 88.7 % for conventional pasta Firmness: 191 N for extrusion cooked pasta, 310 N for conventional pasta	(Marti *et al.*, 2013)
Corn flour, broad beans	Extruder: single screw Temperature: 80 °C, 90 °C, 100 °C Moisture: 28 %, 31 %, 34 % Screw speed: 60 rpm	8-13 min cooking time 28 %, 31 % moisture resulted in <12.5 % cooking loss. 34 % moisture content resulted in 12.5 % cooking loss. 34 % moisture content resulted in an increased in WAC	(Giménez *et al.*, 2013)
Rice flour, yellow pea flour	Extruder: single screw Temperature: 90 °C, 100 °C, 70 °C Moisture: 28 %, 30 %, 32 % Screw speed: 60, 80, 100 rpm	Higher cooking time (9 min) for 32 % moisture and 100 rpm Higher WAC for 32 % moisture, 60 rpm Cooking loss increased as moisture content increased Hardness decreased when moisture content increased	(Bouasla *et al.*, 2016)
Pea starch	Extruder: twin screw Temperature: 40 °C, 70 °C, 85 °C, 90 °C Moisture: 30-40 %, Screw speed: 100-200 rpm	Moisture content increased, cooking loss decreased WAC decreased as temperature and screw speed increased Firmness increased when moisture, temperature and screw speed increased	(Wang *et al.*, 2012)

2.6.1 Optimum cooking time and cooking loss

The cooking time of pasta affects the cooking qualities of pasta as studied by many researchers. Prolonged cooking time may lead to greater cooking loss and cause changes in the percentage share of the particular components in the dry matter at the same time. During cooking of gluten containing pasta manufactured by conventional method, starch absorbs water and gelatinises. The starch granules begin to swell, slowly become soft, elastic, smooth and transparent. Small debris and soluble components of starch granules continuously enter the water due to swelling constituting the cooking loss (Mu *et al.*, 2017). Also, there is leach out of easily soluble components in water and the greater extent among those are gelatinized starch and the products of its hydrolysis, oligosaccharides or simple sugars (Sobota and Zarzycki, 2013). In extruded gluten free pasta, the starch is already gelatinized therefore, pasta absorbs water faster resulting in lower cooking time.

Low amount of leached out materials indicates high quality of cooked pasta (Del Nobile *et al.*, 2005). High cooking loss, which indicates high starch solubility and low cooking tolerance, results in turbid water and a sticky mouth feel (Bhattacharya *et al.*, 1999). The stickiness of pasta surface could be influenced by the surface structure of the pasta strand, the protein content and amylose exuded onto the strand surface during cooking (Dexter *et al.*, 1985; Cunin *et al.*, 1995). Lower amylose and protein content of pasta increases stickiness (Del Nobile *et al.*, 2005; Gianibelli *et al.*, 2005). Gianibelli *et al.* (2005) observed that the stickiness of chick pea-fortified spaghetti was reduced when protein and amylose contents were higher.

Starch and protein exhibit completely different behaviours during cooking. The quality of pasta during cooking depends on the physical competition between protein coagulation into a continuous network and the swelling of starch (Resmini and Pagani, 1983). If protein coagulates first during cooking, it creates a strong network which traps starch materials leading to less swelling, dispersion and solubility resulting in a firmer pasta. On the other hand, the faster the swelling of starch, the slower proteins will coagulate resulting in discrete masses with no continuous network and starch materials will tend to disperse and become partly soluble by leaching out resulting in a softer and sticky pasta (Resmini and Pagani, 1983).

According to Yoenyongbuddhagal and Noomhorm (2002), water absorption capacity (WAC) is an important measured index, which depends on the amount of damaged starch and the weakness

of its granules. Lower water absorption capacity indicates poor quality of cooked pasta as it results in the chewy texture of pasta (Wang *et al.*, 2012). A study by Wójtowicz and Mościcki (2009) reported that there is a significant correlation for water absorption capacity of pasta with starch gelatinization. Thomas and Atwell (1999) explained that in the presence of heat and water, hydrogen bonds of starch granules holding the structure weakens, thus allowing the granules to absorb water and swell. When the high amount of starch is already gelatinized, the swelling power weakens and absorbed water is not held by a pasta strand (Camire *et al.*, 1990; Cunningham *et al.*, 2007). In gluten-free pasta, starch polymers are less able to be entrapped in the protein matrix because of the lack of gluten network, giving a final product with high losses during cooking (Marti and Pagani, 2013).

It is important to consider extrusion conditions to promote starch pregelatinisation to form a stable matrix compared to conditions that promote dextrin formation from starch. High moisture, low temperature and low screw speed promote lower amount of starch depolymerisation to form soluble dextrin compared to higher temperature, higher screw speed and lower moisture during extrusion processing. Marti *et al.* (2013) studied the effect of extrusion process on rice flour pasta. They reported that extrusion temperature at 120 °C with a single screw at 25 rpm used for the production of parboiled rice pasta samples recorded less cooking loss. This was because the parboiled and the extrusion parameters favoured the formation of a strengthened starch network involving the majority of starch macromolecules as exhibited by its low cooking loss. Wang *et al.* (2016) reported that the cooking loss of brown rice pasta decreased sharply with increasing temperature and screw speed. When the temperature was at 120 °C and the screw speed was at 120 rpm, cooking loss ranged from 6.7 % to 7.6 %, a range considered acceptable for semolina pasta (7-8 %) (Doxastakis *et al.*, 2007). They attributed this to the fact that higher temperature strengthened the stability of the retrograded starch network which in turn decreased cooking loss (Susanna and Prabhasankar, 2013).

2.7 Nutritional properties of extruded gluten free pasta

2.7.1 Effect of extrusion on starch digestibility

Thermal treatment in extrusion promotes a high degree of starch gelatinization, rupturing off the starch granules making its substrate more accessible facilitating amylolysis as compared to other

processing methods (Alonso *et al.*, 2000). Work done by Altan *et al.* (2009a) recorded an increase *in vitro* starch digestibility of extruded barley-tomato pomace. The shearing action and kneading in the extruder barrel develops heat through dissipation of mechanical energy causing loss of structural integrity inactivation of enzyme inhibitors, disruption of cellular structure, size reduction and increased starch surface, thus an increase in enzyme susceptibility (Björck and Asp, 1983; Altan *et al.*, 2009a).

On the contrary, Guha *et al.* (1997) reported a decrease *in-vitro* starch digestibility after extruding rice flour. They attributed the reduction in the digestibility to retrogradation or re-association of gelatinized starch, the formation of amylose-lipid complexes and starch-protein complexes. These complexes could reduce the susceptibility of starch to enzyme hydrolysis (Guha *et al.*, 1997).

2.7.2 Effect of extrusion cooking on protein

During extrusion, native proteins are denatured by shear forces (Bhattacharya and Hanna, 1988). When the temperature and shear force within the extruder is increased, the forces which stabilize the tertiary and quaternary structures of the proteins are weakened (Camire, 1991) resulting in individual protein molecules unfolding and aligning themselves with the flow of material towards the die (Harper, 1986). This realignment exposes bonding sites which lead to a cross-linking and reformed expandable structure that creates the chewy texture in fabricated foods. These changes are presented schematically in Figure 2.8.

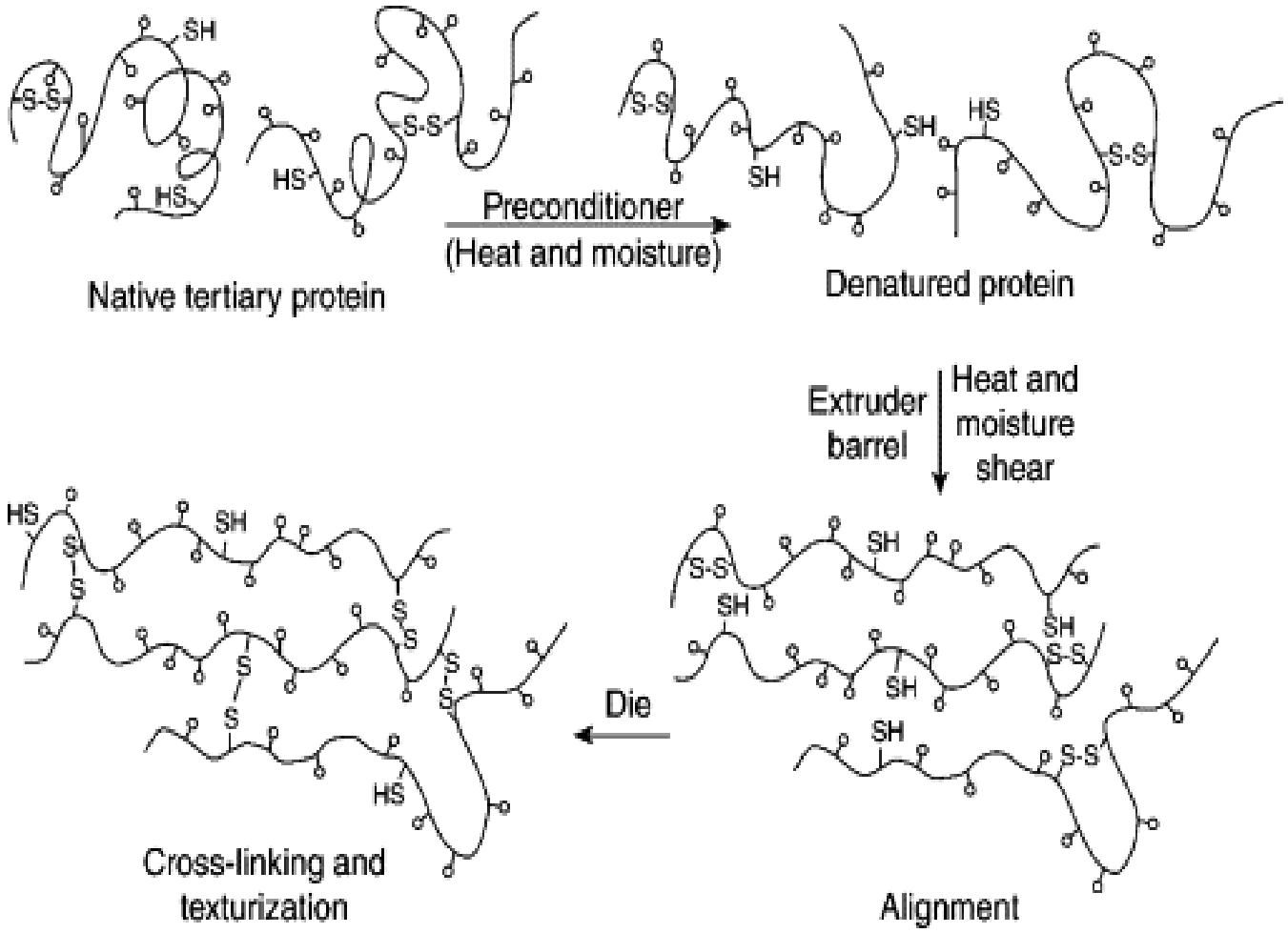

Figure 2.10: Schematic diagram of a protein molecule unfolding, aligning with flow in the extruder barrel and forming new bonds with another molecule.

Adapted from Riaz (2011)

Batterman-Azcona and Hamaker (1998) have shown that maize protein bodies are disrupted by extrusion cooking. A study done on pea flour demonstrated that shear, heat and pressure caused its protein bodies to join, forming a protein matrix during the extrusion process (Ben-Hdech *et al.*, 1991). Chen *et al.* (2011) determined the effect of extrusion cooking at low and high moisture content on the chemical cross-linking and molecular aggregation of soybean protein. The results showed that the structure and interaction of the extrudate were held by hydrophobic interactions, hydrogen bonds, disulphide bonds, regardless of the moisture content in the extruder, and also the contribution of noncovalent bonds outweighed covalent bonds.

Parameters used during extrusion cooking greatly influence protein digestibility. Some of the effects of extrusion variables on protein digestibility is shown in Table 2.4. Singh *et al.* (2007b) reported that extruded products record higher protein digestibility values than non-extruded products which was in agreement with a study by Alonso *et al.* (2000). This might be due to the denaturation of proteins and inactivation of antinutritional factors that impair digestion (Alonso *et al.*, 2000).

Table 2.4: Effect of extrusion variables on protein digestibility

Food source	Processing parameter	Protein digestibility	Reference
Buckwheat	Temperature	Increase in barrel temperature increases digestibility to 81.45 %	(Rayas-Duarte *et al.*, 1998)
Corn, millet , sorghum	Screw speed	Increasing screw speed increases digestibility	(Dahlin and Lorenz, 1993)
Lentil	Feed moisture	Increase in feed moisture increases digestibility to 75.38 %	(Rathod and Annapure, 2016)

Increase in extrusion temperature from 100 -140 °C promoted the inactivation of antinutritional factors that impairs digestion in buckwheat and consequently increased protein digestibility (Rayas-Duarte *et al.*, 1998). The increase in shear during extrusion of maize, millet and sorghum aided in the denaturation of proteins easily, thus facilitating enzymatic hydrolysis (Dahlin and Lorenz, 1993).

Extrusion at low feed moisture of 14 % significantly decreased the in vitro protein digestibility of lentil as compared to extrudate of higher feed moisture at 22 % (Rathod and Annapure, 2016). Lower feed moisture is reported to initiate non-enzymatic Maillard browning reactions (Bastos *et al.*, 2012) which has been reported to have a detrimental effect on protein nutritional value. Due to the protein denaturation during extrusion, previously hidden amino acids become exposed and are free to react with reducing sugars (Camire, 1991). This leads to the reduction of amino acids and subsequently the reduction in protein digestibility (Singh *et al.*, 2007a).

2.7.3 Effect of extrusion cooking on beta carotene content of foods

Carotenoids are very stable in fresh plant tissue, however, processing results in carotenoids becoming very unstable by the action of light, heat, moisture and oxygen (Killeit, 1994). Due to the fact that β-carotene contains a significant number of double bonds, it is very sensitive to degradation by oxidation in a range of reactions which include isomerization, production of radical species and partition of cleavage products (Figure 2.8) (Mordi, 1993; Pénicaud *et al.*, 2011).

Depending on the applied time and temperature conditions during processing, carotenoids can be partially isomerized or totally degraded or can resist degradation (Marx *et al.*, 2003). According to Marty and Berset (1990) the resistance of all-trans- β-carotene to high temperatures depends to a large extent on the conditions of the medium. These authors further stated that, limited breakdown of carotenoid molecules in their pure state is caused by prolonged heating at 180 °C, but the presence of some food constituents such as water and starch combined with mechanical mixing favoring the diffusion of oxygen, can lead to much higher loss.

The kinetics and mechanism of thermally induced isomerization of β-carotene have been studied by Von Doering *et al.* (1995). Thermal treatment of all-trans β-carotene at a temperature slightly below 100 ºC results in the formation of 13- and 15- cis- β-carotene whereas the 9-cis-isomer is formed above 100 °C (Von Doering *et al.*, 1995) as shown in Figure 2.8. According to Olson (1989) two primary oxidative reactions might occur in the conversion of carotenoids into vitamin A. Firstly, one might occur at the central 15, 15´double bond (central cleavage) resulting in two molecules of retinal, whereas the other might occur at one or more of the double bonds (excentric cleavage) which will result in one long and one short β-apo-carotenal by rupturing of the 7´:8´double bond.

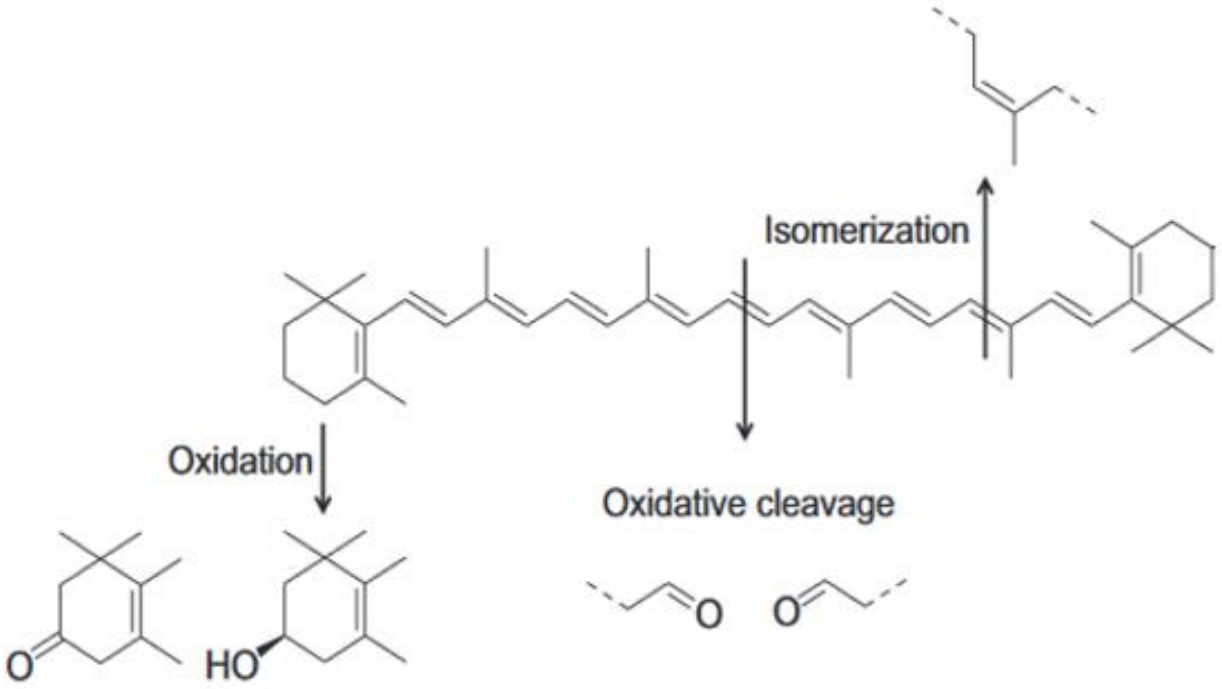

Figure 2.11: Possible degradation of β-carotene during extrusion

Adapted from (Carle and Schweiggert, 2016)

The stability or degradation of beta carotene during extrusion cooking is influenced by the following factors: raw material, mixing, conditioning, moisture, flow rate of the material, temperature, pressure, single screw versus twin screw extruder, screw speed, energy input, die size, expansion, dry extrusion versus wet extrusion etc. (Killeit, 1994; Riaz *et al.*, 2009). Some effects of extrusion conditions on beta carotene content in foods are given in Table 2.5.

Table 2.5: Effect of extrusion cooking variables on beta-carotene content of foods

Food source	Extrusion cooking variables	Effect on beta carotene content	Reference
Orange and cream sweet potato	Feed rate, screw speed	Lower feed rate and lower screw speed led to increased retention of material in the extruder and increased loss of beta carotene	(Fonseca *et al.*, 2008)
Wheat, all trans beta carotene powder	Temperature	Increase in temperature led to increased beta carotene loss	(Guzman-Tello and Cheftel, 1990)
Corn starch-Passion fruit pulp	Feed moisture, temperature	Feed moisture of 27 % and lower barrel temperature led to greater retention of beta carotene	(Cortés *et al.*, 2014)

The high temperatures that are produced during extrusion cooking lead to losses of the stable all trans β-carotene (which exhibit the greatest provitamin A activity) through isomerization to stereoisomers having lower provitamin A activity (cis isomers) (Guzman-Tello and Cheftel, 1990). Similar findings have been reported by Shih *et al.* (2009) who observed a decrease in the β-carotene content of extruded yellow and orange flesh sweet potatoes at high temperature. The lower feed rate and screw speed during extrusion of orange and cream sweet potato resulted in long retention time of material insider the extruder barrel, thereby exposing it to more heat and resulting in the loss of β-carotene (Fonseca *et al.*, 2008).

On the other hand, Waramboi *et al.* (2013) reported that flours from south Pacific sweet potato cultivars, retained carotenoids maximally at a rate of more than 80 % during extrusion at 120 °C, 40 % moisture and 300 rpm screw speed using a twin screw extruder. They further reported that, in order to ensure maximum retention of carotenoids in sweet potato, critical factors such as moisture content and screw speed with fixed screw configuration, barrel temperature profile and formulation should be well ensured. The retention of vitamins in extrusion cooking decreases with decreasing moisture, increasing screw speed, increasing temperature, decreasing throughput, decreasing die diameter and increasing specific energy input as reported by (Killeit, 1994).

2.7.4 Effect of extrusion processing on antioxidant properties of foods

Extrusion processing may positively or negatively affect the antioxidant properties of food products due to its high thermal processing conditions. High temperature with high shearing action inside the extruder barrel changes the structure of food matrices thereby affecting the functionality of antioxidant phytochemicals in the extruded products (Nayak *et al.*, 2015).

A study conducted by Sharma *et al.* (2012) reported an increase in DPPH free radical scavenging activity of extrudates from different cultivars of barley as compared to the control. The authors observed that dark pigmentations were produced during the extrusion process as a result of Maillard browning reactions. According to Manzocco *et al.* (2000) these Maillard reaction products are known to have reducing properties hence the observed increase in antioxidant activity. Thermal processing is also known to alter the antioxidant profile and generate more antioxidants that contribute to antioxidant activity (Sharma *et al.*, 2012). It has been reported that extrusion cooking increases the antioxidant properties of different varieties of sweet potato (Shih *et al.*, 2009), fruit powders from cranberries, raspberries, blueberries and concord grapes (Camire *et al.*, 2007) and cauliflower by-product (Stojceska *et al.*, 2008).

In contrast, reduction in antioxidant properties of extrudates have been reported by other researchers. The effect of extrusion on polyphenol content and antioxidant activity of common bean was investigated by (Korus *et al.*, 2007) who observed a significant decrease in polyphenol content and antioxidant activity. This was in agreement with an observed decrease in total polyphenols and antioxidant activity during extrusion of bean: corn mixture as reported by (Delgado-Licon *et al.*, 2009). Also the antioxidant activity of extruded barley was reduced by 60-68 % as compared to the unprocessed barley flour (Altan *et al.*, 2009b) and similar losses have been reported in extruded sorghum (Dlamini *et al.*, 2007). The loss of antioxidant properties has been mainly attributed to decomposition at elevated temperatures as reported by (Hamama and Nawar, 1991).

2.8 CONCLUDING REMARKS

- Orange flesh sweet potato is an important plant source of beta-carotene content and therefore has great potential to be used to fight vitamin A deficiency.
- Pasta has been successfully produced from maize flour. However, compositing with OFSP flour to increase the nutritional content has not been studied.
- Extrusion processing can cause thermal decomposition of beta-carotene and affect antioxidant properties.
- Gluten free pasta produced by extrusion processing has been shown to exhibit good cooking qualities.
- If used in pasta production, the composition of OFSP such as starch, protein, starch and fibre can affect the cooking qualities of the pasta.
- Several studies have focused on the production of gluten free pasta with cereals using conventional methods and other extrusion technologies but there are limited studies on the use of a high beta-carotene tuber crop (OFSP) and a twin-screw extruder for the production of gluten free pasta.
- Therefore, there is the need to evaluate whether maize: OFSP flour can be extruded to produce pasta with improved nutritional qualities in terms of *in vitro* starch and protein digestibility, beta-carotene content and antioxidant properties while maintaining good cooking qualities of the pasta.
- The cooking quality of gluten free pasta as related to the microstructure is not well understood.

CHAPTER 3: HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

1. An increase in proportions of orange fleshed sweet potato flour in maize-orange fleshed sweet potato composite pasta will have negative effect on the functional properties of the pasta in terms of higher cooking loss, lower water absorption capacity, lower firmness and higher stickiness of the pasta.

   Granule size, internal granular organisation, molecular weight and amylose content all affect gelatinization of starch (Banks and Greenwood, 1975). A major factor controlling swelling is the strength of the internal structure of the granule and the amylose content. The stronger the internal molecular structure, the higher the temperature required for gelatinization (Tian *et al.*, 1991). Maize has a stronger internal structure of starch granules than sweet potato due to the high fibre content in OFSP (Tian *et al.*, 1991). The high fibre content may weaken the internal structure of pasta samples composited with OFSP thus resulting in higher cooking loss, lower firmness and higher stickiness of pasta.

2. Maize – orange-fleshed sweet potato pasta produced using extrusion cooking will have lower beta-carotene content and antioxidant properties.

   High temperature extrusion conditions will lead to cis-trans isomerization (Dutta *et al.*, 2006) and thermal decomposition (Hamama and Nawar, 1991) of beta-carotene which will then reduce antioxidant properties of the pasta. Naturally occurring trans-isomers which are linear are converted to kink cis-isomers during thermal processing (Kalt, 2005) and there can also be thermal fragmentation of beta-carotene as well as oxidative degradation.

3.2 Objectives

1. To determine the effect of compositing maize flour with different levels of orange-fleshed sweet potato flour on the functional properties of extruded pasta with the aim of producing a good quality gluten-free pasta.
2. To determine the effect of extrusion cooking on the beta-carotene content and antioxidant properties of maize – orange-fleshed sweet potato pasta with the aim of producing a gluten-free pasta with enhanced nutritional and health-promoting properties.

CHAPTER 4: MATERIALS AND METHODS

Figure 4.1 shows the experimental design for the production and analysis of extruded pasta composites from maize and orange-fleshed sweet potato flour. The composited flours used were 100 % maize, 80 % maize: 20 % OFSP, 70 % maize: 30 % OFSP and 50 % Maize: 50 % OFSP weight by weight. Commercial pasta made from combination of maize and rice was used a reference. Pasta was manufactured by extrusion process. The proximate composition, physicochemical properties, cooking properties, nutritional properties and microstructure of the cooked and uncooked extruded pasta composites were determined.

4.1 Experimental design

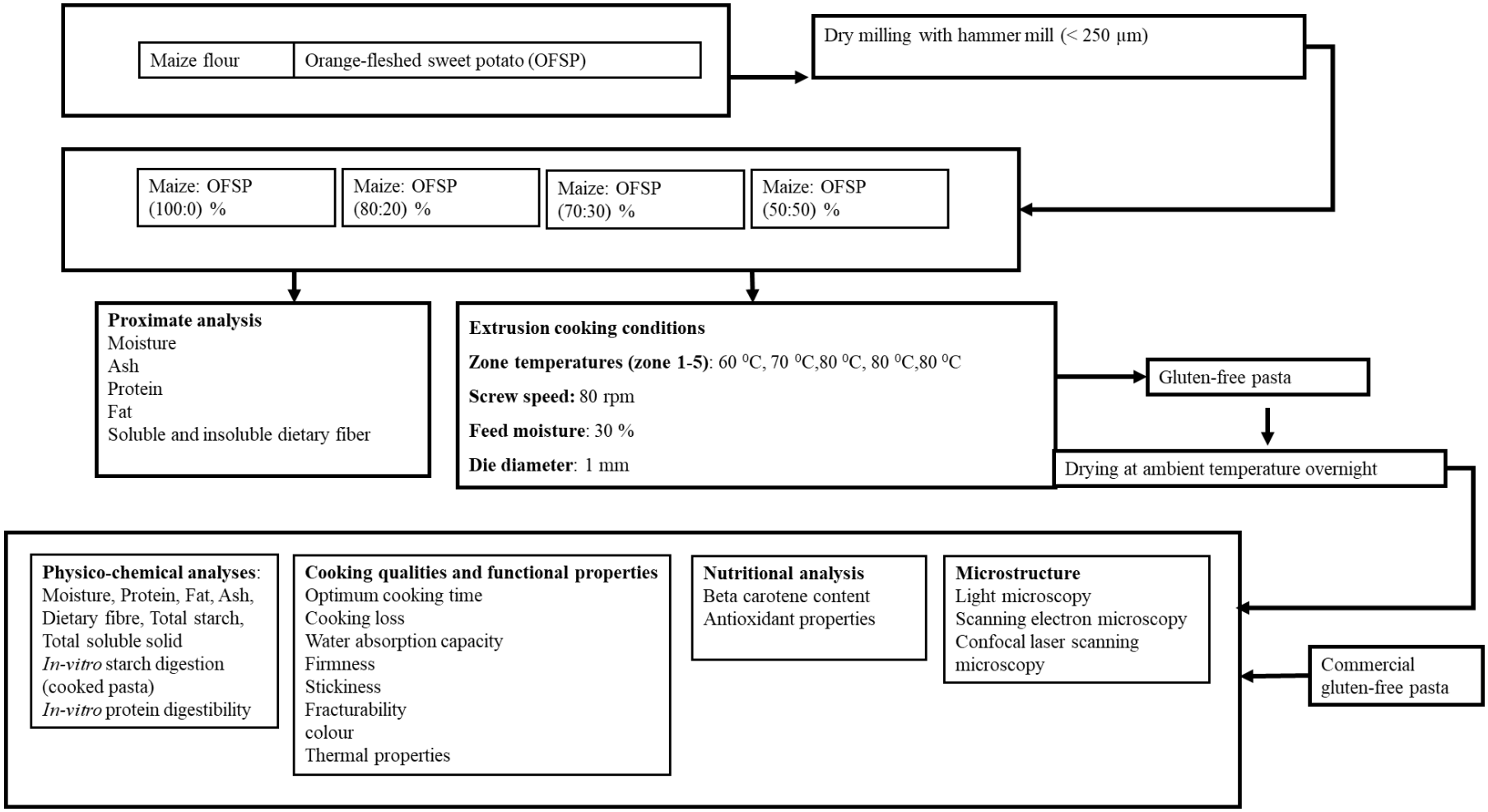

Figure 4.1: Flow diagram of experimental design for the production and analysis of pasta samples

4.2 Materials

White maize flour and orange-fleshed sweet potato flour were used in the study. Super-fine white maize meal was purchased from RCL foods (Pretoria, South Africa) and orange-fleshed sweet potato flour was purchased from Exilite 499 CC in Tzaneen (Limpopo Province, South Africa).

4.3 Methods

4.3.1 Milling

Maize and orange fleshed sweet potato flour were dry milled several times using a Perten Laboratory hammer mill 3170 to pass through a 250 µm screen. The flour samples were vacuum packaged and stored in the dark at – 4 °C for further analyses.

4.3.1 Composites formation

The maize and orange fleshed sweet potato were composited in the ratios 100:0, 80:20, 70:30, 50:50 (w/w) in an industrial bowl mixer (Talsa, Mix 90 ST, Zatamo, Spain). The bowl mixer has a dual blade paddle system that rotates clockwise and anticlockwise. The dual blade stirs and mixes the flour in different directions: one blade stirs the flour from the bottom to the top of the bowl while the other simultaneously stirs the flour from the top to the bottom of the bowl. The flour was mixed for a total of 30 minutes, 15 minutes on a clockwise rotation and another 15 minutes on an anticlockwise rotation. The flour composites were then stored in plastic airtight buckets and stored at a temperature of – 4 °C until further use.

4.3.2 Extrusion processing

Extrusion processing was done by using a TX 32 co-rotating twin-screw extruder (CFAM Technologies (Pty) Ltd, Potchefstroom, South Africa). The pre-prepared formulations (100 % maize flour, 80:20 % maize: OFSP, 70:30 % maize: OFSP, 50:50 % maize: OFSP) were subjected to extrusion processing. The barrel temperatures used were 60, 70, 80, 80 and 80 °C for zone 1, 2, 3, 4 and 5 respectively. The screw speed was set at 80 rpm with raw material feed rate at 5 kg/h. Water flow into the extruder was at a dose rate of 2.5 L/h (Calibration of the flour and moisture feeder was done before running the extruder by measuring the weight output per minute).

The extrudates (gluten-free pasta samples) were dried at ambient temperature overnight. The pasta samples were vacuum packaged and stored in an airtight bucket at room temperature for further analyses.

4.4 Analyses

4.4.1 Physicochemical analyses

4.4.1.1 Moisture

Moisture content was determined according to AOAC (2000), official methods of analysis 925.10 where 2 g of the composite flour and the extrudate samples were weighed into a pre- weighed moisture tin and dried in an oven at 103 °C for 3 hours. The difference obtained between the weight of the sample before oven drying and the weight of the sample after oven drying was used to determine the moisture content of the sample.

4.4.1.2 Protein

Protein in the composite flours and pasta was determined using the Dumas combustion method using the Gerhardt Dumatherm (Königswinter, Germany) according to AACC (2000) method of analysis 46.30. A value of N×6.25 was used as a conversion factor.

4.4.1.3 Ash

Ash content of the composite flour and pasta were determined by weighing 2 g of composite flour and pasta in pre-weighed ashing crucibles according to AOAC (2000), official methods of analysis 923.03. The composite flour and pasta was charred then ignited in a furnace at 550 °C for 6 hours until a light grey ash was obtained. The composite flour and pasta was cooled in a desiccator and the difference obtained from the weight of the sample before ashing and the weight of the sample after ashing were used to calculate the ash content of the samples.

4.4.1.4 Crude Fat

Fat content in the composite flour and pasta was determined by using the Soxhlet extraction method according to AOAC (2000), official methods of analysis 920.39. Crude fat analysis was carried out using petroleum ether as extracting solvent.

4.4.1.5 Soluble and insoluble dietary fibre

Dietary fibre of the composite flour and pasta was determined according to AOAC (2000) official methods of analysis 991.43 using the total dietary fibre Megazyme kit (K-TDFR). Flour sample (1 g) was dissolved in 40 mL of mes-tris buffer (0.05 M, pH 8.2) solution and 50 µl of thermostable α-amylase (3000 U/mL of ceralpha reagent at pH 6.5 and 40 ºC) was added to hydrolyze starch to dextrin at a temperature of 100 °C. Protease (100 µl) with an activity of 350 tyrosine U/ml (E-BSPRT) was added to solubilize protein. Amyloglucosidase (AMG) (200 µl), with an activity of 3,300 U/ml **(**E-BLAAM) was used to hydrolyze starch to glucose. The enzyme mixture and sample were filtered and residue washed with acetone and ethanol to obtain the insoluble dietary fibre (IDF) portion. Four volumes of ethanol heated at 60 °C was added to the filtrate and left to stand for 60 minutes to form the soluble dietary fibre (SDF) precipitate after which it was filtered. The soluble dietary fibre residues were washed with 78 % and 95 % (v/v) ethanol and acetone respectively. The IDF and SDF residues were dried overnight at 103 °C. One part of the soluble dietary fibre and insoluble dietary fibre residues were used to determine protein and the other part used to determine ash content to be used for the final calculations of soluble dietary fibre and insoluble dietary fibre values.

4.4.1.6 Soluble solids

A Digital Hand-held "Pocket" Refractometer PAL-3 (Atago co. Ltd, Tokyo, Japan) was employed to measure the total soluble solids of 10 % (w/v) sample suspension of raw flours and uncooked extruded pasta.

4.4.1.7 Total starch content

Total starch content of raw flours and uncooked extruded pasta was determined using the Megazyme Total starch Assay procedure K-TSTA 08/16 (Megazyme Ltd, Bray, Ireland, and Wicklow) which adopted the AOAC (2000) method 996.11. The Megazyme method (c) determines the total starch content of sample containing resistant starch, but no D-glucose and/or maltodextrins (KOH Format). 0.1 mL of thermostable α-amylase (3000 U/mL on ceralpha reagent at pH 6.5 and 40 ºC) (EC 3.2.1.1, CAS 9000-90-2) and 0.1 mL of amyloglucosidase (3,300 U/mL on soluble starch) (EC 3.2.1.3, CAS 9032-08-0) were used for the starch hydrolysis to glucose. Glucose was then quantified colorimetrically by glucose oxidase-peroxide reaction and the

absorbance was read at 510 nm using a spectrophotometer (T80+UV/VS) against a reagent blank. Total starch was then expressed as starch as a proportion (%) of total sample weight.

4.4.2 Determination of cooking quality parameters of cooked and uncooked extruded pasta

4.4.2.1 Optimal Cooking Time

The optimal cooking time (OCT) was determined according to Giménez *et al.* (2013). Dried pasta (25 g) was boiled in 250 ml distilled water. At 30-second intervals, a small portion (3-4 strands) of the pasta was removed from the boiling water and squeezed between two glass slides. Pasta was considered cooked when the white centre core disappeared.

4.4.2.2 Cooking Loss and Water Absorption Capacity

Water absorption (WAC) and cooking loss (CL) was determined according to Giménez *et al.* (2013) with slight modifications. For cooking loss determination, 25 g dried pasta sample was cooked in 250 ml boiling water. The cooking water was collected in a tared and pre-dried beaker; the content was dried to constant weight in an air oven at 100 °C. The residue was weighed and the loss during cooking was calculated as a percentage of the starting material.

For water absorption capacity, 25 g dried pasta samples was weighed and boiled in 250 ml water. During the cooking time previously determined, pasta was then removed and weighed; the weight difference before and after cooking was used to calculate the water absorption using the following formula:

$$\text{WA (\%)} = \frac{CPW - DPW}{DPW} \times 100$$

Where CPW = cooked (wet) pasta weight (g) and DPW = dried pasta weight (g)

4.4.2.3 Expansion ratio

Expansion ratio was determined as the diameter of extrudates divided by the diameter of the die exit (1 mm) (Gujska and Khan, 1991). Diameter of the pasta was measured using a vernier caliper.

4.4.2.4 Texture analysis

Texture Profile Analysis (TPA) was done according to Curiel *et al.* (2014) with slight modification. Firmness and stickiness of the cooked pasta samples was determined using an EZ Test (Model: EZ-L, Shimadzu, Tokyo, Japan) texture analyser equipped with a mini Kramer shear cell with a square probe (3 mm× 3 mm). Pasta samples were cooked till the optimal cooking time. The texture analyser was calibrated for a load cell at 200 N. Cooked pasta (20 g) was compressed once at rate of 30 mm/min at a ratio of 50 % with the compression probe. Firmness was measured at the maximum force (N) during compression and stickiness (N) was measured as the minimum force of the curve obtained. The test was repeated five times.

The fracturability of the cooked and uncooked pasta samples were analysed using two different methods. Raw pasta fracturability was evaluated using a texture analyzer with 3-point break rig. A pasta strand of 5 cm long was compressed at a test speed of 30 mm/min and the fracturability (N) was determined as the maximum peak force until the pasta strand fractures. Cooked pasta sample was evaluated using a mini compression Kramer shear cell with 5 blades. The test was repeated five times on each pasta sample.

4.4.3 Colour evaluation

The colour of the extruded raw and cooked pasta was instrumentally measured according to the method of Giuberti *et al.* (2015) with some modification using a Chroma meter CR-400/410 (Konica Minolta Sensing, Inc. Osaka Japan) model. A relatively large amount of each composite flour and pasta was finely ground before sampling to minimise variation. The results are expressed in the CIE *L*a*b**. The value *L** (100=white. 0=black) is an indication of lightness, *a** measures chromaticity, with positive values indicating redness and negative values indicating greenness. The *b** value also measures chromaticity, with positive values indicating yellowness and negative values indicating blueness. Reference blank measurements were done on a plain whiteboard. For each composite flour and pasta, five readings were taken.

4.4.4 *In-vitro* protein digestibility (Pepsin method)

Protein digestibility of cooked pasta was determined according the method described by Hamaker *et al.* (1987) with some modifications. 200 mg of milled pasta was weighed in centrifuge tubes, suspended in distilled water. Citrate buffer (5 ml at pH 2.0) was added and was vortexed vigorously. Citrate buffer (28 ml at pH 2.0) containing pepsin from porcine (131 mg pepsin/ 100 ml buffer) (EC. 3.4.23.1, ≥250 Units/mg solid) (Sigma-Aldrich, St Louis, MO) was used for hydrolysis of pasta for 2 hours at a temperature of 37 °C. Digestion was stopped using 2 ml of 2 M sodium hydroxide. The clear supernatant was carefully pipetted off without removing the sediment using a Pasteur pipette. The residue was washed with distilled water, centrifuged and clear supernatant was pipetted off again. A forced draft oven (100 °C) was used to dry off the residue overnight. The residual material was weighed and the protein content determined by Dumas combustion. *In-vitro* protein digestibility was calculated as the difference between the initial total weight of protein and the residual weight of protein after digestion expressed as a percentage of the total protein as shown below:

% protein digestibility = (X – Y/ X) × 100

X= Initial total weight of protein (mg) (Initial weight of protein × protein content of material A)

Y= Residual weight of protein (mg) (Residual weigh of protein × % protein content of residual material B).

4.4.5 *In-vitro* starch digestibility

The method according to Goñi *et al.*, (1997) was used with slight modification. Raw and extruded samples (50 mg) were used per assay and 1 ml of boiling water was added to each sample for easy dispersion in 1 ml boiled water before 10 mL of HCl-KCl buffer (pH 1.5) and 0.2 ml of a solution containing 1 mg pepsin enzyme from porcine gastric mucosa (Sigma Aldrich P7000-100G) was added, followed by incubation at 40 °C for 60 minutes with constant agitation. Tris-maleate buffer (10 ml at pH 6.9) was added and pH adjusted with 1 M NaOH. The volume was made up to 25 ml with tris-maleate buffer (pH 6.9); and for 0 min, aliquot of 0.1 ML was taken before the addition of 5 mL tris- maleate buffer containing 2.6 IU of pancreatic α-amylase from porcine pancreas with activity of 19.6 units/mg (Sigma-Aldrich A-3176) followed by incubation at 37 °C with constant

agitation. Aliquots of 0.1 ml were taken at 5 minutes and then at intervals of 30 minutes until 3 hours into Eppendorf tubes. The tubes containing the aliquots (0.1 ml) were placed in boiling water for 15 minutes to inactivate the α-amylase enzyme. Sodium-acetate buffer (1 mL of 0.4 M at pH 4.75) and 90 µL of amyloglucosidase with an activity of 64.7 U/mg **(**Megazyme E-AMDDF) was added and the aliquots incubated at 60 °C for 45 minutes in a shaking water bath. The aliquots were centrifuged at 5292 RCF for 5 minutes. Aliquot of 0.1 mL of the supernatant obtained was taken and 3 mL of GOPOD (Glucose Oxidase Peroxidase, Megazyme Ltd, Bray, Ireland, and Wicklow) was added and incubated in shaking water bath for 20 minutes at 50 °C. To determine glucose released, the colour developed was measured in terms of absorbance at 510 nm using a Spectrophotometer (T80+UV/VS) against a reagent blank. The percentage of the glucose released was converted to starch by multiplying the glucose percentage with 0.9.

$$\text{Total starch hydrolysed (\%)} = \frac{\text{mg starch digested}}{\text{mg starch in porridge}} \times 100$$

Starch hydrolysis kinetics were described using the non-linear model by Goñi *et al.* (1997). The non-linear starch hydrolysis kinetics are described by $C = C_\infty (1-e^{-kt})$

Where C is the percentage of starch hydrolysed at time t, C_∞ is the percentage of starch hydrolysed after 180 min, k is the kinetic constant (min^{-1}) and t is the time (min). Hydrolysis index (HI) was the area under the curve of the treated pasta divided by the area of the reference (white bread). The area under hydrolysis curve (AUC) was calculated using the equation;

EGI = 39.71 +0.549HI

$AUC = C_\infty (t_f - t_0) - (C_\infty / k)(1-\exp(-k(t_f - t_0)))$ where t_f is the final time (180 min) and t_0 is the initial

Glycaemic index (GI) was calculated according to Goñi *et al.* (1997) using the equation:

GI=39.71+0.549HI

0.9: Factor for converting glucose to starch

Resistant starch (RS), rapidly digestible starch (RDS) and slowly digestible starch (SDS) were calculated using the following equations (Englyst *et al.*, 1992):

RS= TS – (RDS + SDS)

RDS = (G30) × 0.9

SDS= (G120 – G30) × 0.9

Where G30 is glucose released after 30 minutes, TS is total starch and G120 is the glucose released after 120 minutes.

4.4.6 Differential scanning calorimetry (DSC)

The thermal properties of raw and cooked milled pasta samples were determined according to the method described by Cappa *et al.* (2016) with some modifications using the Mettler High Pressure Differential Scanning Calorimeter. Indium (T_p=156.6, ΔH=28.45 Jg^{-1}) was used as a standard. The samples were tested under a nitrogen flow of (60 ml/minute) and a pressure of 40 bars. Pasta sample (10 mg) was mixed with 30 µl of distilled water in a stainless steel pan and hermetically sealed and equilibrated at room temperature for 4 hours. Each pasta sample was heated from 25 ºC to 120 ºC at a heating rate of 10 °C/minute. An empty hermetically sealed aluminium tin was used as a reference. The results were analysed using STARe software (Mettler Toledo) to get onset temperature (T_o), endset temperature (T_c) and peak temperature (T_p).

4.4.7 Nutritional and antioxidant properties analysis

4.4.7.1 Determination of β-carotene content

β-Carotene was extracted from about 2 g of raw flours and uncooked milled extruded pasta samples with 10 ml tetrahydrofuran (THF) in a small beaker by magnetic stirring for 30 minutes. The mixture was centrifuged at 1149 RCF for 10 minutes and the supernatant set aside. The extraction was repeated three times with a fresh 10 ml THF aliquot and separation of the supernatant after centrifugation until the pasta flour residue was colourless. The THF was evaporated to dryness using a rotary evaporator at 27 ºC. The crude carotene extract was dissolved in 10 ml toluene. The carotene extract in toluene was filtered using 0.45 µm PTFE membrane filters directly into amber vials in preparation for chromatography.

Chromatographic analysis of β-carotene content was done using a Prominence ultra-fast liquid chromatograph (UFLC) (Shimadzu, Tokyo, Japan) equipped with a SIL-20A Prominence auto-sampler, a DGU-20A3 Prominence degasser, a CTO-10AS VP Shimadzu column oven and a SPD-

M20A Prominence diode array detector. UV/Vis spectra of carotenoids was recorded between 200 to 600 nm with detection of β-carotene at 450 nm. The separation of carotenoids was performed at 30 °C on a C18 Waters Nova-Pak carotenoid column (300 x 3.9 mm, 4 µm particle size) by isocratic elution with acetonitrile (58%), HPLC grade methanol (35%) and THF (7%) as mobile phase, at a flow rate of 1.0 ml/min for 30 min. The quantification of β-carotene was done using a calibration curve of β-carotene standard, and the vitamin A content approximated as retinol activity equivalents (RAE) using a conversion factor of 12 µg β-carotene to 1 µg retinol (Van Jaarsveld *et al.*, 2006).

4.4.7.2 Determination of ABTS•+ radical scavenging capacity

A modification of the 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay described by Awika *et al.* (2003) was used. The mother solution was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate to 2 ml in an eppendorf tube. The mother solution was incubated for a maximum of 12 hours in the dark. A working solution was prepared in an aluminum foil wrapped bottle. Serial dilutions of trolox standard were prepared by diluting trolox with PBS. The sample extract or trolox standard and working solution was mixed in the wells of a 96-well microplate. The absorbance was read at 570 nm after incubation for 30 minutes and the radical scavenging capacity was expressed as µmol Trolox equivalents per gram of samples on dry basis.

4.4.8 Microstructure of uncooked and cooked pasta

4.4.8.1 Bright field Light microscopy

Milled extruded pasta and cooked pasta samples were visualized with a VS3 Series Biological Trinocular Light Microscope from Micromet Scientific with a Biowizard Image Analysis Software (Delhi, India) equipped with a Polarising filter lens. The milled extruded and cooked pasta samples (10 mg) were suspended in 30 % glycerol. One drop of the suspension was placed onto the specimen glass slide and covered with a glass cover slip. The structural integrity was analysed with both the light microscope without a polarising filter and a light microscope with a polarising filter. To stain starch, iodine solution was added. Images were taken with 20× magnification and evaluated with the ImageJ®software package.

4.4.8.2 Scanning Electron Microscopy (SEM)

Scanning electron microscopy of raw and cooked pasta (wet) was done by first freezing the pasta using liquid nitrogen and a freeze fracture of a small fraction was taken. The small fractions were mounted on aluminium stubs with the aid of double-sided carbon tape, followed by coating with carbon of about 20 nm in thickness. The coated pasta was viewed and photographed using the Zeiss Crossbeam 540 FE 6 Scanning Electron Microscope (Carl Zeiss Microscopy, 6mbH, Germany) at an accelerating voltage of 5.0 kV.

4.4.8.3 Confocal laser scanning microscopy

Confocal laser scanning microscopy of raw and cooked pasta was done by cutting cross sections and longitudinal sections of the pasta. Five pieces of each section (cross or longitudinal) were placed on a concave microscope slide. Two drops of 0.1 % Safranin O dye (Sigma-Aldrich, St. Louis, MO, USA) were added to the pasta in the slides and covered with a coverslip. Proteins are preferentially stained by safranin O dye (Ogundele *et al.*, 2017). A Zeiss LSM 880 META Confocal Laser Scanning Microscope (Carl Zeiss Microscopy, 6mbH, Germany) was used to observe and capture images of the pasta. The pixel time was 12.8 µs and picture size was 512 ×512 pixels. The Safranin O dye excitation was 488 nm and emission was 540 nm.

4.4.9 Statistical Analysis

Multivariate analysis was used to analyse the interaction between the effect of extrusion cooking and compositing maize flour with orange fleshed sweet potato flour of the physical, functional and nutritional characteristics. Two-way analysis of variance (ANOVA) was used to analyse the data using SPSS version 22. Means were compared at $p \leq 0.05$ using Fisher's Least Significant Difference (LSD) test. Experiments were conducted in triplicate.

CHAPTER 5: RESULTS

5.1 Proximate composition of raw flours and pasta samples

The proximate composition of raw flour samples and extruded pasta samples is shown in Table 5.1. Moisture content of pasta samples ranged from 6.29 to 9.21 %. The protein content in 100 % maize flour was significantly ($P<0.05$) higher than the protein content in 100 % OFSP flour. The different composite flours and pasta samples showed similar protein content. Commercial pasta showed the highest protein content of 9.64 %.

A progressive increase in ash content was observed with increasing proportion of OFSP in maize: OFSP composite flours and pasta samples most likely due to the fact that ash content of OFSP was 10 times higher than that of maize. The insoluble and soluble dietary fibre content of the composited flours increased with increasing proportions of OFSP flours. Insoluble dietary fibre decreased as soluble dietary fibre increased after extrusion cooking for all pasta samples. Commercial pasta exhibited the lowest soluble dietary fibre content.

There was no significant difference ($p>0.05$) in the crude fat content between 100 % maize flour and 100 % OFSP flour resulting in no significant differences in fat content of their composite pasta samples after extrusion. However, extrusion cooking decreased the crude fat content in the pasta samples. 100 % maize pasta showed the highest fat content (0.91 %) and commercial pasta showed the lowest (0.42 %).

Table 5.1: Effect of compositing maize flour with orange-fleshed sweet potato flour (OFSP) on the proximate composition of flour and extrusion made pasta (%)

Sample	Composite	Moisture[a]	Protein[b]	Ash[b]	Fat[b]	IDF[b]	SDF[b]
Flour	100 % Maize	12.0 h (0.04)	8.37 c (0.08)	0.44 b (0.02)	1.51 f (0.07)	1.27 c (0.03)	0.45 b (0.05)
	80 % Maize: 20 % OFSP	11.0 g (0.06)	8.04 b (0.10)	1.24 c (0.00)	1.45 f (0.13)	2.95 e (0.13)	2.48 d (0.03)
	70 % Maize: 30 % OFSP	9.19 f (0.16)	8.22 bc (0.02)	1.74 e (0.07)	1.15 e (0.08)	2.83 e (0.08)	2.46 d (0.02)
	50 % Maize: 50 % OFSP	7.64 e (0.15)	8.11 b (0.02)	2.54 g (0.10)	1.24 e (0.01)	6.03 g (0.04)	4.10 f (0.19)
Pasta	100 % Maize	6.64 c (0.12)	8.15 b (0.10)	0.53 b (0.01)	0.91 d (0.03)	0.34 a (0.06)	0.77 c (0.03)
	80 % Maize: 20 % OFSP	6.29 b (0.05)	7.66 a (0.01)	1.42 d (0.09)	0.69 cb (0.02)	1.92 d (0.04)	2.86 e (0.10)
	70 % Maize: 30 % OFSP	6.63 c (0.05)	7.67 a (0.05)	1.97 f (0.00)	0.80 cd (0.02)	1.97 d (0.07)	2.74 e (0.02)
	50 % Maize: 50 % OFSP	7.15 d (0.05)	8.03 b (0.17)	2.92 h (0.05)	0.59 b (0.04)	3.32 f (0.08)	4.23 f (0.13)
Commercial pasta		9.21 f (0.20)	9.64 d (0.06)	0.27 a (0.03)	0.42 a (0.01)	0.74 b (0.07)	0.22 a (0.02)
100 % OFSP		4.05 a (0.06)	7.77 a (0.09)	4.36 i (0.05)	1.15 e (0.07)	10.5 h (0.22)	5.04 g (0.19)

Means within a column with different letters are significantly different ($p<0.05$)
Standard deviations are given in parenthesis
[a]In as is basis
[b]In dry matter basis
IDF- Insoluble Dietary Fibre
SDF- Soluble Dietary Fibre
Commercial gluten-free pasta made from maize and rice

5.2 Total starch and total soluble solids of composited flours and pasta samples

Table 5.2 shows the total starch content and the ºbrix of maize flour composited with OFSP flour and their extruded pasta. It was observed that 100 % maize flour showed the highest total starch content of about 88.7 % with 100 % orange-fleshed sweet potato flour showing the lowest percentage of starch (56.8 %). Thus as the ratio of maize flour: orange-fleshed sweet potato flour increased, the total starch content also increased. Extrusion cooking did not statistically affect the starch content in the pasta compared to raw flour.

There was a significant difference ($p<0.05$) between the ºbrix of both flours and extruded pasta samples with 100 % OFSP flour showing the highest ºbrix (4 ºbrix) and 100 % maize flour showing the lowest ºbrix (0.23 ºbrix). There was progressive increase in ºbrix with increasing proportion of OFSP in the maize: OFSP composite flour and pasta samples. Extrusion cooking increased the ºbrix of the pasta samples. Commercial pasta was significantly ($P<0.05$) lower in ºbrix.

Table 5.2: Effect of compositing maize flour with orange-fleshed sweet potato flour (OFSP) on the starch content and soluble solids of their flours and their extrusion made pasta samples

Sample	Composites	Starch % (db)	Soluble solids (°brix)
Flour	100 % Maize	88.7 d (5.12)	0.23 a (0.06)
	80 % Maize: 20 % OFSP	81.5 cd (2.78)	1.23 c (0.06)
	70 % Maize: 30 % OFSP	80.7 c (3.35)	1.53 d (0.06)
	50 % Maize: 50 % OFSP	69.7 b (1.36)	2.50 f (0.00)
Pasta	100 % Maize	81.5 cd (2.35)	0.60 b (0.00)
	80 % Maize: 20 % OFSP	78.3 c (2.47)	1.30 c (0.00)
	70 % Maize: 30 % OFSP	82.6 cd (4.14)	1.90 e (0.00)
	50 % Maize: 50 % OFSP	68.6 b (1.79)	2.93 g (0.06)
Commercial pasta		77.1 c (0.96)	0.23 a (0.06)
100 % OFSP		56.8 a (1.42)	4.00 h (0.10)

Means within a column with different letters are significantly different ($p<0.05$)
Standard deviations are given in parenthesis
Commercial gluten-free pasta made from maize and rice

5.3 Cooking time, firmness and stickiness of pasta samples

Cooking time, firmness and stickiness of pasta samples are shown in Table 5.3. Pasta made with 100 % maize, 70 % maize: 30 % OFSP and 80 % maize: 20 % OFSP pasta showed no significant difference ($p>0.05$) in cooking time. Commercial pasta exhibited the highest cooking time of 10 min and extruded 50 % maize: 50 % OFSP pasta exhibited the lowest cooking time of 2.17 min.

Figure 5.1 shows the firmness and stickiness of the cooked pasta samples. Pasta made from 80 % maize: 20 % OFSP pasta exhibited higher firmness value which showed no significance difference ($p>0.05$) with 100 % maize pasta. There was no significance difference ($p>0.05$) in the stickiness between the different extruded pasta samples.

Table 5.3: Effect of compositing maize flour with orange-fleshed sweet potato flour (OFSP) on the cooking properties of extruded pasta

Pasta samples	Cooking time (min)	Firmness (N)	Stickiness (N)
100 % Maize	4.53 b (0.40)	21.1 bc (0.43)	-0.43 bc (0.09)
80 % Maize: 20 % OFSP	4.20 b (0.17)	22.1 c (1.95)	-0.54 bc (0.04)
70 % Maize: 30 % OFSP	4.00 b (0.00)	11.6 a (0.41)	-0.46 bc (0.07)
50 % Maize: 50 % OFSP	2.17 a (0.29)	8.69 a (1.99)	-0.37 c (0.03)
Commercial pasta	10.0 c (0.00)	17.9 b (0.73)	-0.58 a (0.08)

Means within a column with different letters are significantly different ($p<0.05$)
Standard deviations are given in parenthesis
Commercial gluten-free pasta made from maize and rice

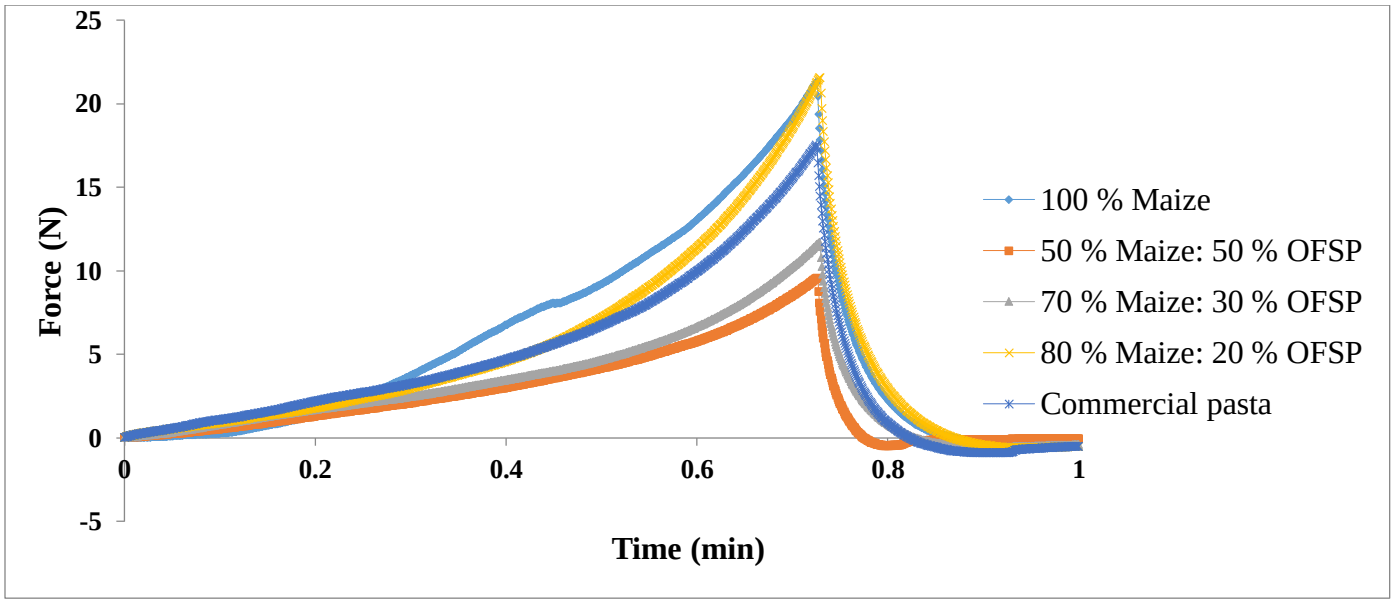

Figure 5.1: Effect of compositing maize flour with Orange fleshed sweet potato flour (OFSP) on the firmness and stickiness of extruded pasta

5.4 Cooking loss, water absorption capacity and fracturability of pasta samples

Figures 5.2 and 5.3 show the cooking loss and water absorption capacity of extruded pasta samples. The cooking loss ranged from 4.36 to 12.5 %. There was progressive increase in cooking loss with increase in proportion of OFSP in maize: OFSP composite pasta samples. The water absorption capacity ranged from 95.3 % - 123 %. Commercial pasta exhibited higher water absorption capacity of 123 % and pasta samples composited with OFSP statistically ($p>0.05$) showed lower water absorption capacity.

Figures 5.4 and 5.5 show the maximum force required to break the raw pasta and cooked pasta respectively. Statistically, there was no significant difference ($p>0.05$) in the force required to break 100 % maize raw pasta and raw commercial pasta. There was progressive decrease in fracturability with increasing proportion of OFSP in the maize: OFSP composite raw and cooked pasta samples. There was a significant difference ($p<0.05$) in the forces required to break the cooked pasta samples. Cooked commercial pasta showed a highest fracturability force.

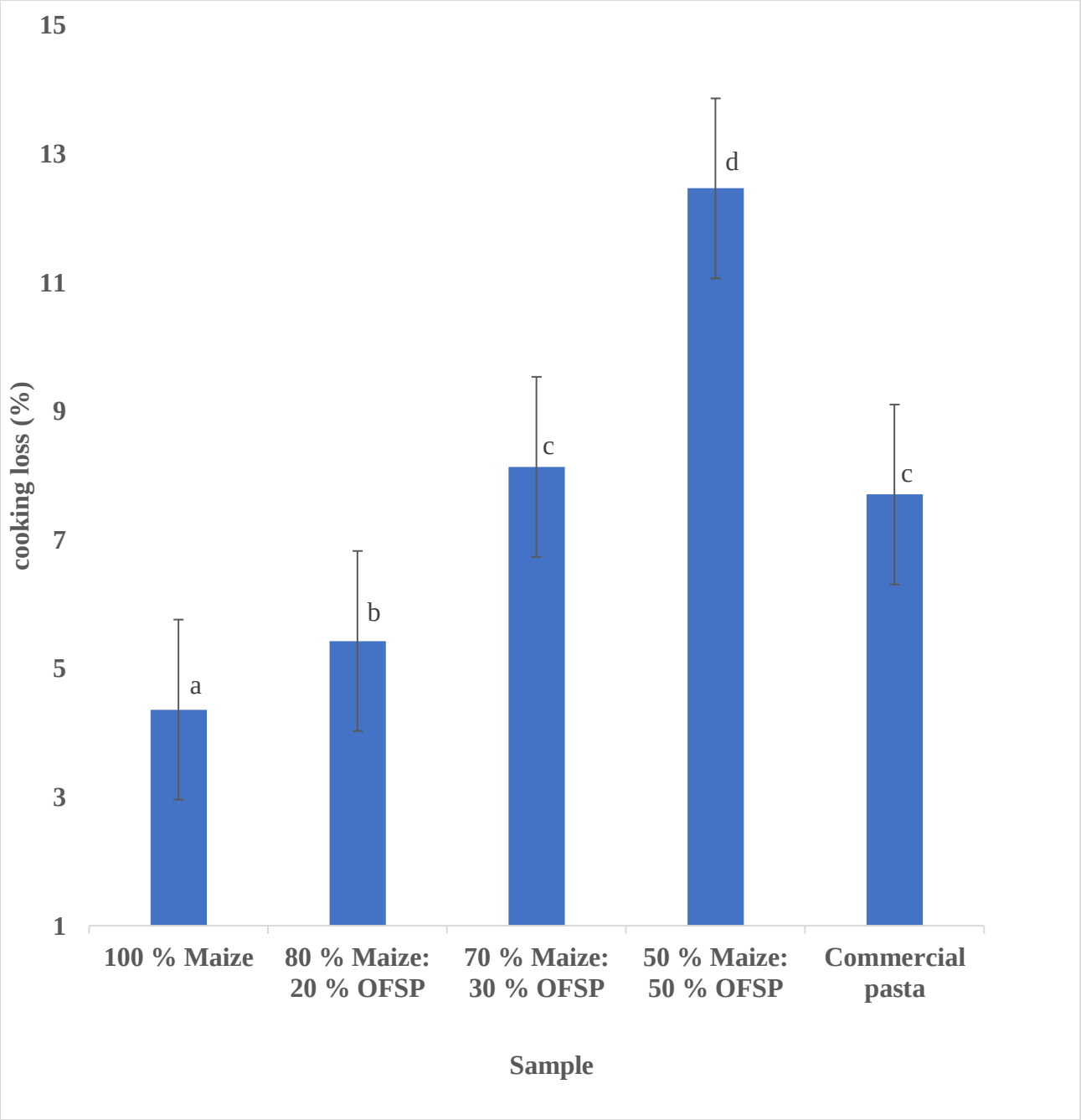

Figure 5.2: Effect of compositing maize flour with Orange-fleshed sweet potato flour (OFSP) on the cooking loss of extruded pasta

Error bars represents standard deviations
Bar charts with different letters are significantly different ($p<0.05$)
Commercial gluten-free pasta made from maize and rice

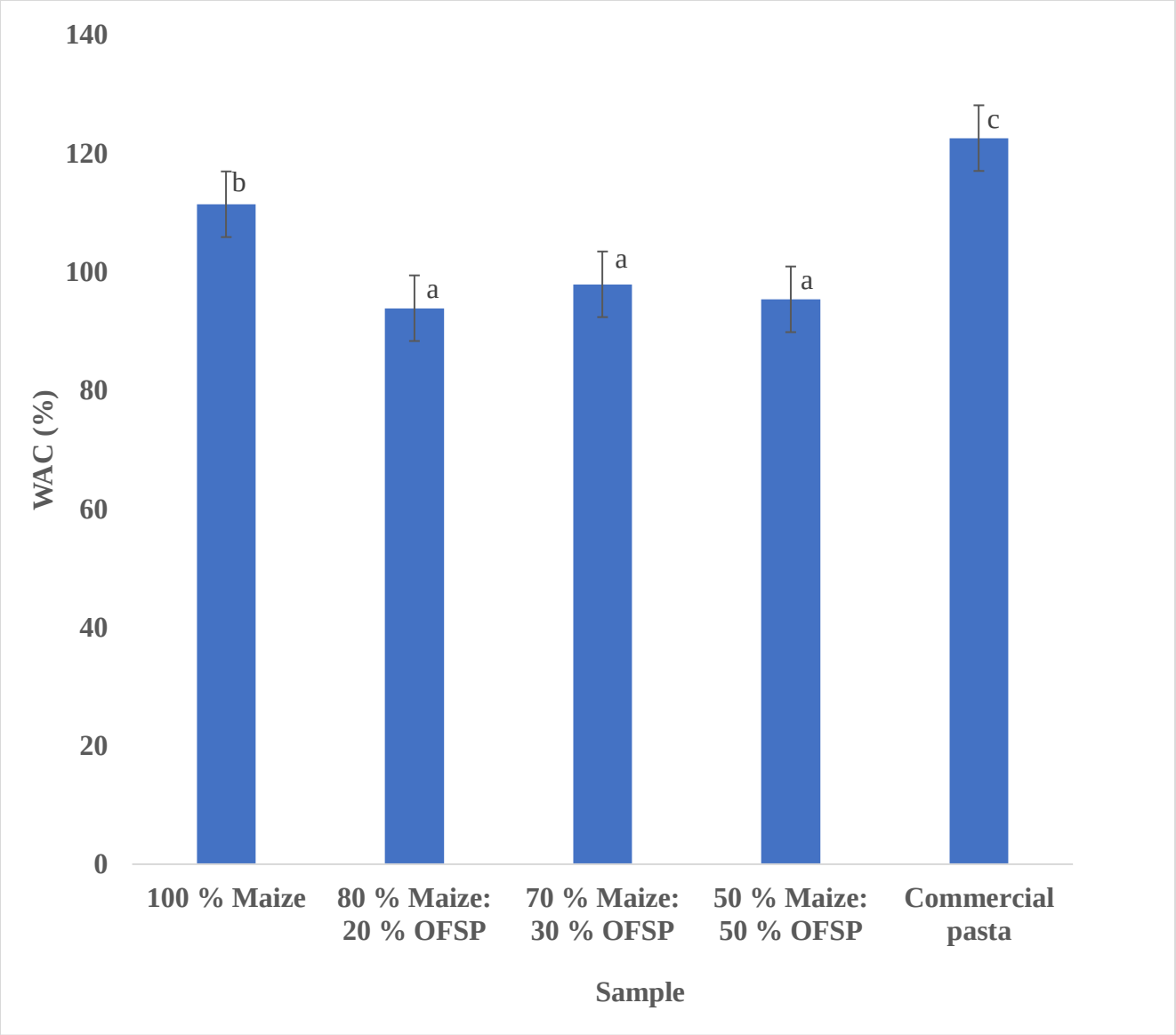

Figure 5.3: Effect of compositing maize flour with Orange-fleshed sweet potato flour (OFSP) on the water absorption capacity (WAC) of extruded pasta

Error bars shows standard deviations
Bar charts with different letters are significantly different ($p<0.05$)
Commercial gluten-free pasta made from maize and rice

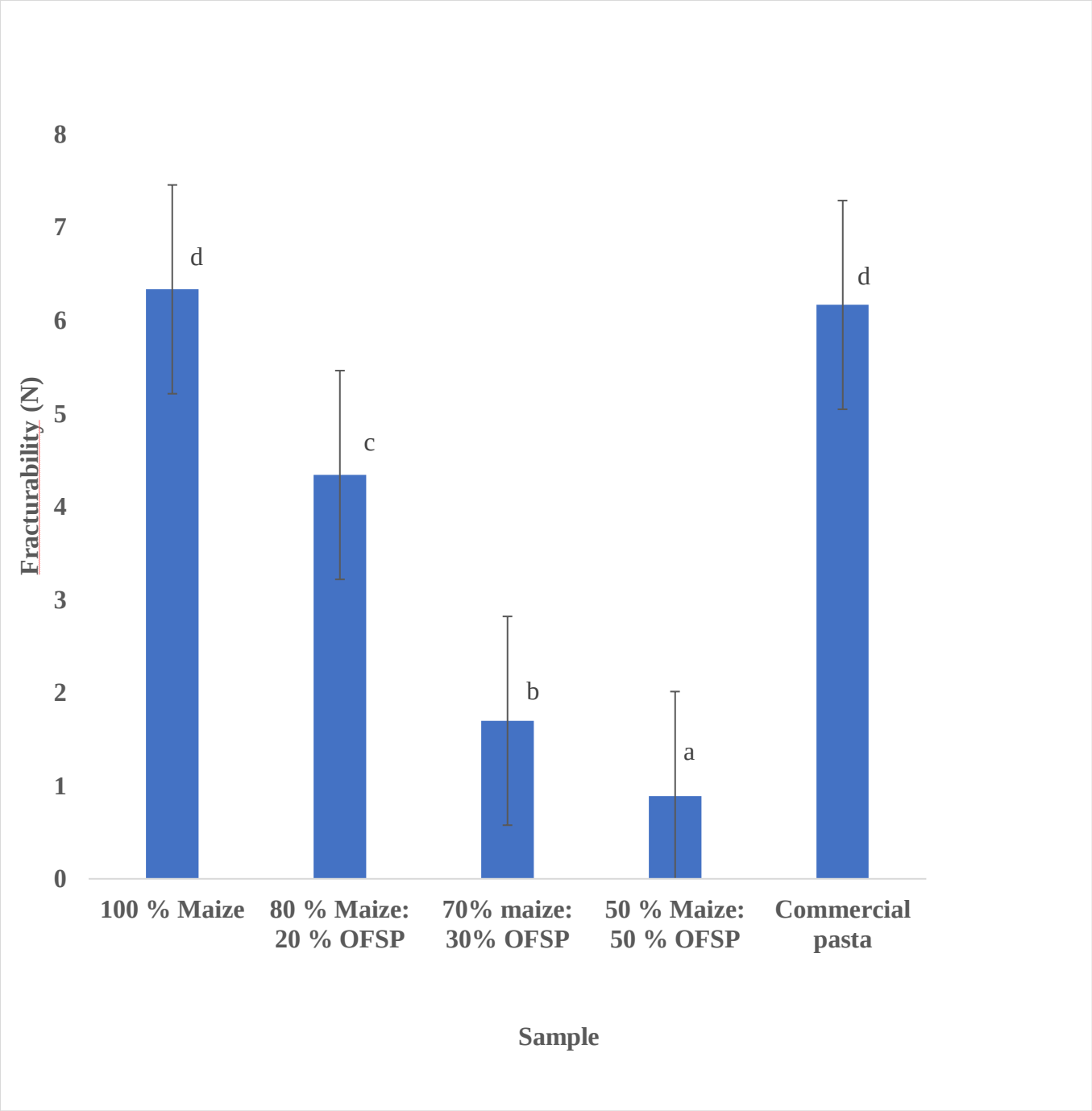

Figure 5.4: Effect of compositing maize flour with Orange-fleshed sweet potato flour (OFSP) on the fracturability of extruded raw (uncooked) pasta

Error bars shows standard deviations
Bar charts with different letters are significantly different ($p<0.05$)
Commercial gluten-free pasta made from maize and rice

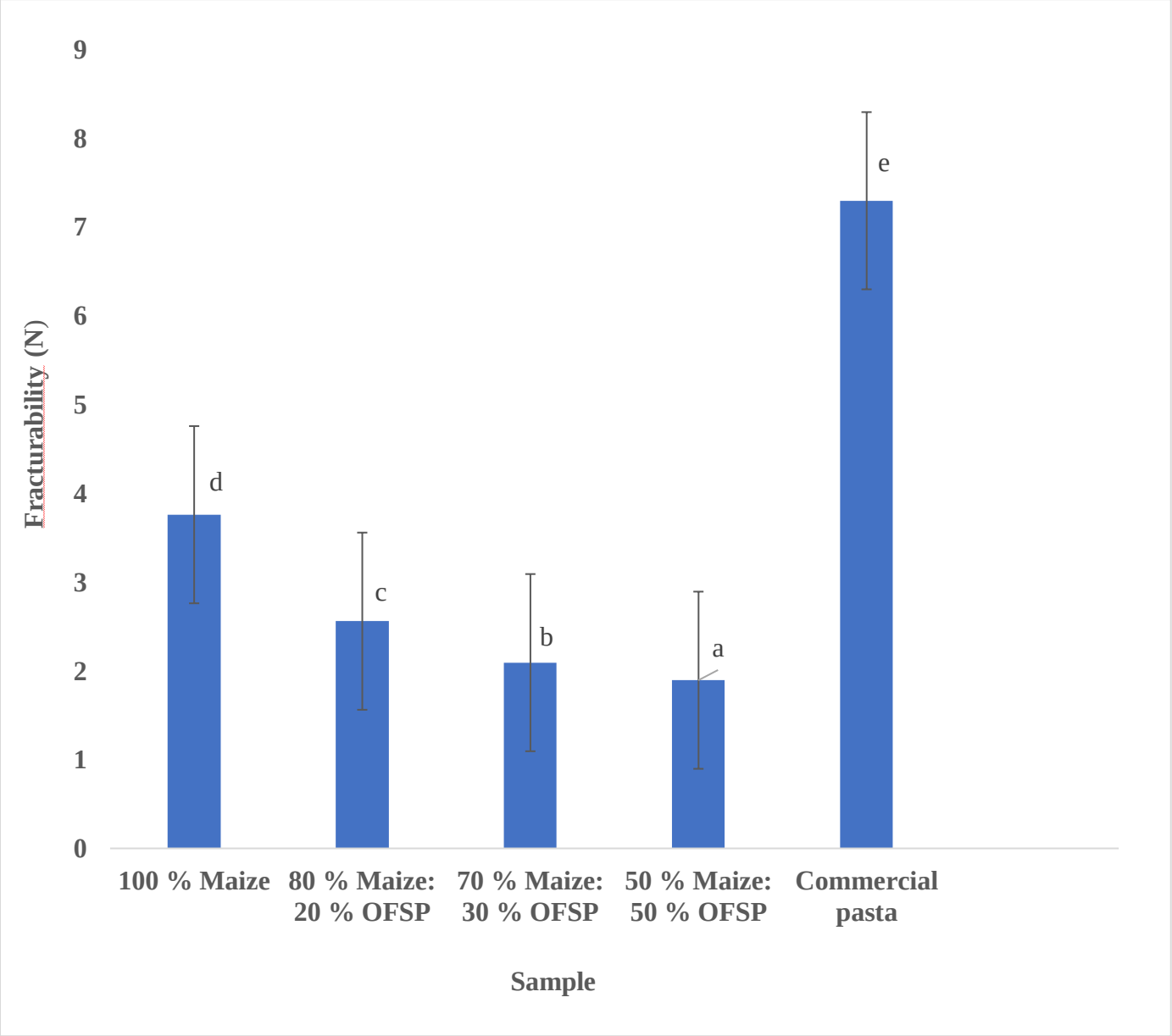

Figure 5.5: Effect of compositing maize flour with Orange-fleshed sweet potato flour (OFSP) on the fracturability of extruded and cooked pasta

Error bars shows standard deviations
Bar charts with different letters are significantly different ($p<0.05$)
Commercial gluten-free pasta made from maize and rice

5.5 Expansion ratio of raw and cooked pasta samples

Figure 5.6 shows the expansion ratio of raw and cooked pasta samples. There was an expansion in pasta samples as compared to the 1 mm die used. There was no significant difference ($p>0.05$) in the expansion ratio between the raw and cooked pasta samples. There seemed to be a non-significant ($P>0.05$) increase in expansion ratio from raw to cooked pasta.

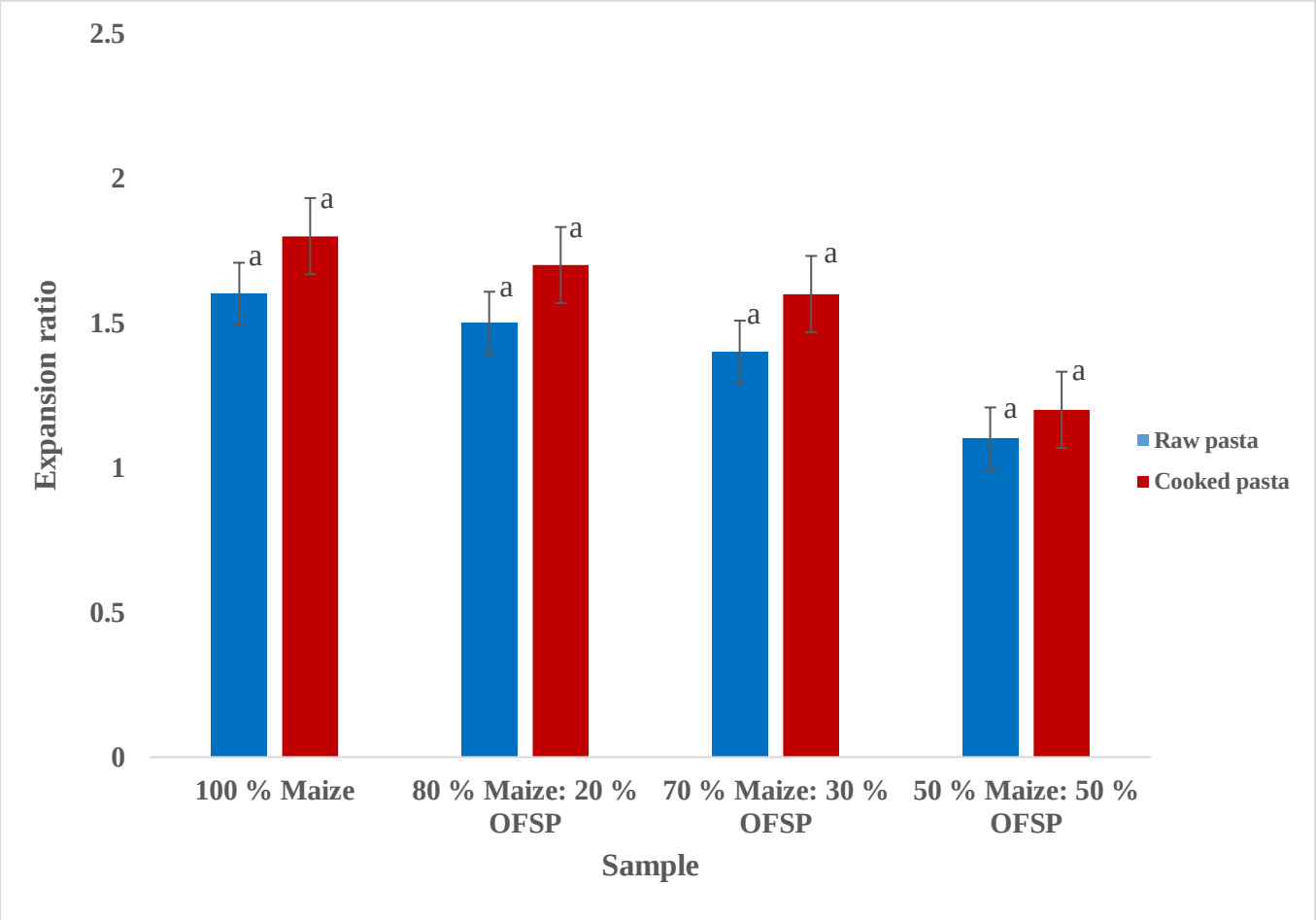

Figure 5.6: Effect of compositing maize flour with orange-fleshed sweet potato flour (OFSP) on the expansion ratio raw and cooked pasta

Error bars shows standard deviations
Different Bar chart colours with the same letters are not significantly different ($p>0.05$)

5.6 Colour values of raw flours and pasta samples

Colour values (L*, b* and a*) of raw and extruded pasta samples are presented in Table 5.4. The L^* value indicating whiteness was significantly different ($p<0.05$) among the raw flours and extruded pasta samples. The whiteness values decreased with increasing proportion of OFSP in the maize: OFSP composite flours.

The a^* value indicating redness was higher in 100 % OFSP flour. Redness values decreased significantly ($p<0.05$) with increasing proportion of maize flour in the composites. The redness values of 100 % maize pasta and commercial pasta were not significantly different.

There was no significant difference in yellowness (b*) between the extruded pasta samples composited with orange fleshed sweet potato. Commercial pasta showed the highest value for yellowness value and this can visually be seen in Figure 5.7.

Table 5.4: The effect of compositing maize flour with orange-fleshed sweet potato (OFSP) flour on the L*, b* and a* colour values of extruded uncooked and extruded pasta

		colour		
Treatment	**Composites**	**L***	**a***	**b***
Raw flour	100 % Maize	96.1 h (0.05)	-0.42 a (0.01)	6.47 a (0.13)
	100 % OFSP	84.9 d (0.13)	10.5 g (0.12)	29.4 g (0.16)
	80 % Maize: 20 % OFSP	89.0 f (0.46)	4.88 d (0.06)	22.6 de (1.03)
	70 % Maize: 30 % OFSP	88.4 ef (0.36)	6.59 e (0.04)	23.0 c (0.28)
	50 % Maize: 50 % OFSP	87.3 e (0.19)	7.51 f (0.10)	25.1 d (0.70)
Extruded and uncooked	100 % Maize	90.5 g (0.40)	-0.85 a (0.04)	10.5 b (0.43)
	80 % Maize:20 % OFSP	80.3 c (0.54)	1.26 b (0.28)	27.2 ef (0.74)
	70 % Maize:30 % OFSP	78.5 b (0.48)	2.20 c (0.27)	27.9 fg (0.79)
	50 % Maize:50 % OFSP	75.9 a (0.46)	4.85 d (0.22)	27.8 fg (0.44)
	Commercial pasta	89.5 fg (0.51)	-1.49 a (0.12)	33.6 h (1.04)

Means within a column with different letters are significantly different ($p<0.05$)
Standard deviations are given in parenthesis
Commercial gluten-free pasta made from maize and rice
L*- Whiteness
a*- Redness
b*- Yellowness

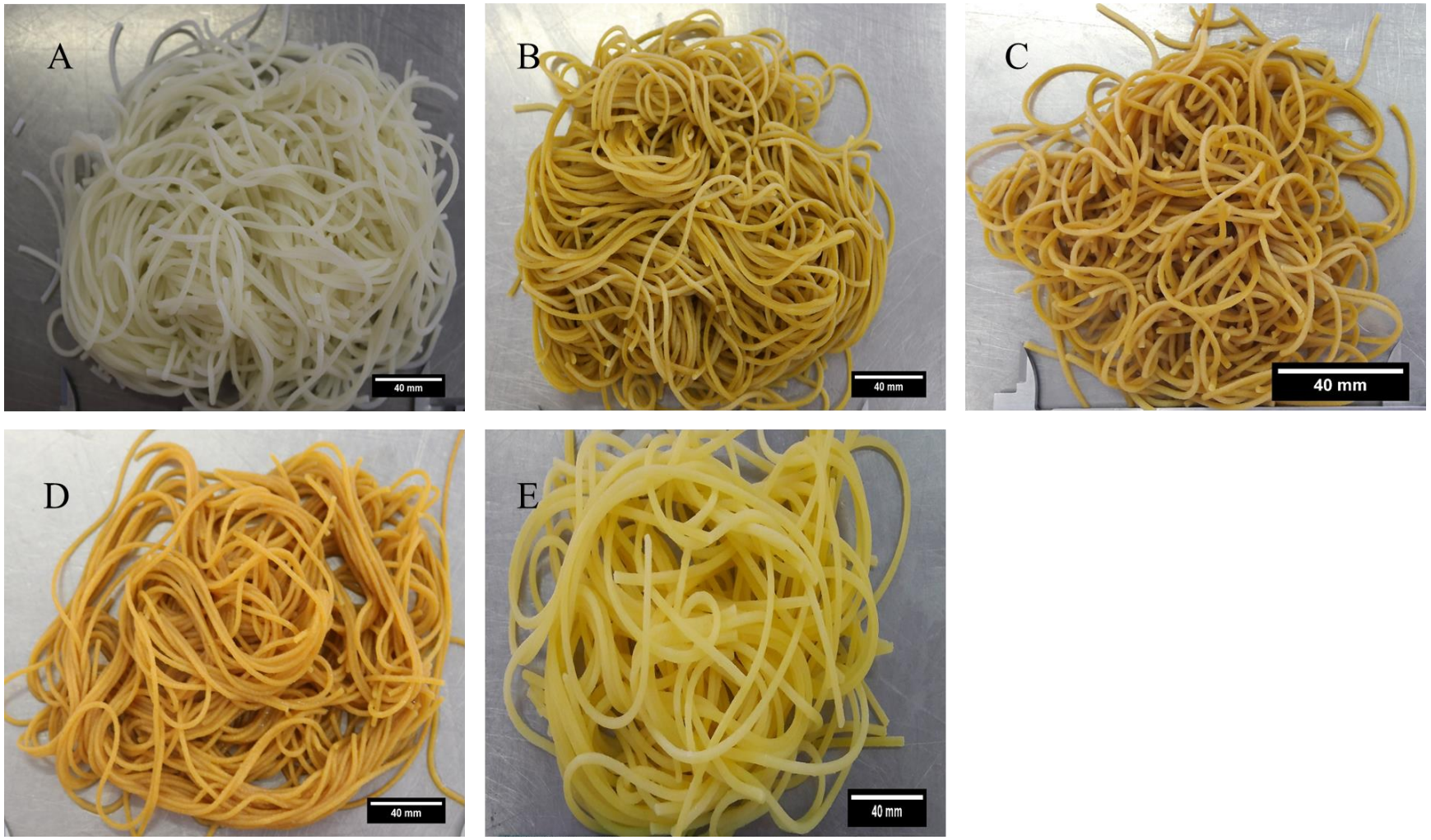

Figure 5.7: Images of extruded and cooked pasta samples.

A: 100 % Maize pasta, **B:** 80 % Maize: 20 % OFSP pasta, **C**: 70 % Maize: 30 % OFSP pasta, **D:** 50 % Maize: 50 % OFSP pasta, **E:** Commercial pasta

5.7 Beta carotene content and antioxidant properties of raw flours and extruded pasta samples

The chromatograms of the β-carotene extracts from raw flour and the extruded pasta samples are shown in Figures 5.8 and 5.9 respectively. The β-carotene peak eluted with a retention time of about 24 minutes. Table 5.5 shows the effect of extrusion cooking and compositing with orange flesh sweet potato flour on β-carotene content and antioxidant properties of maize flour and pasta samples. β-carotene was not detected in 100 % maize flour and pasta. There was a progressive increase in β-carotene content of raw flour and extruded pasta samples with increasing level of incorporation of orange flesh sweet potato flour. Extrusion cooking decreased the β-carotene content of the pasta samples significantly ($p<0.05$) relative to the flours.

In a similar trend to the β-carotene content, there was a progressive increase in antioxidant activity of the raw flours with increasing levels of incorporation of orange flesh sweet potato flour. Extrusion cooking to produce pasta led to a significant increase in antioxidant activity of composite pasta samples relative to their flours. However, the composite pasta samples did not differ from each other in terms of antioxidant activity.

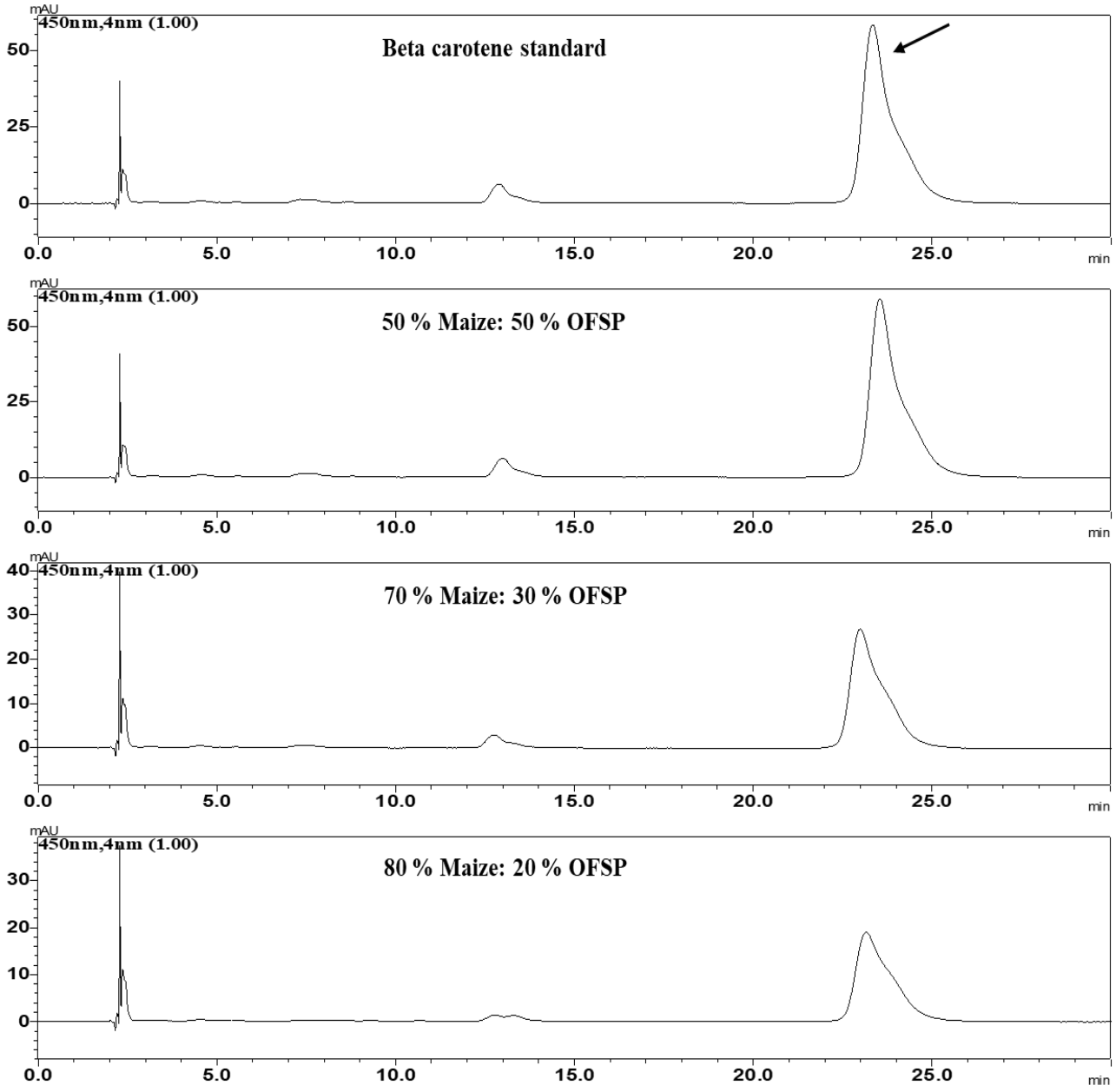

Figure 5.8: HPLC chromatograms of β-carotene extracts from maize-orange-fleshed sweet potato composite flours

Arrow in the figure points to β-carotene peak

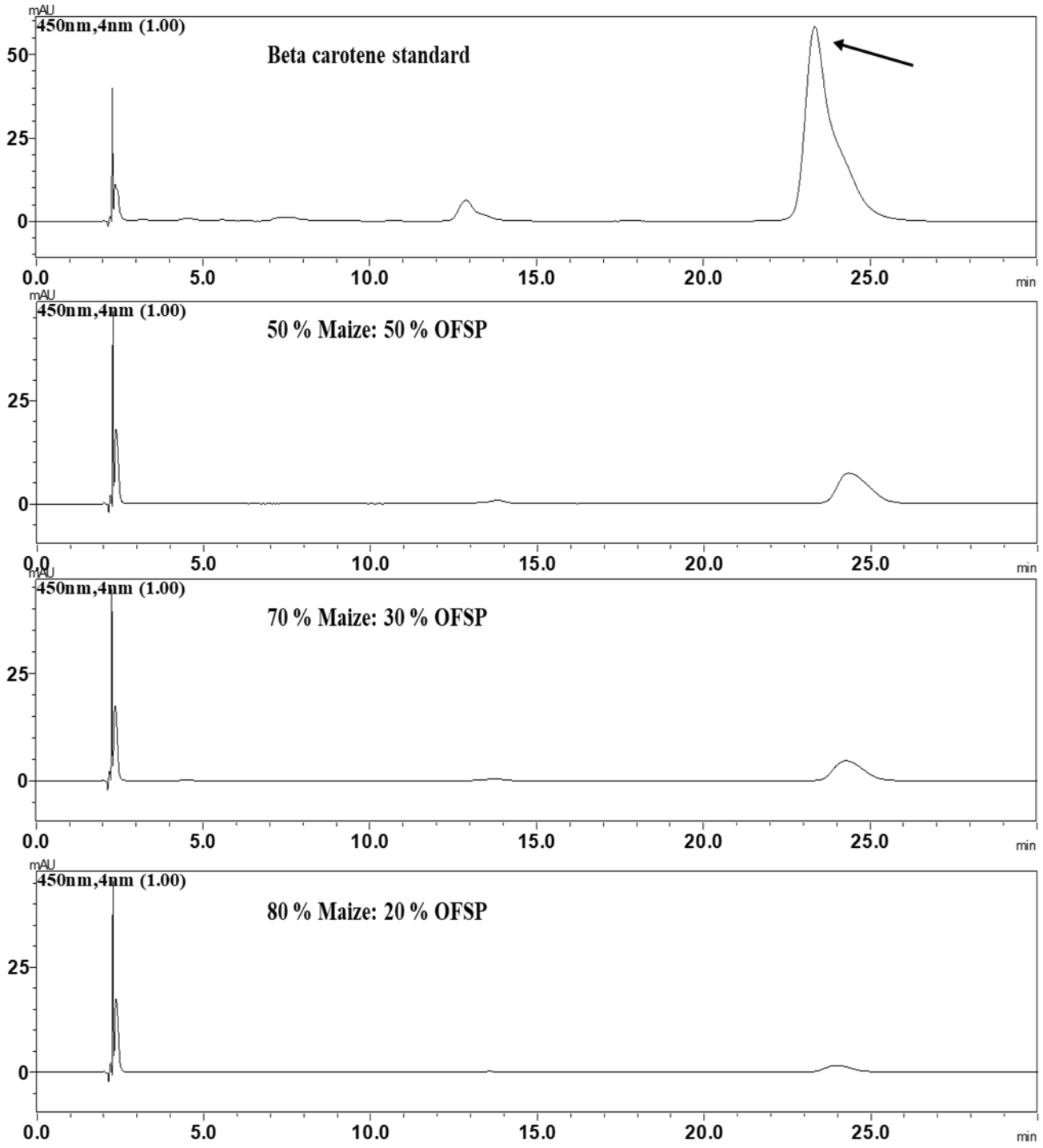

Figure 5.9: HPLC chromatograms of β-carotene extracts from maize-orange fleshed sweet potato composite extruded pasta

Arrow in the figure points to β-carotene peak

Table 5.5: Effect of extrusion cooking and compositing with Orange-fleshed sweet potato (OFSP) flour on the β-carotene and antioxidant activity (ABTS radical scavenging) of maize flour and extruded gluten-free pasta

Treatment	Composites	β-carotene (µg/g)	Antioxidant activity (µmol TE/g)
Raw flour	100 % Maize	ND	3.38 a (0.18)
	100 % OFSP	611 g (37.0)	23.5 e (1.98)
	80 % Maize: 20 % OFSP	126 d (13.9)	9.91 b (0.52)
	70 % Maize: 30 % OFSP	167 e (10.0)	13.3 c (0.92)
	50 % Maize: 50 % OFSP	339 f (38.5)	16.7 d (0.05)
Extrusion cooked pasta	100 % Maize	ND	1.42 a (1.22)
	80 % Maize: 20 % OFSP	7.88 a (1.79)	51.4 f (0.82)
	70 % Maize: 30 % OFSP	27.7 b (4.72)	52.2 f (0.78)
	50 % Maize: 50 % OFSP	43.4 c (7.26)	52.0 f (0.94)

Means within a column with different letters are significantly different ($p<0.05$)
Standard deviations are given in parenthesis
ABTS- 2, 2'-Azinobis-3-Ethylbenzothiazoline-6-Sulphonic acid
TE- Trolox Equivalent
ND- Not detected

5.8 Recommended dietary allowance for vitamin A in different groups of the composited extruded pasta samples

Table 5.6 shows the contribution of the extruded composite pasta to the recommended dietary allowances for vitamin A in different groups. Increasing levels of orange-fleshed sweet potato flour in pasta samples resulted in progressive increases in the contribution of the pasta samples to the RDA requirements for vitamin A for all the different groups. The 50:50 maize-OFSP pasta could contribute at least 42 % of the RDA requirements for vitamin A for all the groups on a 100 g pasta sample basis.

Table 5.6: Contribution (%) of extruded Maize: orange fleshed sweet potato (OFSP) pasta to the recommended dietary allowance for vitamin A in different groups

Pasta sample	Vitamin A (µg RAE/100 g of pasta	Children 3-10 years (RDA = 400 µg RE/day)	Adolescents 10-18 years (RDA = 600 µg RE/day)	Adults females (RDA = 500 µg RE/day)	Adults males (RDA = 600 µg RE/day)	Pregnant women (RDA = 800 µg RE/day)	Lactating women (RDA = 850 µg RE/day)
80 % Maize: 20 % OFSP	65.7 a (1.50)	16.4 a (0.37)	10.9 a (0.25)	13.1 a (0.30)	10.9 a (0.25)	8.21 a (0.19)	7.72 a (1.76)
70 % Maize: 30 % OFSP	231 b (3.93)	57.8 b (0.98)	38.5 b (0.66)	46.2 b (0.79)	38.5 b (0.66)	28.9 b (0.49)	27.2 b (0.46)
50 % Maize: 50 % OFSP	362 c (6.05)	90.5 c (1.51)	60.3 c (1.01)	72.4 c (1.21)	60.3 c (1.00)	45.3 c (0.76)	42.6 c (0.71)

Means within a column with different letters are significantly different ($p<0.05$)
Standard deviations are given in parenthesis
The retinol activity equivalency factor of 12:1 was used for β-carotene

5.9 *In-vitro* protein digestibility

In-vitro protein digestibility of extruded and cooked pasta are presented in Table 5.6. Composited raw and cooked pasta samples with OFSP showed higher protein digestibility values as compared to the 100 % maize pasta. Further cooking of pasta slightly increased the *in-vitro* protein digestibility values of pasta samples with commercial pasta showing the highest value and statistically not different ($p>0.05$) from 50 % maize: 50 % OFSP pasta.

Table 5.7: Effect of compositing maize flour with orange flesh sweet potato flour on *in-vitro* protein digestibility (IVPD) (%) of extruded and cooked pasta using pepsin method

Sample	Composite	Protein digestibility (%)	Amount of digestible protein per 100 g pasta
Raw pasta	100 % Maize	66.5 b (1.16)	8.57 b (0.82)
	80 % Maize: 20 % OFSP	80.6 ef (2.81)	11.3 e (0.39)
	70 % Maize: 30 % OFSP	75.9 d (3.30)	10.2 d (0.44)
	50 % Maize: 50 % OFSP	77.1 de (3.05)	10.3 d (0.41)
	Commercial pasta	60.9 a (2.39)	6.95 a (0.27)
Cooked pasta	100 % Maize	71.2 c (1.17)	9.37 c (0.15)
	80 % Maize: 20 % OFSP	77.9 de (1.76)	10.9 de (0.24)
	70 % Maize: 30 % OFSP	78.8 de (1.80)	10.8 de (0.63)
	50 % Maize: 50 % OFSP	80.6 ef (1.68)	11.2 e (0.14)
	Commercial pasta	83.4 f (1.05)	9.22 bc (0.19)

Means within a column with different letters are significantly different ($p<0.05$)
Standard deviations are given in parenthesis
Commercial gluten-free pasta made from maize and rice

5.10 *In-vitro* starch digestibility of cooked freeze dried pasta

The effect of compositing maize flour with OFSP flour and extrusion cooking on the *in vitro* starch digestibility are shown in Figure 5.10. The kinetics of in vitro starch digestibility was monitored from 0-180 minutes for the various pasta samples. The total starch hydrolysed at the end of the 180 minutes for maize pasta and its composited pasta with OFSP flour and the commercial pasta showed no significant difference except for 80 % Maize: 20 % OFSP pasta (table 5.7). Starch digestion was higher as the proportion of maize flour decreased in the maize: OFSP composited pasta samples. Higher total starch digestibility was recorded for the various pasta samples, with corresponding higher estimated glycemic index, higher rapidly digestible starch and lower percentage of resistant starch values. Addition of OFSP seemed to increase the starch digestibility of the extruded pasta.

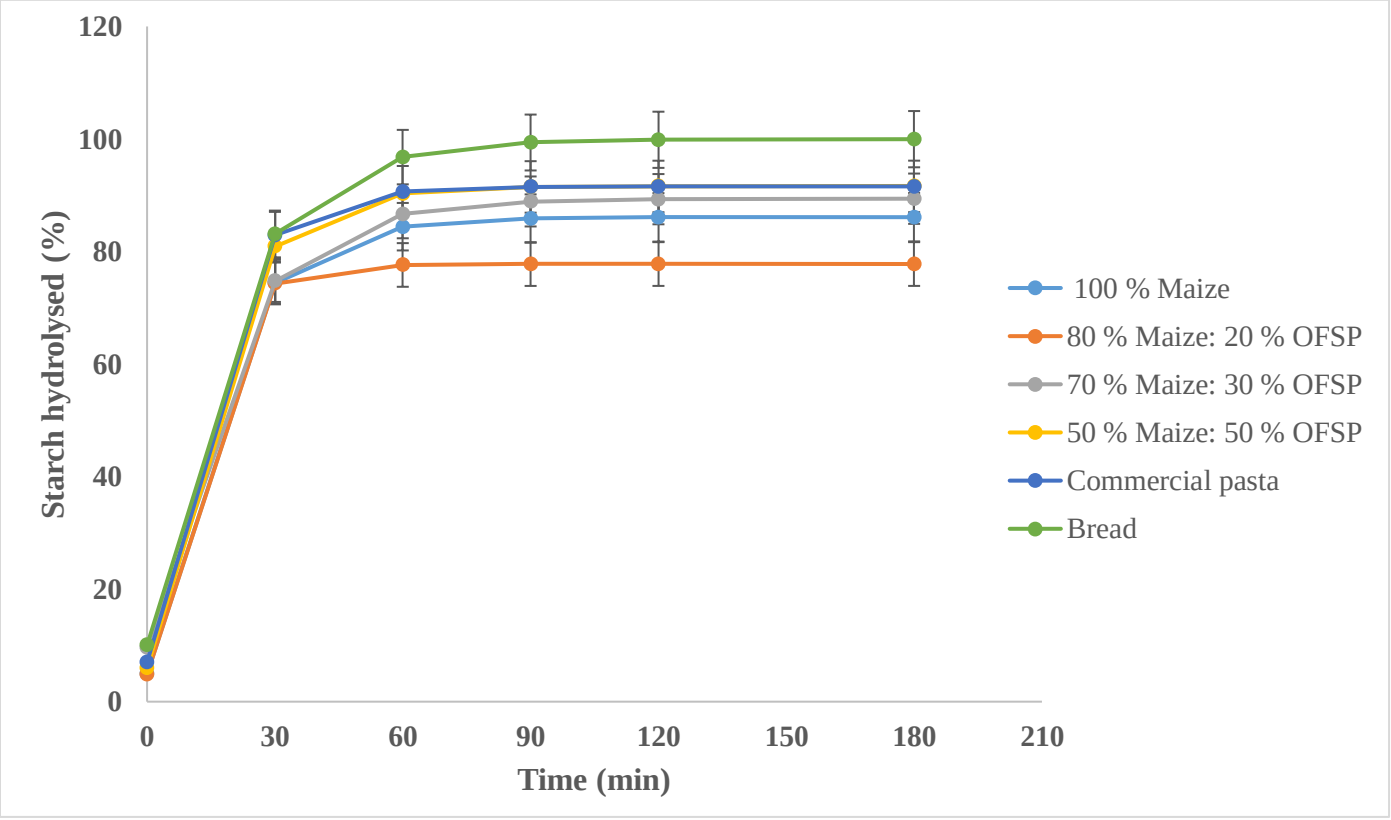

Figure 5.10: Effects of compositing maize flour with orange-fleshed sweet potato flour and extrusion cooking on *in-vitro* starch hydrolysis of extruded pasta.

Table 5.8: Effect of compositing maize flour with orange fleshed sweet potato flour and extrusion cooking on the percent starch hydrolysed after 180 min, hydrolysis constant, RDS, SDS, and estimated glycaemic index of pasta

Pasta sample	[1]C∞ (%)	[1]K(min)[ab]	[1]EGI[a]	HI	RDS (%)	SDS (%)	RS (%)
100 % Maize	83.2 b (0.97)	0.25 a (0.03)	87.5 b (0.49)	87.1 b (0.88)	75.1 a (2.54)	4.17 a (0.78)	23.4 d (1.29)
80 % Maize:20 % OFSP	76.6 a (1.87)	0.89 b (0.12)	85.3 a (0.37)	83.1 a (0.67)	72.0 a (1.06)	4.65 a (0.17)	22.3 d (1.92)
70 % Maize:30 % OFSP	87.8 b (3.56)	0.28 a (0.03)	90.9 c (1.89)	93.2 c (3.45)	82.3 b (1.12)	8.86 b (0.69)	8.98 b (0.56)
50 % Maize:50% OFSP	88.5 b (1.17)	1.26 c (0.06)	92.8 c (1.32)	96.7 ef (2.41)	82.4 b (1.90)	15.6 c (1.85)	9.32 b (0.20)
Commercial pasta	88.4 b (5.43)	12.1 d (0.17)	91.7 c (0.88)	94.7 d (1.59)	83.6 b (1.50)	4.03 a (0.29)	13.1 c (0.96)
Bread	96.8 c (0.29)	0.12 a (0.00)	94.9 d (0.44)	99.7 f (0.58)	84.4 b (2.96)	16.3 c (1.08)	0.38 a (0.09)

Means within a column with different letters are significantly different ($p<0.05$)
Standard deviations are given in parenthesis
[a]EGI (estimated glycaemic) was calculated using the equation (39.71+0.549HI) according to Goni *et al*., 1997
[ab]C∞ (percentage of starch hydrolysed after 180 min) and K (Kinetic hydrolysis) were calculated using the equation $C= C\infty (1-e^{-kt})$
White wheat bread was used as the reference to calculate the EGI
RSD- rapidly digested starch
SDS- slowly digested starch
RS- resistant starch

5.11 Thermal properties of raw and cooked pasta samples

Figure 5.11 shows the DSC thermogram of raw pasta and cooked pasta samples. All raw and cooked extruded pasta samples did not exhibit any endotherm associated with starch gelatinization. Only raw commercial pasta recorded a first endotherm and from Table 5.8 it was observed that the temperature ranged from 69.1 °C to 80.2 °C with an enthalpy (ΔH) of 0.99 J/g.

All raw and cooked pasta samples exhibited a second endotherm with temperatures ranging from 88.7 – 104 °C and 91.8 – 105 °C respectively, and this correspond to amylose-lipid complexes. The enthalpy change (ΔH) decreased for all extruded pasta samples after cooking.

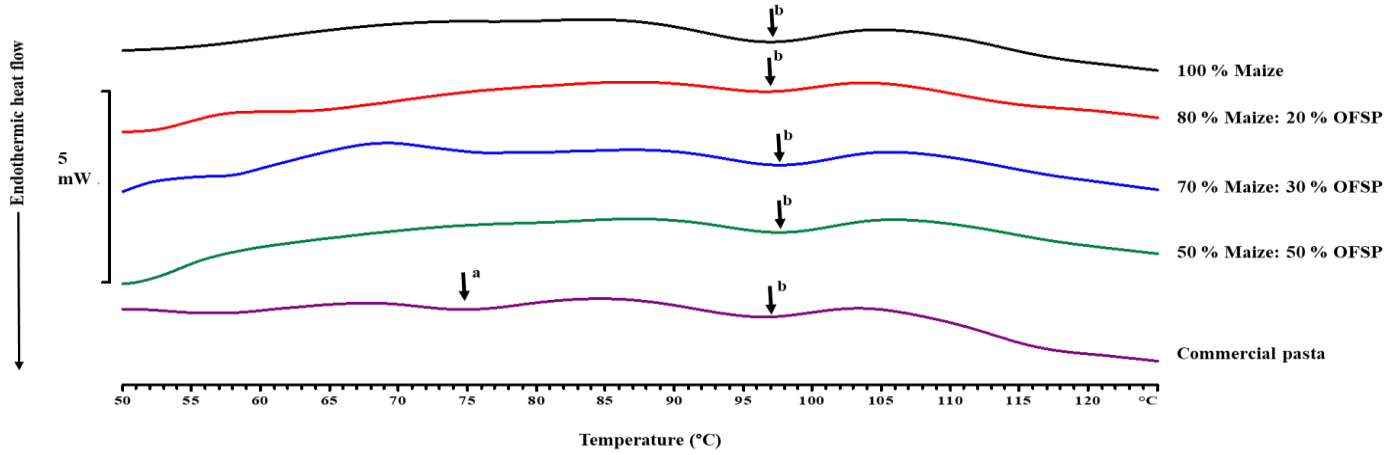

Figure 5.11: Effect of compositing maize flour with OFSP flour on the thermal properties of raw pasta samples.

Commercial gluten-free pasta made from maize and rice
[a] Endotherm for gelatinization temperature
[b] Endotherm for type 1 amylose-lipid complex.

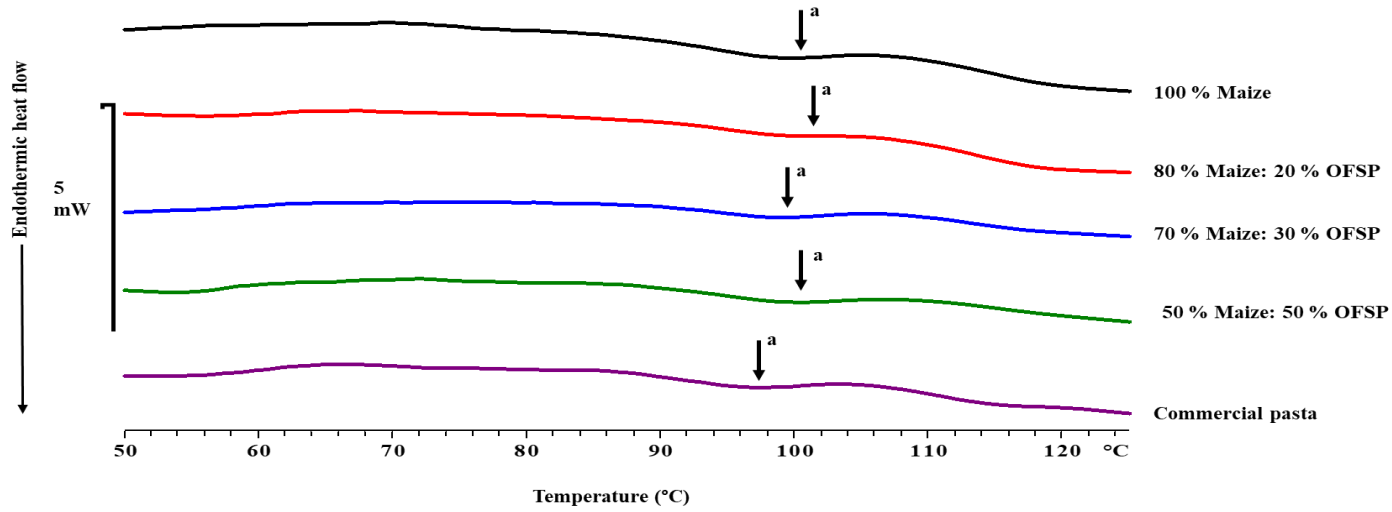

Figure 5.12: Effect of compositing maize flour with OFSP flour on the thermal properties of cooked pasta samples.

Commercial gluten-free pasta made from maize and rice
[a] Endotherm for type 1 amylose-lipid complex

Table 5.9 Effect of compositing maize flour with OFSP flour on the thermal properties of raw and cooked pasta sample

		Gelatinization endotherm				Amylose lipid complex endotherm			
Sample	**Composite**	**To (°C)**	**Tp (°C)**	**Tc (°C)**	**ΔH (J/g)**	**To (°C)**	**Tp (°C)**	**Tc (°C)**	**ΔH (J/g)**
Raw	100 % Maize	ND	ND	ND	ND	90.2 b (0.48)	96.0 ab (0.15)	102 abc (0.23)	1.28 e (0.22)
	80 % Maize: 20 % OFSP	ND	ND	ND	ND	90.0 ab (0.62)	96.0 ab (0.21)	101 a (0.37)	0.97 cd (0.09)
	70 % Maize: 30 % OFSP	ND	ND	ND	ND	91.9 c (0.06)	97.4 cd (0.75)	104 cde (1.16)	1.12 de (0.03)
	50 % Maize: 50 % OFSP	ND	ND	ND	ND	91.9 c (0.26)	97.6 cd (0.95)	103 bcd (1.19)	1.13 de (0.17)
	Commercial pasta	69.1 (0.48)	74.4 (0.17)	80.2 (0.30)	0.99 (0.15)	88.7 a (0.36)	95.4 a (0.29)	102 ab (0.05)	1.40 e (0.17)
Cooked	100 % Maize	ND	ND	ND	ND	93.0 c (0.51)	97.6 cd (0.80)	103 abcd (0.74)	0.79 bc (0.06)
	80 % Maize: 20 % OFSP	ND	ND	ND	ND	92.3 c (0.17)	98.2 cd (0.28)	104 de (1.05)	0.48 a (0.03)
	70 % Maize: 30 % OFSP	ND	ND	ND	ND	94.7 d (0.58)	99.0 de (0.72)	104 de (0.36)	0.42 a (0.16)
	50 % Maize: 50 % OFSP	ND	ND	ND	ND	95.2 d (1.33)	100 e (1.51)	105 e (0.86)	0.63 ab (0.03)
	Commercial pasta	ND	ND	ND	ND	91.8 c (0.30)	96.9 abc (0.78)	101 a (0.14)	0.64 ab (0.00)

Means within a column with different letters are significantly different ($p<0.05$)
Standard deviations are given in parenthesis
Commercial gluten-free pasta made from maize and rice
ND is not detected
To is onset temperature, Tc is conclusion temperature and Tp is peak temperature
ΔH is heat flow

5.12 Microstructure of raw and cooked pasta samples

Figures 5.13 and 5.14 show the microstructure of raw and cooked pasta respectively, visualized using the light microscope. Disrupted and no intact starch granules were observed in both extruded uncooked and cooked pasta samples, but more disrupted granules occurred in cooked pasta samples. Birefringence was hardly seen under polarized light in the raw and cooked pasta samples, except for commercial pasta. Blue/violet stains indicate starch granules. Extruded uncooked and cooked pasta from 50 % maize: 50 % OFSP had less starch granules being stained with iodine.

Figures 5.15 and 5.16 show the scanning electron micrographs of the cross section of extruded uncooked and cooked pasta respectively. A smooth and a compact surface was observed in 100 % maize cooked pasta without distinct starch granular structure. Addition of OFSP seemed to make the pasta surface microstructure more porous. The commercial pasta sample revealed its starch granules embedded in the protein matrix as compared to the extruded pasta sample. There were visible cracks in both the raw and cooked pasta samples.

The longitudinal sections of raw and cooked pasta are shown in Figures 5.17 and 5.18 respectively. It was observed that, all the uncooked pasta samples had smooth surface and cooking made them rough. Extruded pasta samples showed continuous starch-protein network as there was no discrete starch granular structure. Starch granules were more visible in the commercial pasta samples than the extruded pasta samples.

Figures 5.19 and 5.20 show images of stained fluorescing red spots as proteins (Ogundele *et al.* (2017) in the cross sectional and longitudinal view respectively viewed under the confocal laser scanning microscope (CLSM). More visible red spots were seen in the cross sectional view of cooked pasta samples compared to the raw pasta samples. It was observed that increasing proportions of maize in pasta samples increased the red spots. Increasing the OFSP seems to increase the black spot in the micrographs. Most pasta seems to have a continuous starch and protein matrix as there was a continuous red colour in the micrograph. Cooked 100 % maize pasta showed more red spots with continuous protein network. The longitudinal view (Figure 5.20) showed less red spots in both raw and cooked pasta samples with discontinuity in the protein network.

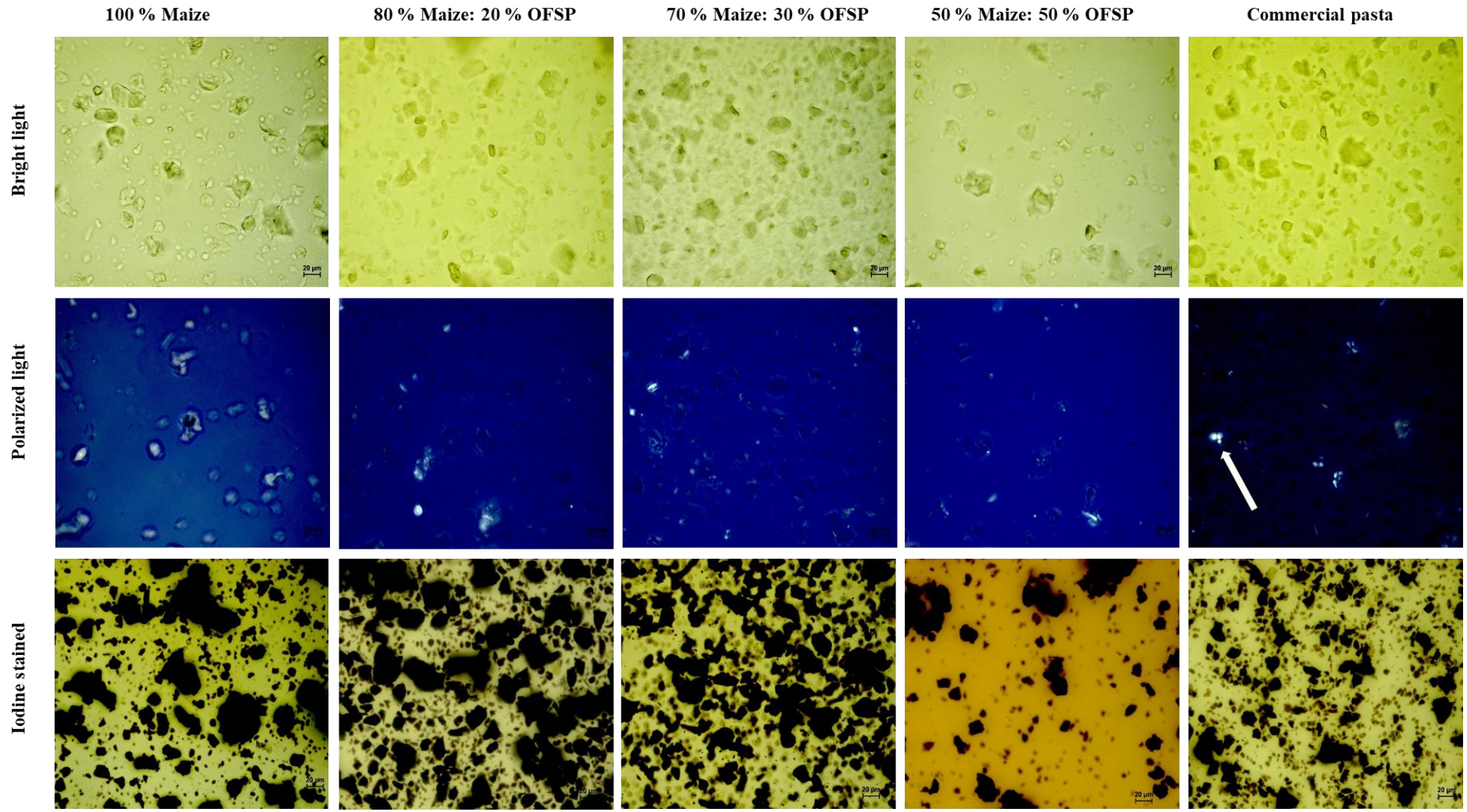

Figure 5.13: Light micrographs of milled extruded pasta: viewed under light microscope, polarized lens and stained with iodine and viewed under light microscope.

Starch was stained blue\violet. Bar 20 µm. Arrow indicates birefringence of un-gelatinized starch

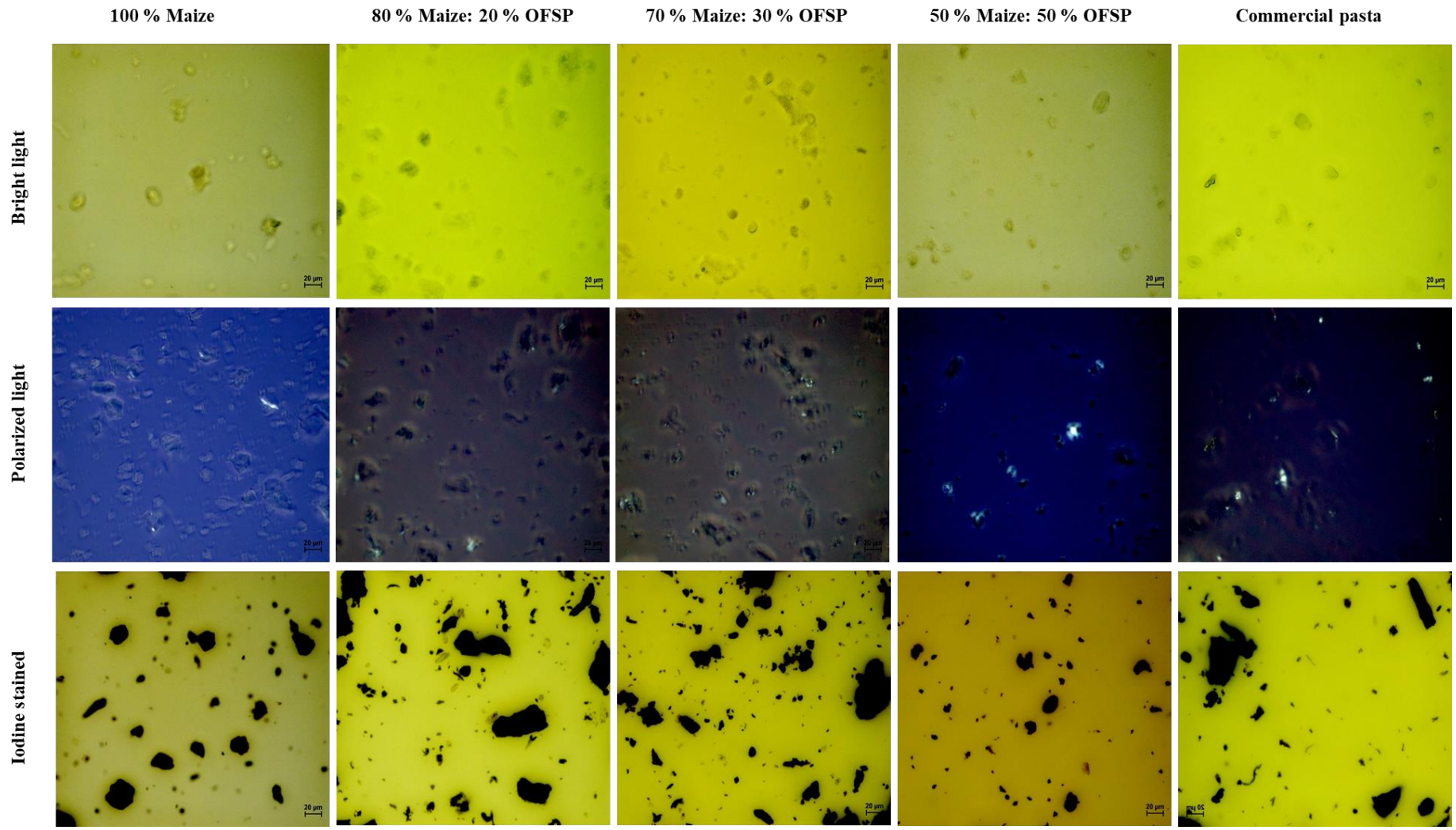

Figure 5.14: Light microscopy of cooked pasta: viewed under light microscope, polarized lens and stained with iodine and viewed under light microscope.

Starch was stained blue\violet. Bar 20 µm.

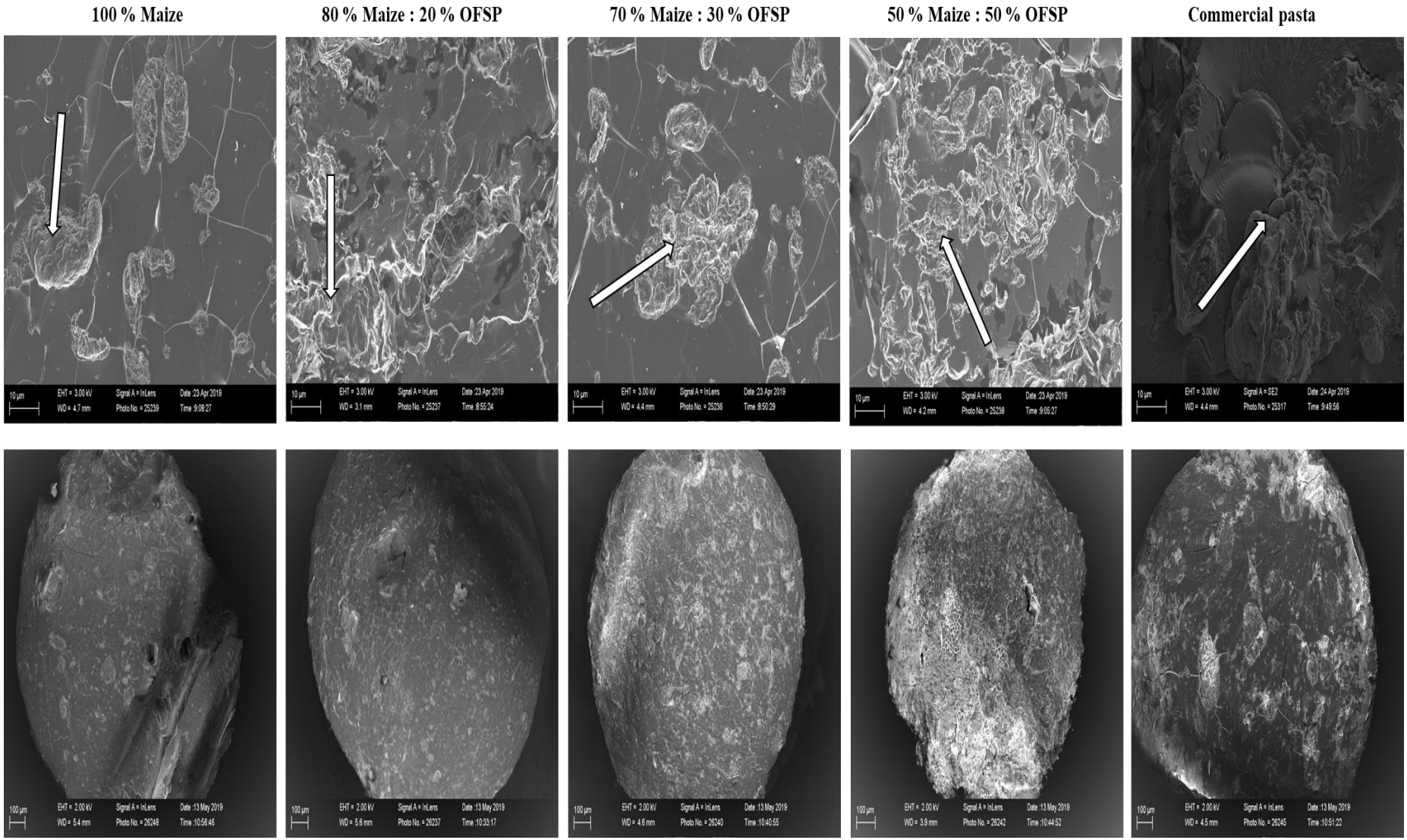

Figure 5.15: Scanning electron microscopy images of raw pasta. Surface images showing cross section of pasta.

Bar: 10 µm, 100 µm. Arrows pointing on starch protein network

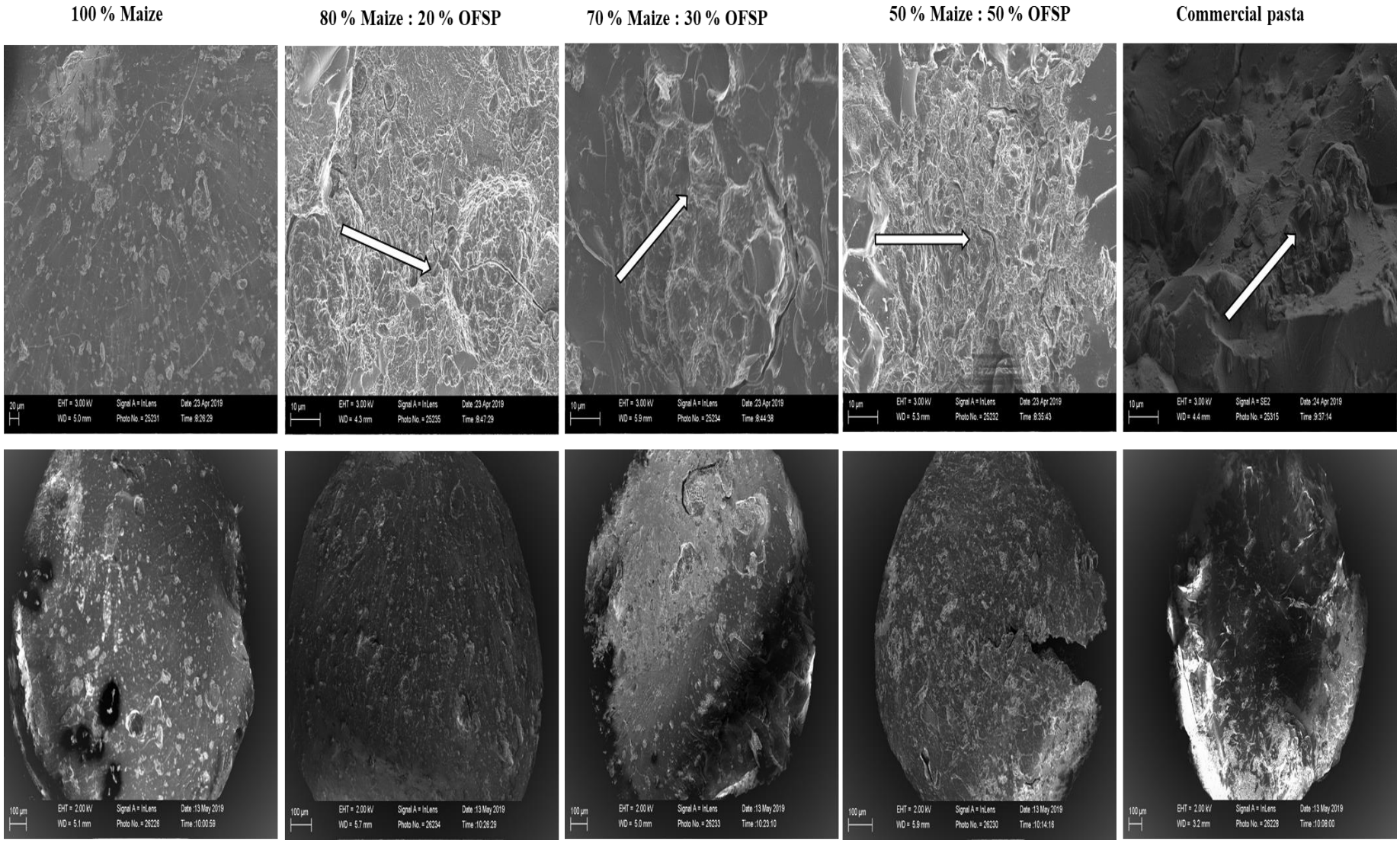

Figure 5.16: Scanning electron microscopy images of cooked pasta. Surface images showing cross section of pasta.

Bar: 10 µm, 100 µm Arrows pointing on starch protein network

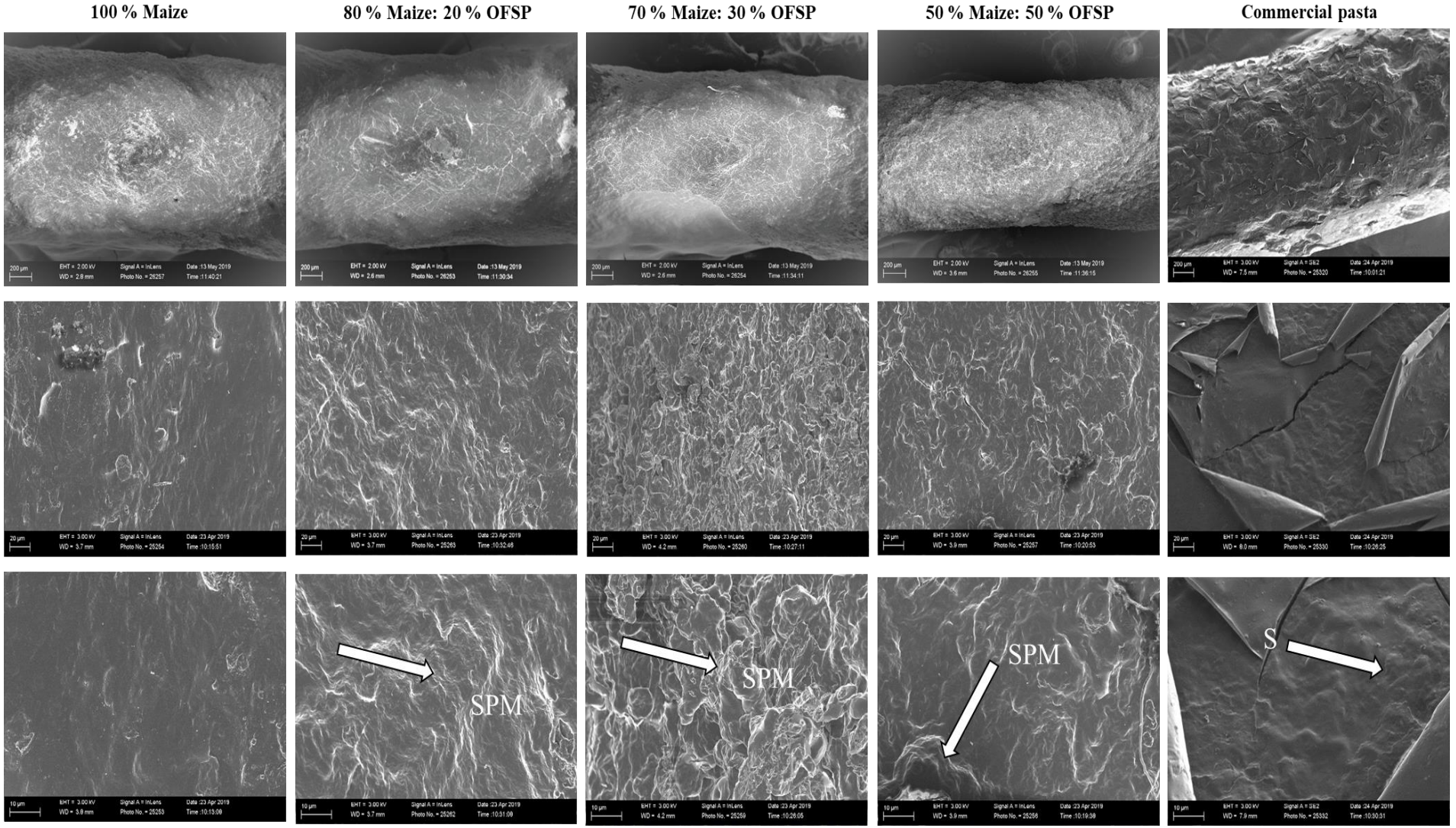

Figure 5.17: Scanning electron microscopy images of raw pasta. Surface images showing longitudinal section of pasta.

Bar: 10 µm, 20 µm, 200 µm. SPM- Starch protein network S- Starch

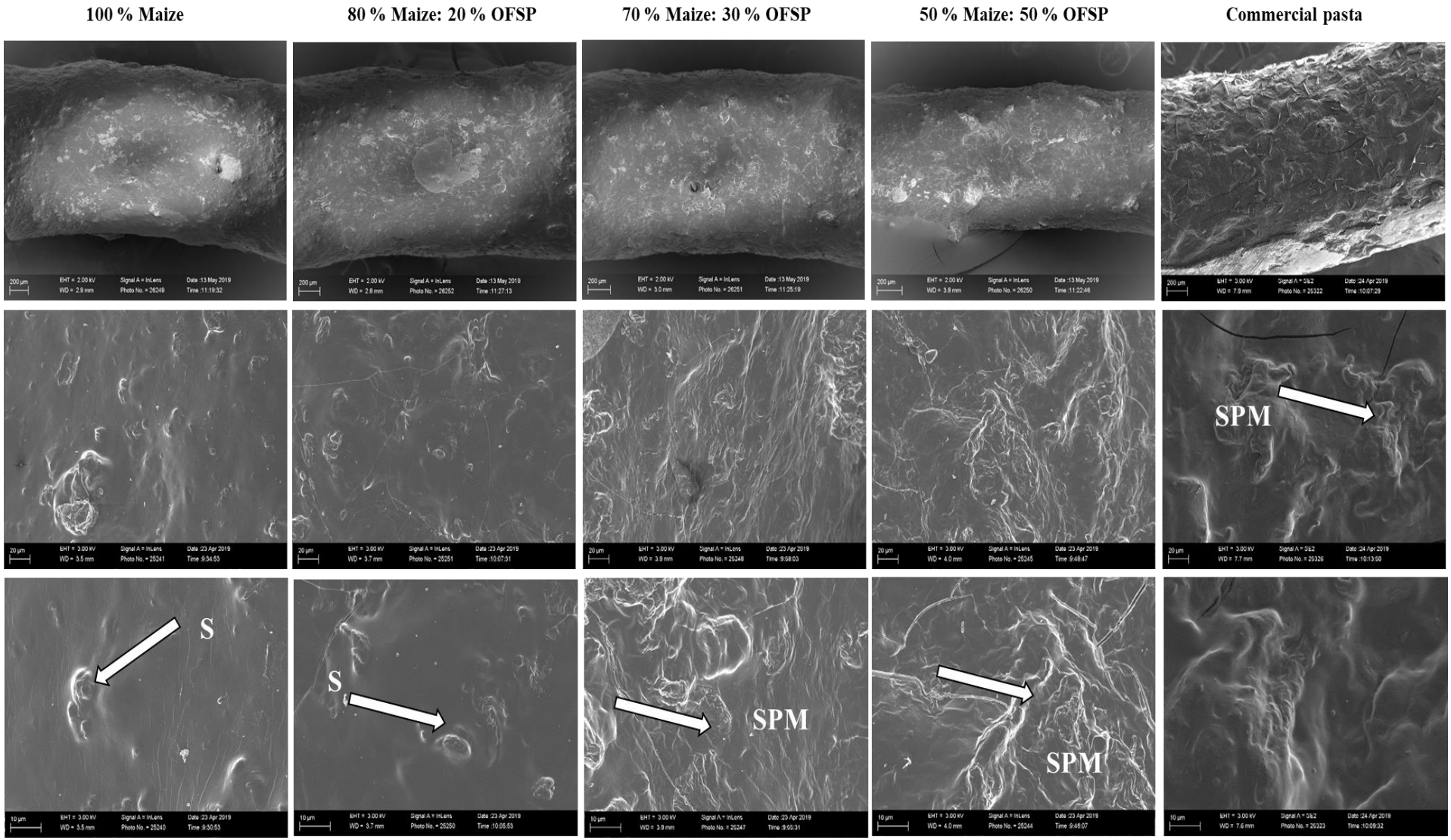

Figure 5.18: Scanning electron microscopy images of cooked pasta. Surface images showing longitudinal section of pasta.

Bar: 10 µm, 20 µm, 200 µm. SPM- Starch protein network, S –Starch

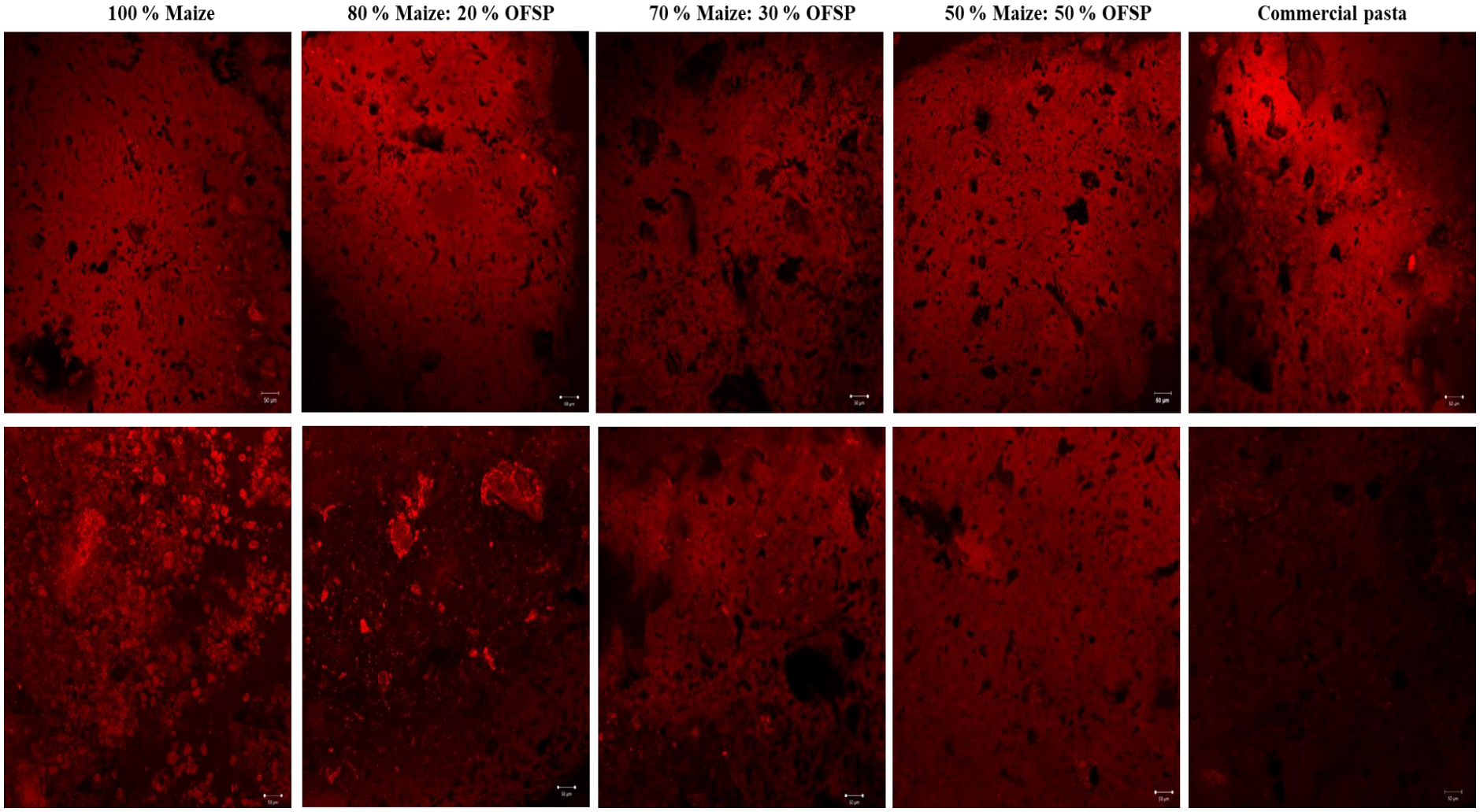

Figure 5.19: Confocal laser scanning microscopy images showing cross section of raw and cooked pasta.

Bar: 50 µm. Pasta stained with Safranin O for protein. Black spots inside the fluorescing red stained indicate starch and the red indicates protein matrix.

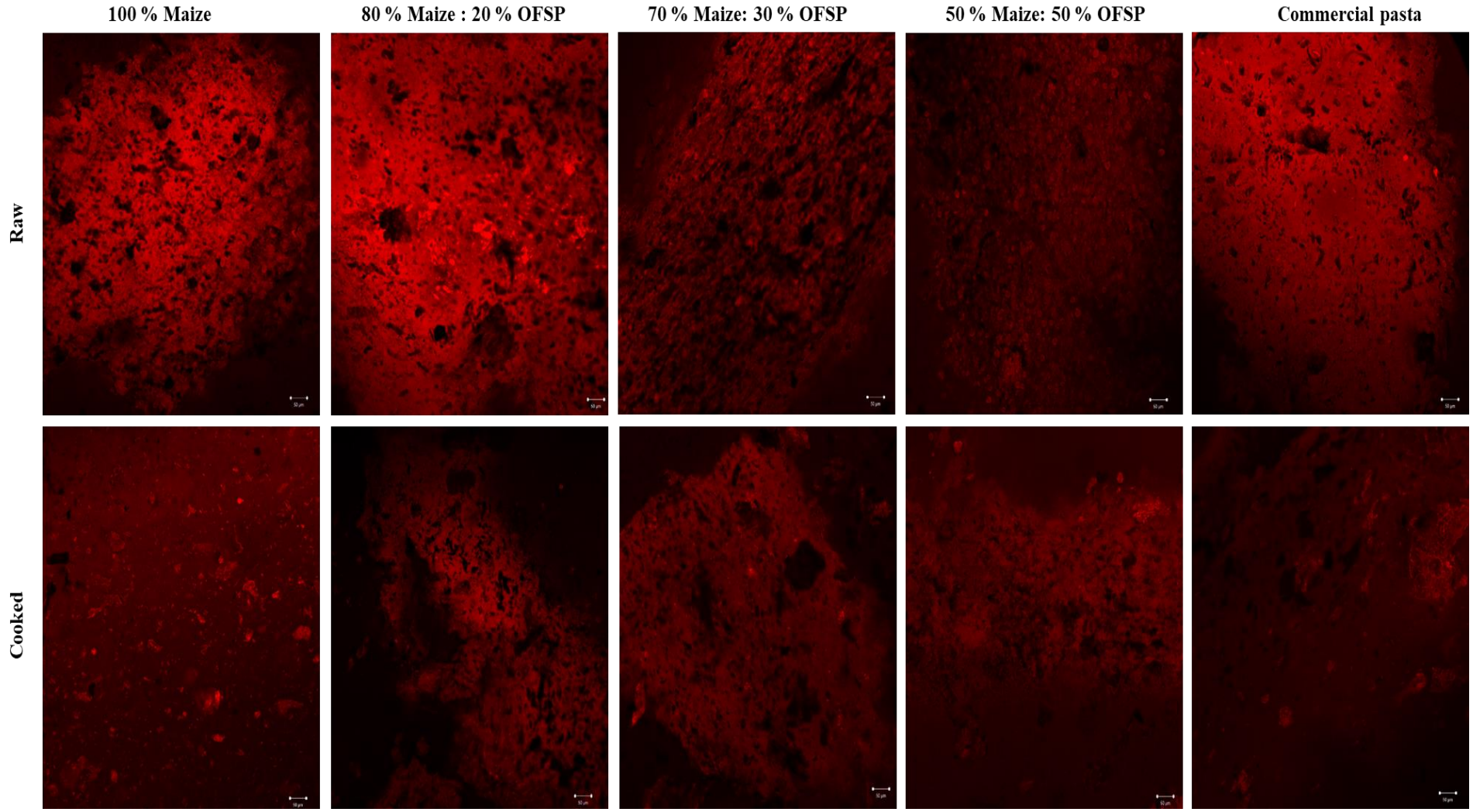

Figure 5.20: Confocal laser scanning microscopy images showing longitudinal section of raw and cooked pasta.

Bar: 50 µm. Pasta stained with Safranin O for protein. Black spots inside the fluorescing red stained indicate starch and the red indicates protein matrix.

CHAPTER 6: GENERAL DISCUSSION

This chapter is divided into two sections. The first is a critical review of the methodology, considering the strengths and weaknesses in the research done. The second section is a discussion of the major findings with scientific explanations on the effect of compositing maize flour with orange-fleshed sweet potato flour on the cooking qualities, nutritional and antioxidant properties of the gluten free pasta.

6.1 Critical review of methodology

6.1.1 Extrusion processing

Extrusion processing is one of the suitable technologies for the production of gluten-free pasta where native flour is treated with water or steam and extruded at high temperature- short time to promote starch gelatinization directly inside the extruder (Padalino *et al.*, 2016). Extrusion conditions were maintained as a constant for the production of pasta in order to determine the effects of compositing maize flour with OFSP. In order not to produce an expanded product, a low screw speed, and lower zone temperatures were used compared to those used for expanded products. Generally, a temperature of >120 °C, a moisture content of <30 %, a screw speed of >180 rpm used for expanded products. In this research, zone temperatures ranging from 60 to 80 °C, 80 rpm screw speed, low shear screw configuration and 30 % moisture were used. Lower shear and lower temperature will prevent the build-up of pressure in the extruder barrel preventing expansion of product as there will be hardly any pressure difference in the barrel and the atmospheric pressure.

6.1.2 Cooking qualities

The cooking method used for determination of cooking quality was representative of how pasta is normally cooked in various households with the exception of the addition of sodium chloride. Raw pasta (25 g) was immersed in an aluminium pot with 250 mL boiling distilled water. The pasta: water ratio was important because lower cooking water would create less space between pasta strands for movement which will lead to collision and pasta surface sticking together. The use of distilled water was for standardization of the procedure. Distilled water is free from certain minerals which could prevent pasta from recording higher stickiness and cooking loss values (Malcolmson and Matsuo, 1993). A glass slide used to determine the cooking time was for easy

visualization of the white core to confirm if pasta was not cooked well. The pasta broke easily because it was not a straight strand.

6.1.3 Texture qualities

The firmness, stickiness and fracturability of the pasta was determined using the texture analyser. The texture analyser applies scientific method to measure and analyse product texture. The equipment was used to understand consumer's acceptance criteria by using it to evaluate and control the factors affecting quality, processing and handling. The principle was to physically deform a test sample in a controlled manner and measure the response.

The firmness and stickiness was analysed using a compression test that uses the Kramer shear cell with a probe applying a compression force. Pasta was put in the Kramer shear cell and the probe performed a double compression. Figure 6.1 shows a general chart of texture profile analysis and how it correlates with human eating process. The firmness represented the hardness on the graph, which is the force necessary to deform a product at a given distance; for example, to compress it between the molars, to cut it with the incisors, or to compress it between the tongue and the palate. Stickiness was related to adhesiveness on the graph. It is the force necessary to overcome the forces of attraction between the surface of the product and the surface of the material (probe) with which the product comes into contact.

The fracturability of the raw and cooked pasta samples were not determined using the same test cell. The fracturability of the raw pasta was determined using the snap, bend and break method using the 3-point break rig. The pasta was supported by 2 "fulcrums" on both side, and a third, centre fulcrum comes down to apply the bending force. The distance between the two side fulcrums was maintained for all different samples. This method was not useful for the cooked pasta because it was just bending and not breaking due to its moist nature. Therefore, the shear compression Kramer cell was used to measure the fracturability of the cooked pasta.

The test involved bringing down the 5 blades along the grooves in the cell and, depending on the speed selected, compressing and partially extruding the pasta through the holes in the base of the cell. One disadvantage is that higher force was being recorded because of the number of pasta stands placed at a time. Furthermore, the disadvantage of using the texture analyser was that, the test destroys the sample and involves lengthy measuring times since each time the probe is used it

has to be disassembled, meticulously cleaned as pieces of pasta get jammed between the blades and reassembled.

Even though there is a correlation between sensory and texture profile method, (Paula and Conti-Silva, 2014), sensory evaluation could be conducted to help predict consumer response as well.

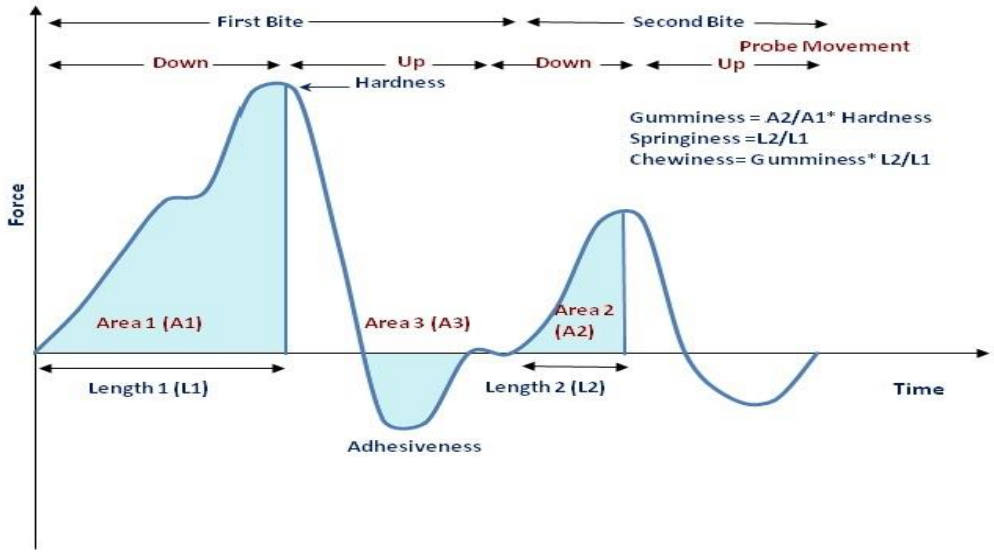

Figure 6.1: Chart of Texture Profile Analysis (TPA).

Adapted from (Banjare *et al.*, 2015)

6.1.4 *In-vitro* starch digestibility

An *in-vitro* starch digestibility method was used to determine starch hydrolysis to predict the glycemic index of the pasta samples. Different researchers have used different methods to determine *in vitro* starch digestibility (Goñi *et al.*, 1997; Amadou *et al.*, 2014; Brou *et al.*, 2014). In this study, the method by Goñi *et al.* (1997) was used. The method simulates the conditions of the stomach and intestines and measures the release of glucose at different times. Starch was digested using α-amylase and amyloglucosidase enzyme and pepsin enzyme was used to disrupt any starch-protein interactions by digesting the protein. The dextrin and oligosaccharides obtained after hydrolysis by α-amylase are further broken down by amyloglucosidase to release glucose (Goñi *et al.*, 1997).

Some methods of digestion require digestion of the test food as it is eaten (Englyst *et al.*, 2000). The chewing process is then simulated. Englyst *et al.* (1999) simulated the chewing process using mincers. The Goñi *et al.* (1997) method of analysis does not require the realistic "as eaten" food particles (Woolnough *et al.*, 2008). Therefore, the pasta was milled before being digested but this does not give the exact particle size of the food when eaten. The Goñi *et al.* (1997) method also has the limitation of not considering the influence of the transit time and absorption time in the gastrointestinal tract which could affect digestibility of starch even though it is said to have a strong correlation with *in vivo* method.

6.1.5 *In-vitro* protein digestibility

Protein digestibility can be determined using *in-vivo* or *in-vitro* assays. Pepsin and the multienzyme method of digestion are widely used *in-vitro* protein digestibility determination (Boisen and Eggum, 1991; Thomas *et al.*, 2004). The pepsin method was used in this study. The pepsin method involves one step incubations with a single enzyme. The use of a single enzyme allows for standardization of *in-vitro* digestion models enabling consistent comparison (Hur *et al.*, 2011). The method requires the protein content of the test food to be determined first before digestibility. The pepsin enzyme method of digestion simulates conditions of digestion in the stomach (Hur *et al.*, 2011). The determined protein content in the digested food is expressed as a percentage of the initial protein content (Hamaker *et al.*, 1987). A drawback of using a single enzyme method of digestion is that it may be less reproducible than the multienzyme method of digestibility (Hur *et al.*, 2011). A single enzyme of digestion that is specific to a peptide bond may give different results for proteins with different amounts of the specific amino acid (Hsu *et al.*, 1977). Thus the results are relative rather than absolute values.

6.1.6 Thermal properties

The differential scanning calorimetry (DSC) has been extensively used to study thermal properties of food. The method determines quantitatively the amount of gelatinized starch in processed foods (Biliaderis and Galloway, 1989). DSC has been used in studies of phase transitions of aqueous starch systems because of gelatinisation being an endothermic process (Biliaderis and Galloway, 1989). The DSC has the advantage of detecting characteristic temperatures and enthalpies of different transitions (Biliaderis and Galloway, 1989). It also allows for the study of gelatinisation over a wide range of starch water/ratio (Biliaderis *et al.*, 1980). It has the ability to analyse a wide

range of starch concentrations (Biliaderis and Galloway, 1989). There is difficulty in detecting glass transition of the amorphous region of starch granules when the DSC is used (Xie *et al.*, 2010). This is due to the small discontinuity heat capacity at glass transition that is too small to be measured for the native starch when heated in excess water (Xie *et al.*, 2010). The difference of the glass transition temperature and the processing material temperature is important in extruded products because it determines its characteristics (Núñez *et al.*, 2009). In samples where there is a limited amount of native starch, the DSC endothermic peak will be at or below the sensitivity threshold of the equipment. Furthermore, the water: solid ratio influences the thermal properties under DSC. That is, the higher the water content, the lower the gelatinization temperature.

6.1.7 β- carotene

Carotenoid extraction has no standard or accepted method. Thus making the choice of extraction method very important since errors associated with extraction process are potentially significant (Kimura and Rodriguez-Amaya, 1999). Organic solvents such as, chloroform, hexane, tetrahydrofuran, methanol, dichloromethane acetone, ethanol and petroleum ether are the widely accepted solvents used in carotenoid extraction (Su *et al.*, 2002; Meléndez-Martínez *et al.*, 2007). Tetrahydrofuran (THF) was used for the extraction of beta carotene in this study because of its high capacity to solubilize carotenoids (Rivera and Canela, 2012). Aside its capabilities, THF can form peroxides which could degrade carotenoids by auto-oxidation and cis-trans isomerization therefore extraction was carried out rapidly under dark conditions to avoid exposure to light, heat and oxygen (Rivera and Canela, 2012). Minimizing the degradation of β-carotene in this study was not carried out well. According to Saini and Keum (2018) to minimize the degradation of carotenoids during extraction, storage and analysis these factors should be taken into consideration: i) the neutralization of acids liberated from plant samples during extraction by using neutralizers such as, magnesium carbonate or calcium carbonate. If not neutralized the acids can cause potential isomerization and rearrangement of 5,6-epoxy- to 5,8-epoxy-carotenoids (e.g. neoxanthin and violaxanthin); ii) the use of antioxidant , such as butylated hydroxytoluene (BHT), tert-butlylhydroqinone (TBHQ), or pyrogallol in the extraction solvents; iii) minimizing the time lag between sample maceration and extraction to prevent enzymatic oxidation; also, viable extraction, a short extraction time with appropriate temperature is recommended; iv) flushing sample tubes with nitrogen gas during extraction to eliminate oxygen and provide an inert

environment; and v) preventing direct exposure of ultraviolet light on samples, as this can promote trans-cis photoisomerization and photodestruction. Therefore, the values obtained from this study may be regarded more as relative values. Nonetheless, the fact that the extractions were carried out rapidly under the dark conditions could mitigate any losses of β-carotene due to the factors mentioned above.

6.1.8 Antioxidant properties

Different assays have been used to measure antioxidant capacity of foods and biological samples. In general, antioxidant capacity assays measure the ability of antioxidant components in foods and biological systems to scavenge free radicals in what are essentially redox reactions. The most popular spectrophotometric assays being used are the 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays. The ABTS radical scavenging assay was used in this study. The principle is that, a redox-active compound or synthetic coloured radical (in this case, the ABTS radical) is generated; and the ability of a biological sample to reduce the redox-active compound or scavenge the ABTS radical is monitored using spectrophotometer, applying an appropriate standard to quantify antioxidant capacity, e.g. as Trolox equivalent antioxidant capacity (Floegel *et al.*, 2011).

The ABTS method is preferred for its rapid reaction of the samples with the ABTS in the aqueous buffer solution reaching a steady state within 30 min and also its extra flexibility in that, it can be used at different pH (Ou *et al.*, 2002; Shalaby and Shanab, 2013). The drawback is that, ABTS assay is time consuming because, the ABTS radical needs to be prepared first and it takes 12 hours to be ready. Using this method, antioxidant capacity may be overestimated, due to both a scavenger effect and an effect on the rate of ABTS oxidation (Strljbe *et al.*, 1997). Furthermore, the radicals in this assay do not occur in biological systems and therefore the antioxidant activities obtained may be of little physiological relevance (Prior *et al.*, 2005). Nonetheless, the ABTS radical scavenging is useful as a convenient method of measuring the ability of bioactive components of foods to scavenge free radicals.

6.2 Major findings

6.2.1 Proximate composition of extruded pasta

Generally, moisture content of pasta is lowered from approximately 30 % in the extruder barrel to less than 12.5 % moisture after drying (Czaja *et al.*, 2018). During extrusion process, materials being produced under high temperature results in some evaporation of water as a result of pressure drop and temperature difference of the material exiting the die and this results in lower moisture content (Alonso *et al.*, 2001). In addition, the moist pasta was air dried for 24 hours. The low moisture content of less than 12 % suggests that the pasta will be shelf stable. The moisture content of the extruded pasta in this study ranged from 6.64 % to 7.15 % which is an indication of a shelf stable pasta. At such moisture level, the pasta will not support the growth of moulds, yeast or other spoilage microorganisms (Doster and Kahn, 1986).

The protein content of maize flour shown in this study was similar to the value obtained by Onyango *et al.* (2004). Shukla and Cheryan (2001) also reported that maize flour contains 6- 8 % protein content. The higher ash content in orange-fleshed sweet potato suggests higher mineral content as the proportions of OFSP flour in the maize: OFSP flour and pasta samples increased. This is in agreement confirms with other researchers (Haile *et al.*, 2015; Rodrigues *et al.*, 2016) who have also reported a higher ash content in orange-fleshed sweet potato flour.

The low fat content of OFSP agrees with previous reports by Zakaria-Rungkat *et al.* (2000) and Aina *et al.* (2009) who showed low fat content of OFSP, 0.61 % (wet basis) and (0.23-1.83 % dry weight basis) respectively. Extrusion processing during pasta manufacturing decreased the fat content in the pasta samples. Björck and Asp (1983) reported that, extrusion cooking decreases the extractable fat due to higher shear, steam distillation, thermal degradation and complexation. Free fatty acids can form complexes with amylose therefore resulting in difficulty in extracting with organic solvent (Mercier *et al.*, 1980). The formation of amylose-lipid complexes in pasta samples will be discussed in detail in a later part of this text.

As the proportions of OFSP in the maize: OFSP composite flour and pasta samples increased, insoluble and soluble dietary fibre increased essentially due to the high levels of insoluble and soluble dietary fibre in the OFSP flour. The decrease in insoluble dietary fibre and increase in soluble dietary fibre after extrusion to produce pasta suggests thermal and mechanical

decomposition of insoluble dietary fibre to soluble ones (Lue *et al.*, 1991). The mechanical shear and high temperature during extrusion leads to the breakage of polysaccharide glycosidic linkages. This leads to the redistribution of insoluble to soluble dietary fibre content (Oladiran and Emmambux, 2018).

The alteration in the composition of dietary fibre in extrudate could have led to the increase in soluble solids of pasta samples as a result of changes in molecular structures of dietary fibre during extrusion processing. During extrusion cooking, shear forces and high temperature disrupt the glycosidic bonds of carbohydrate biopolymers to form more soluble lower molecular weight polymers (Sarifudin and Assiry, 2014). Soluble fibre forming viscous fluid with water and increasing its viscosity as a result of fibre fragmentation and solubilization has a physiological benefit (Oladiran and Emmambux, 2018). According to Dartois *et al.* (2010) high viscosity in the gut lumen can reduce post prandial glucose response by reducing glucose absorption.

6.2.2 Principal component analysis (PCA) of extruded gluten free maize pasta with and without OFSP and commercial pasta

Principal component analysis (PCA) was used to understand the relationship between the composited pasta samples and the measured variables and to explain the relationship between the variables (Figure 6.2). The two principal components contributed about 57.18 % and 24.21 % of the total variation respectively. Principal component 1 separated raw maize with and without orange-fleshed sweet potato flour. The clusters in the principal component plot of scores were identified and labelled based on the high loadings of respective variables. The cluster of high percentage of OFSP in pasta samples was identified based on high cooking loss, soluble solids, stickiness, insoluble and soluble dietary fibre, *in-vitro* protein digestibility, slowly digestible starch and estimated glycemic index. The cluster for 80 % maize: 20 % OFSP pasta sample was identified based on high total starch, resistant starch, fracturability and firmness. The commercial pasta was identified based on cooking time and water absorption capacity.

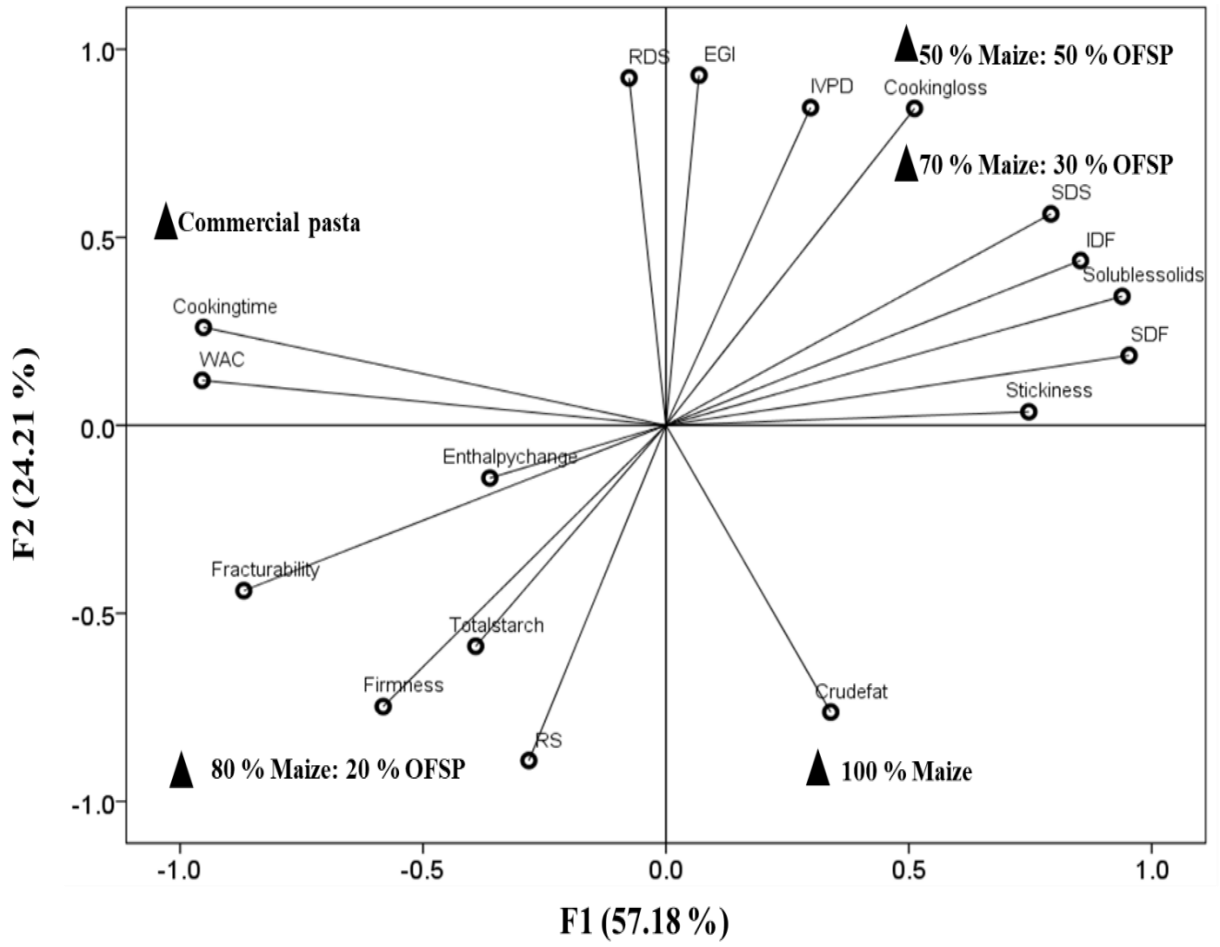

Figure 6.2: Principal component analysis (PCA) plots for loading of measurable and scores of extruded gluten free maize pasta with and without OFSP and commercial rice and maize pasta

(RDS- Rapidly digestible starch, EGI- Estimated glycemic index, IVPD- In vitro protein digestibility, SDS- slowly digestible starch, IDF- Insoluble dietary fibre, SDF- Soluble dietary fibre. RS- Resistant starch, WAC- Water absorption capacity)

6.2.3 Cooking qualities of pasta samples

Several phenomena that occur during cooking such as starch hydration and interaction with non-starchy materials contribute to the overall cooking and textural characteristics of pasta (Giuberti *et al.*, 2015). Expansion ratio, cooking loss and water absorption capacity of pasta samples increased with decrease in proportion of maize flour in maize: orange-fleshed sweet potato extruded composite pasta samples.

Extruded pasta samples statistically ($p>0.05$) showed lower cooking time as compared to the commercial pasta. This could have been due to the lower diameter of the extruded pasta strands (1.1-1.6 mm) as compared to the commercial pasta (1.8 mm) resulting in a faster penetration of water to the core during cooking. The dietary fibre component in orange fleshed sweet potato flour tends to disrupt the physical structure of the pasta and limit its ability to expand (Haralampu, 2000) as compared to the 100 % maize and commercial pasta. Furthermore, the lower cooking time for extruded pasta samples could have been due to lower molecular weight compounds. The starch content decreased as a result of OFSP addition. During extrusion process, starch is gelatinized, disrupted and partially depolymerized resulting in the production of lower molecular weight compounds (Altan and Maskan, 2011) thus leading to rapid hydration of compounds during cooking, resulting in lower cooking time.

The light microscopy (Figure 5.13) showed indistinct starch granules and lack of birefringence in the extruded pasta samples as compared to the commercial pasta. This could be due to the pre-gelatinization process that occurs during extrusion process which could also cause lower cooking time. The minimal preparation time for the pasta samples in this study was similar to other researchers who reported a minimal preparation time for extruded gluten-free pasta from yellow pea ranging from 5.5-7.0 mins (Wójtowicz and Mościcki, 2014), rice pasta composited with lentil ranging from 8-9 mins (Bouasla *et al.*, 2017), rice pasta composited with yellow pea ranging from 7 to 8 mins (Bouasla *et al.*, 2016).

From the PCA plot, there was a positive correlation between the WAC and cooking time. The water absorption capacity ranged from 95.3 % - 122.5 %. Commercial pasta exhibited higher water absorption capacity compared to the composited pasta samples with OFSP flour. OFSP has lower content of biopolymers such as starch and protein compared to maize flour. According to Köber *et al.* (2007) WAC is a function of the amylose/amylopectin ratio affected by starch gelatinization as well as protein hydration. The higher cooking time and WAC for the commercial pasta could probably be due to its compact starch-protein network as a result of low temperature used in conventional pasta production. The SEM microscopy (Figure 5.18) for commercial pasta showed visible starch granules embedded in proteins which indicated that, there was less gelatinization of starch and protein damage resulting in longer hydration increasing the cooking time and subsequently resulting in higher uptake of water during cooking.

Cooking loss is due to the loosening of the compact structure of the pasta and the leach out of soluble materials (Petitot *et al.*, 2010b). High cooking loss is undesirable as it represents high solubility of starch, resulting in turbid cooking water and pasta having sticky mouthfeel. The cooking loss of the extruded pasta samples ranged from 4.36 to 12.46 %. According to Khan *et al.* (2013) the acceptable cooking loss of pasta considered desirable for good quality pasta should be less than or equal to 8 %, thereby making pasta samples in this current study acceptable except for 50 % maize: 50 % OFSP pasta.

This increase in cooking loss with pasta samples composited with OFSP flour could probably be due to the higher total soluble solids which leached out during cooking. The higher solubilization in OFSP could be due to shorter chain length of starch with a corresponding weakening of the hydrogen bonds holding the granules together. Furthermore, the fibre content in OFSP could have caused the higher cooking loss in the pasta samples. Extrusion process and dietary fibre may disrupt the compact protein-starch matrix causing weakness in its structure leading to loss of solids during cooking (Tudorica *et al.*, 2002). Figure 6.3 shows how the dietary fibre content in the OFSP and the extrusion processing would have caused higher cooking loss for the composited pasta samples. During the mixing and kneading of the flour samples in the extruder barrel, the fibre in the OFSP flour may have disrupted the compact starch-protein network that would have been formed to keep the pasta structure continuous and intact. This disruption might have caused a discontinuity in the matrix of the pasta thereby upon cooking in water, the loosely bound starch to proteins leached out faster resulting in cooking loss. During high temperature processing the glycosidic linkages in dietary fibre polysaccharides may be broken. A decreased association between fibre molecules, and/or a depolymerization of the fibre, results in a solubilization (Chindapan *et al.*, 2015). The SEM microscopy (Figure 5.15) for the composited pasta with OFSP showed what could be a discontinuity of protein matrix and damage of starch granules further exposing it to leaching during cooking in water.

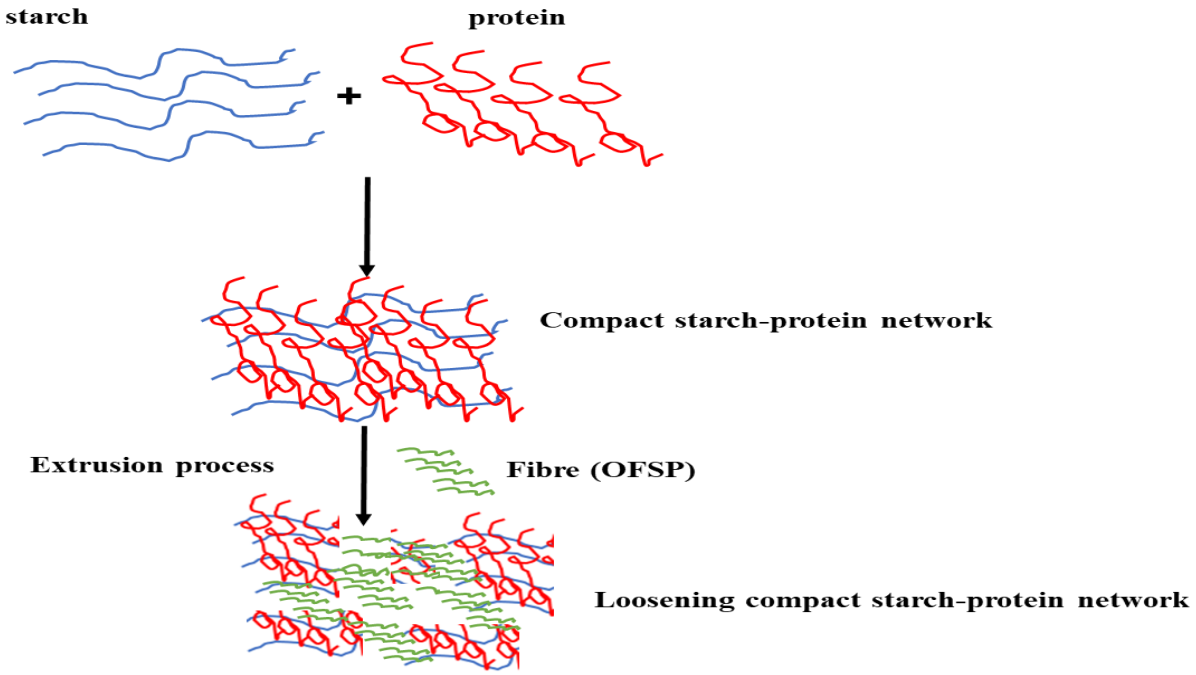

Figure 6.3: An illustration of the effect of dietary fibre on the strength of the starch-protein network formed during extrusion

In a study on the inclusion of high fibre legume flour to rice in a gluten free pasta using a high temperature single screw extrusion process, Bouasla *et al.* (2017) showed similar results to those in this study and attributed the higher cooking loss to the fact that, cooking loss was due to the weakness of starch network by the presence of fibre. Foschia *et al.* (2015) also reported a similar study on the effect of dietary fibre on the physicochemical characteristics of conventionally made pasta and proposed that, the increased cooking loss may be related to the presence of water-soluble components and the disruption of protein-starch matrix and uneven distribution of water within the pasta matrix due to the competitive hydration tendencies of fibre.

The starch, protein and fibre content present in the composite flours influenced the firmness and the stickiness of the pasta produced. The stickiness of pasta is caused by the surface structure of the strand and the leach out of starch unto the surface of the strand during cooking (Susanna and Prabhasankar, 2013). It was observed that, the firmer the pasta the less sticky it was. The decrease in proportions of maize flour in maize: OFSP composites decreased the pasta firmness and increased its stickiness. The increase in stickiness may be due to higher cooking loss. There was a

negative correlation between firmness and cooking loss from the PCA plot (Figure 6.2). Decreasing proportions of maize flour in the maize: OFSP composites led to higher cooking loss and lower firmness of the pasta samples. It was observed that, pasta samples with higher cooking loss showed lower firmness and pasta samples with lower cooking loss showed higher firmness. The discontinues starch-protein network seen in the SEM (Figure 5.15), may have led to the excessive leach of amylose or loosely bound starch during cooking onto the surfaces of the pasta strands causing adherence of pasta to each other resulting in a stickiness. According to Tudorica *et al.* (2002), higher cooking loss of pasta brings about the leach out of soluble solids into the cooking water making the pasta less firm. This is in agreement with work done by Phongthai *et al.* (2017) who found a significant correlation between higher cooking loss and higher stickiness of pasta. Phongthai *et al.* (2017) stated that the cooking loss was mainly caused by fibre content. The fibre content in OFSP flour may have disrupted the protein matrix and allowed excess leach out of starch onto the surface of the pasta (Tudorica *et al.* 2002) resulting in a sticky pasta.

Figures 5.4 and 5.5 show the maximum force required to break the raw pasta and cooked pasta respectively. The tensile testing assessed the breaking strength as this gives an indication of how samples can hold together during cooking and also their resistance to fracture during packaging or transportation operations. Fracturability force value required to break 100 % raw maize pasta and commercial pasta was significantly ($p>0.05$) not different and higher probably due to the strong matrix present in maize and rice (raw materials used for the pasta). However, composited pasta samples with OFSP flour required less force to rupture probably due to the fibre content. Fibre is known to disrupt continuous matrix of biopolymers weakening pasta structure (Petitot *et al.*, 2010b).

Figure 6.4 shows a diagram of how the cooking qualities relate to each other in extruded gluten free pasta. In extruded gluten free pasta, there are already pregelatinized starches which absorb water faster upon cooking resulting in lower optimum cooking time. When the internal starch-protein network is very compact, there is lower cooking loss, higher water absorption capacity, high firmness and higher fracturability of pasta. On the other hand, when the starch-protein network is not very compact, there is higher cooking loss, lower water absorption capacity, higher stickiness and lower fracturability of pasta.

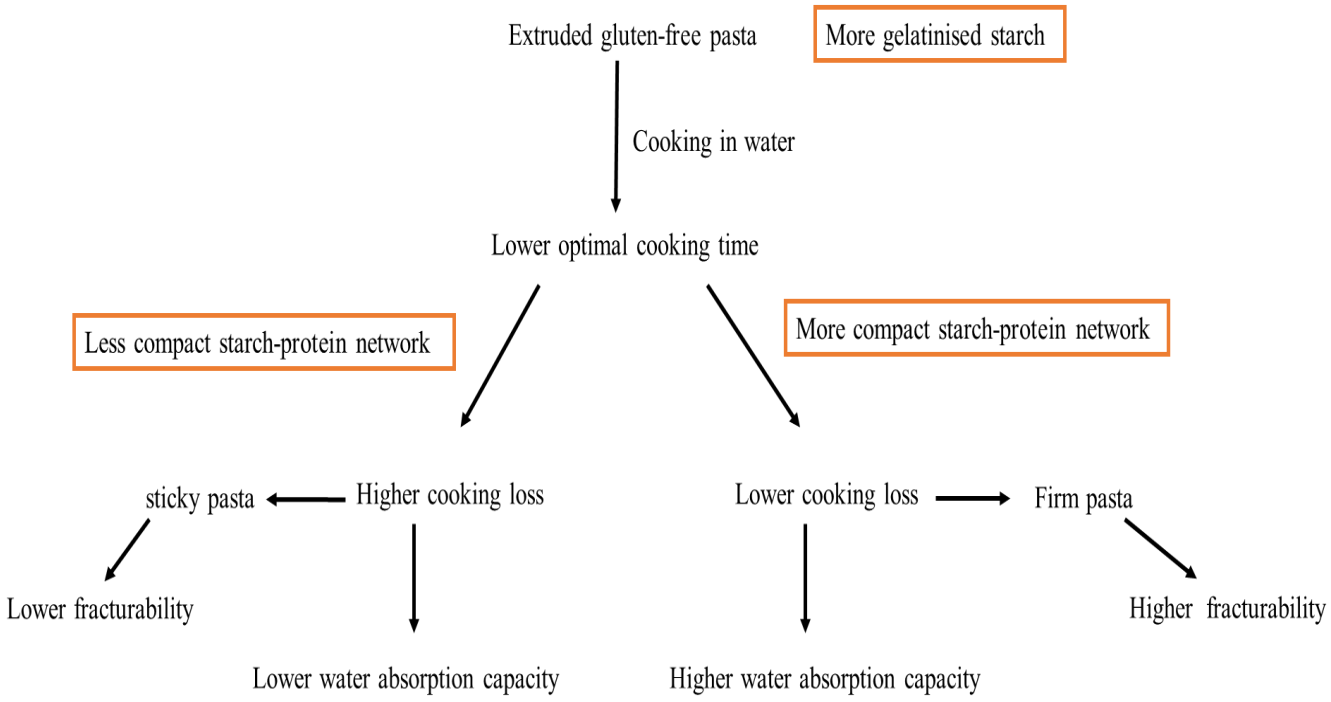

Figure 6.4: Relationship between cooking qualities of extruded pasta

6.2.4 Nutritional quality of pasta samples

The major observation from this research was the significant decrease in β–carotene content of the pasta samples after extrusion cooking. Similar observations have been reported by Shih *et al.* (2009) who recorded a decrease in the β-carotene content in orange fleshed sweet potato after extrusion cooking. During extrusion cooking, the pasta samples are exposed to conditions such as light, oxygen and high temperature. Under these conditions, β–carotene is labile and could be lost through three main mechanisms: cis-trans isomerization, fragmentation and oxidation (Ogunlesi and Lee, 1979). These modes of degradation of β–carotene are illustrated in Figure 6.5.

During cis-trans isomerization, the trans forms of β–carotene are converted to cis forms (collectively known as neo-β–carotenes) such as 15-15'-di-cis-β-carotene, 13-cis-β-carotene and 9-cis-β-carotene (Pénicaud *et al.*, 2011). The cis isomers have significantly reduced vitamin A activity (Deming *et al.*, 2002) and furthermore, they are susceptible to further degradation.

The very high temperatures used during extrusion cooking to produce the pasta samples could lead to the fragmentation of trans β–carotene resulting in the formation of aromatic compounds such as

toluene, *m*-xylene and 2,6-dimethylnaphthalene (Rios *et al.*, 2008). A significant amount of trans β–carotene could be lost through this mechanism.

Oxidation of β-carotene results in production of its diradical which can easily be attacked by oxygen to produce epoxides. Other compounds like apocarotenones and apocarotenals could also be formed from the epoxides (Pénicaud *et al.*, 2011).

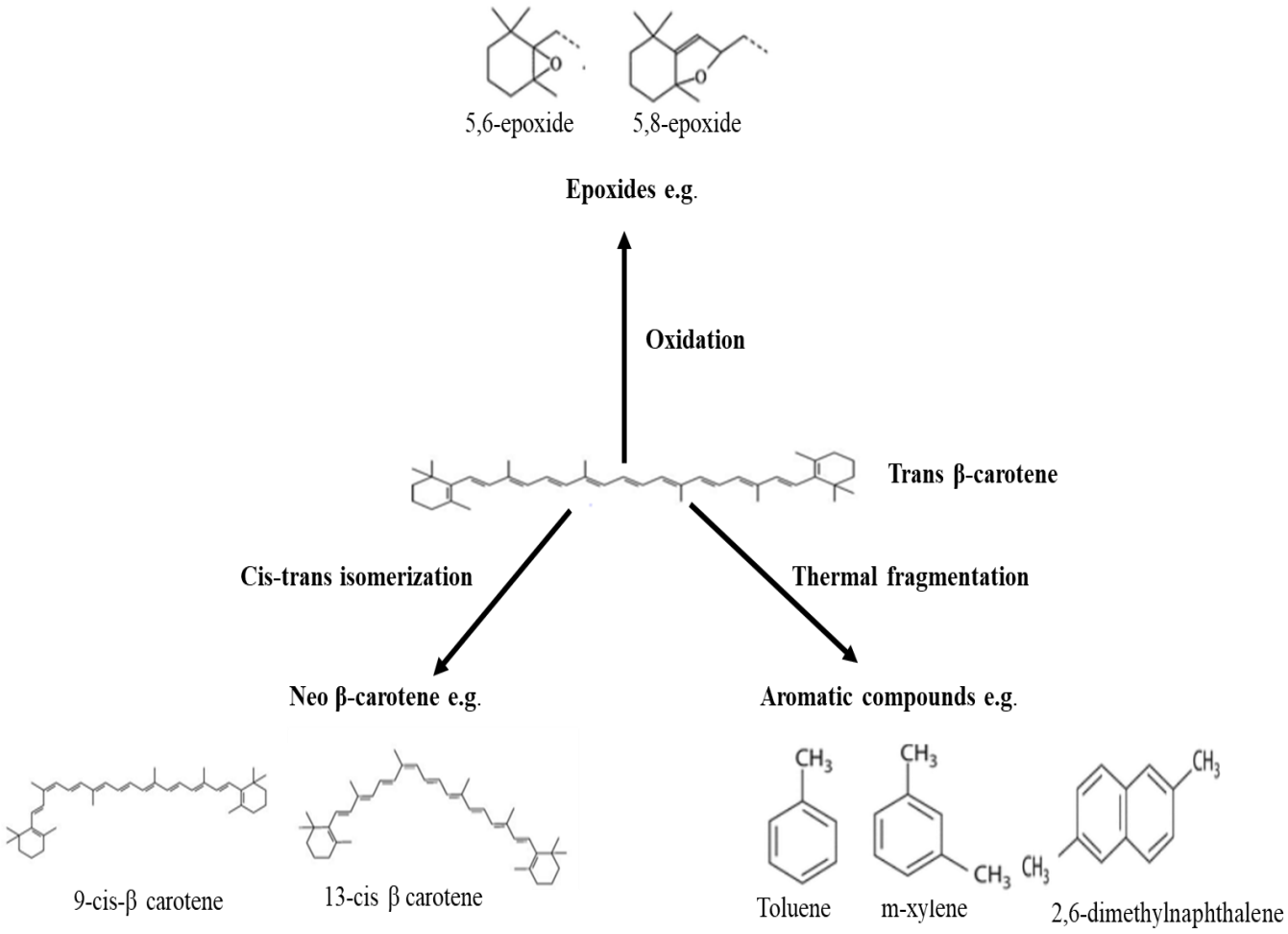

Figure 6.5: Degradation of β-carotene during extrusion processing

The observed progressive increase in antioxidant activity of the raw flours with increasing levels of incorporation of orange flesh sweet potato flour suggests that β-carotene was a major contributor to the antioxidant properties of the flours. However, that was not the case with the extruded pasta sample where there was a significant increase in antioxidant activity of composite pasta samples

relative to their flours and the different pasta samples had similar antioxidant activity. This suggests that for the pasta samples, in addition to β-carotene, other compounds could have contributed to the antioxidant activity. These compounds are likely to be products of the Maillard reaction and caramelization of sugars. The high temperature conditions of the extrusion process can promote the Maillard reaction (between amino groups of proteins and reducing sugars) and caramelization of sugars. The products of these reactions are well known to have reducing properties and therefore could contribute to the observed antioxidant activity of the pasta samples (Benjakul *et al.*, 2005; Chawla *et al.*, 2009).

The characteristic dark colour of the composited pasta samples as evident in Figure 5.7, could have been due to a combination of the orange colour of the OFSP flour from the carotenoid pigments present and the Maillard browning reactions which occurred during high temperature extrusion cooking. According to Chia and Chong (2015) higher temperatures used during extrusion promote Maillard browning reactions or caramelization of sugars present in the OFSP flour, both of which can produce melanoidin pigments resulting in dark colour of the composite pasta samples.

The results of *in-vitro* protein digestibility are presented in Table 5.6. Further cooking of the pasta samples in water, increased the *in-vitro* protein digestibility. This could be attributed to the further inactivation of protease inhibitors and / or further opening up of the protein structure through denaturation (Sagum and Arcot, 2000). This was in agreement with work done by Kiran and Padmaja (2003) who reported a steady decline in the protease activity in different cultivars of sweet potato after 10 minutes of cooking. Protease inhibitors are distributed in plants and it has been recognised that the nutritive value and protein digestibility of many plant proteins are very poor unless they are cooked or subjected to some thermal processing (Kiran and Padmaja, 2003).

Extruded pasta samples showed lower *in-vitro* protein digestibility values compared to the conventional commercial pasta. Extrusion process leads to protein insolubility, which is associated with lower protein digestibility (Kaczmarek *et al.*, 2014). The temperature used during extrusion process leads to the disruption of protein structures by unfolding, re-organization and polymerization through the formation of disulphide bonds. Disulphide bonds can result in reduction in protein solubility (Arêas, 1992; Kaczmarek *et al.*, 2014) resulting in lower *in-vitro* protein digestibility.

Furthermore, Maillard reactions in extruded pasta could have led to the production of free sugars from starch hydrolysis which react with lysine and other amino acids with free terminal amines leading to lower *in vitro* protein digestibility (Singh *et al.*, 2007b). A study done by Beaufrand *et al.* (1978) reported a loss of available lysine in extrusion processed cereal mixture ranging from 32 % to 80 % at 170 ºC temperature, 10 -14 % feed moisture and 60 rpm.

During the early stages of the Maillard reaction, protein-bound lysine reacts with reducing sugars to give deoxyketosyl-lysine. Other amino acids do not react at this stage and there is no colour development. At the advanced Maillard reaction stage, the deoxyketosyl decomposes to give premelanodins which may react with other amino acid side chains leading to the destruction of essential amino acids, cross-link formation between protein chains and an overall reduction in protein digestibility. The premelanodins polymerise to give melanoidins and colour develops (Hurrell, 1990; Singh *et al.*, 2007b).

In-vitro starch digestibility allowed for the determination of starch fractions in the pasta samples (Figure 5.10, Table 5.7), that is, slowly digestible starch (SDS), rapidly digestible starch (RDS) and resistant starch (RS). The kinetics of *in-vitro* starch digestibility was monitored from 0-180 minutes for the various pasta samples. The total starch digested for the commercial pasta was significantly higher than that of the extruded pasta samples. The lower total digestible starch in the extruded pasta could have been due to the formation of amylose-lipid complexes. The DSC results indicated that commercial pasta showed lower peak temperature (Table 5.8) as compared to the extruded pasta samples that could partly be used to explain a formation of lower amounts of amylose-lipid complexes.

According to Guha *et al.* (1997) lower starch digestibility in extruded starch foods could be attributed to the formation of amylose-lipid complexation which prolongs starch digestibility during enzymatic hydrolysis. During extrusion processing of starchy foods, there is gelatinization of starch, and the formation of complexes between starch and lipids (De Pilli *et al.*, 2008). The formation of complexes between starches and lipids is due to the ability of the amylose to bind lipids such as fatty acids (De Pilli *et al.*, 2008). The strong stable complex formed limits cross-linking and double helical structure formation between amylose molecules. Amylose lipid complex can then inhibit starch hydrolysis due to reduced accessibility of the glycosidic bonds by α-amylase enzyme (Figure 6.6) (Ye *et al.*, 2018b).

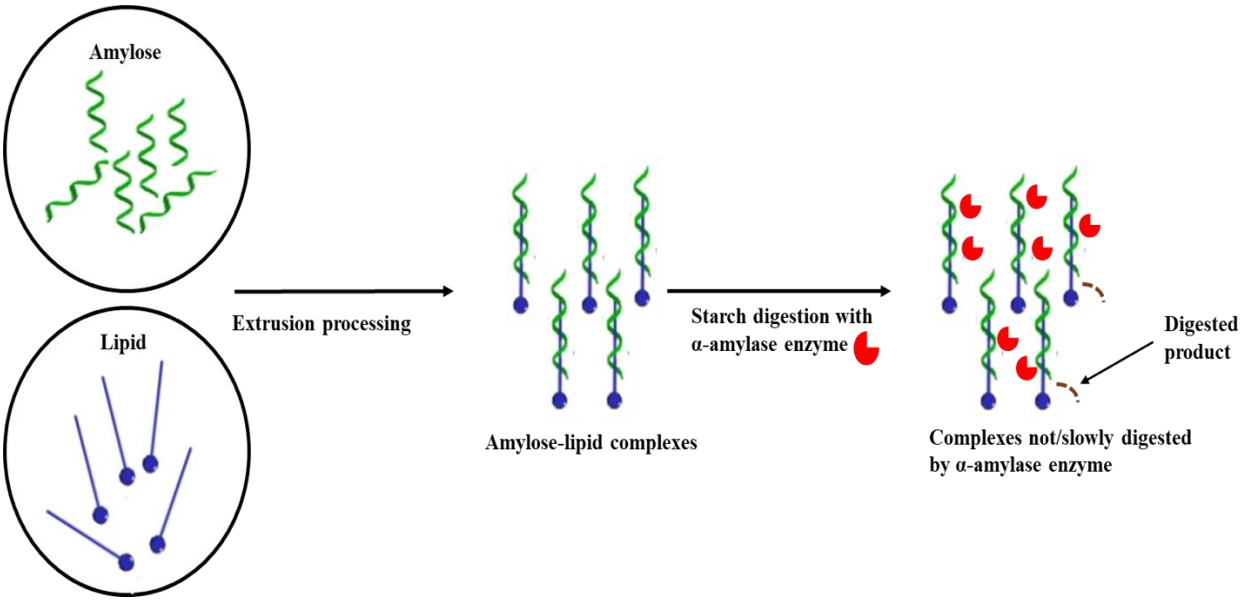

Figure 6.6: Illustration of how amylose-lipid complexes formed during extrusion processing resist starch digestion. Adapted from (Li *et al.*, 2019).

The higher starch digestibility recorded for the pasta samples, led to a corresponding higher estimated glycemic index (EGI) values, higher rapidly digestible starch (RDS) and lower percentage of resistant starch (RS). 100 % maize pasta showed higher resistant starch value indicating lower starch digestibility which could be as a result of retrograded starch forming RS resistant starch type 3 which mostly happens in cereal food products (Haralampu, 2000). Gelatinized starch upon cooling re-associates and forms tightly packed structures stabilized by hydrogen bonding (Colonna *et al.*, 1992). Amylose acts as a cross-linking agent to increase intermolecular association and continuity and firmness of a gel network (Jane, 2009). Amylose is preferentially leached out from granules as a random coil. The random coil tends to form double amylose-amylose helices by self-association (Jane and Robyt, 1984). Retrograded amylose with a double-helical structure is suggested to be resistant to amylolytic hydrolysis (Haralampu, 2000; Jane, 2009).

The thermal properties of the raw extruded pasta samples did not exhibit any first endotherm except for raw commercial pasta which showed a first endotherm with a temperature range of 69.1-80.2 °C, a peak temperature of 74.4 °C and ΔH of 0.99 J/g indicating starch gelatinization temperature. This confirms the non-occurrence of birefringence under polarized light for the

extruded pasta samples (Figure 5.13). This report was in agreement with work down by Bhatnagar and Hanna (1994) who recorded no endothermic transition from 60-70 °C for extruded corn starch indicating complete gelatinization of starch. Comparing the extruded pasta samples with the commercial pasta which was made by using the conventional method, extrusion cooking brings about starch damage and gelatinization of starch granules which required less energy to melt (Petitot *et al.*, 2010a) which could explain the results. The incomplete gelatinization of the commercial pasta could have also resulted in the longer cooking time of the pasta.

The raw extruded pasta samples showed a single endotherm with temperature ranging from 90.0 - 103 °C which was higher than the commercial raw pasta which showed a second endotherm with temperatures ranging from 88.7 - 102 °C, indicating the formation of type I amylose lipid-complex. The high temperature during extrusion could have resulted in more amylose-lipid complexes formed which slowly inhibit the starch digestibility. Many researchers have confirmed the formation of amylose-lipid complexes with a twin-screw extruder. The formation of the complex could probably be due to the amylose content present in the flours used. According to Panyoo and Emmambux (2017), the degree of lipid binding depends on amylose content. Merayo *et al.* (2011) stated that, during extrusion cooking, the native structure of amylose is partially destroyed, and new crystalline ones, corresponding to the amylose-lipid complex, are formed. De Pilli *et al.* (2008) further explained that the higher temperature and high moisture content used during extrusion processing promotes the gelatinization of starch to increase the amylose availability for complexation.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

This study shows that quick cooking gluten free maize and OFSP composite pasta can be produced using extrusion processing. The pre-gelatinized starch of the extruded pasta results in faster water absorption and heat dissemination during cooking resulting in lower cooking time. The progressive increase in proportion of orange-fleshed sweet potato flour affects the cooking qualities and the nutritional properties of the pasta. The maize: OFSP pasta is characterized by higher cooking loss, stickiness and lower firmness with increase in OFSP addition and this is related to the microstructure as a more discontinuous matrix is formed when OFSP is composited with maize. Extrusion promotes the conversion of insoluble fibre to soluble fibre in the OFSP flour. The fibre in the OFSP disrupts the compact starch-protein networks in the pasta samples and increases the leach out of materials resulting in stickiness.

The nutritional qualities of the maize: OFSP flour increase with increase in addition of OFSP flour in terms of β-carotene content and antioxidant properties. Although there is a decrease in the β-carotene content after extrusion, pasta samples still possess some radical scavenging properties with potential health benefit. This study demonstrates that, pasta can be produced from OFSP using extrusion which might appeal to consumers as it can meet an appreciable amount of the recommended daily allowance of vitamin A, has appreciable antioxidant properties and has quick cooking time**.**

For further studies, it can be recommended to use a different extraction process for the determination of the beta-carotene content and also determine the β-carotene bio-accessibility of the pasta. Also consumer acceptability is required as this will provide valuable information on the commercial potential of the pasta.

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