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Incorporation of mushroom powder into cereal food products

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy in Food Science

at
Lincoln University
by
Xikun Lu

Lincoln University
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Declaration

Some aspects of this thesis have been published or submitted for publication and presented at conferences.

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- Vallée, M., Lu, X., Narciso, J. O., Li, W., Qin, Y., Brennan, M. A., & Brennan, C. S. (2017). Physical, predictive glycaemic response and antioxidative properties of black ear mushroom (*Auricularia auricula*) extrudates. *Plant Foods for Human Nutrition*, 72, 301-307.
- Lu, X., Brennan, M. A., Serventi, L., & Brennan, C. S. (2018). Incorporation of mushroom powder into bread dough: effects on dough rheology and bread properties. *Cereal Chemistry*. DOI: 10.1002/cche.10043

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- Lu, X., Brennan, M. A., Narciso, J., Guan, W., Zhang, J., Yuan, L., Serventi, L., & Brennan,

- C. S. Mushroom bioactive compounds enhance the nutritional profile of breads: The association between the predictive glycaemic response of breads and phenolic contents. Submitted to Journal of Agricultural and Food Chemistry, October 6th 2017.
- Lu, X., Brennan, M. A., Narciso, J., Guan, W., Zhang, J., Yuan, L., Serventi, L., & Brennan, C. S. Matrix transformation of fortified extruded snacks: effect of different mushroom powders on texture, macrostructure, nutrition, microstructure and digestibility. Submitted to Journal of Cereal Science, November 3th 2017.
 - Lu, X., Brennan, M. A., Narciso, J., Guan, W., Zhang, J., Yuan, L., Serventi, L., & Brennan, C. S. Effect of cooking processing on the benefits of mushroom (*Auricularia auricula*) supplementation in cereal snack products. Submitted to Nutrients, October 19th 2017.

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- ◆ 18th New Zealand Institute of Food Science and Technology Annual General Meeting
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- ◆ 50th International Journal of Food Science and Technology Conference
 - Poster presentation about PhD project introduction
- ◆ 66th Australian Cereal Chemistry Conference, Tamworth, 14th-16th September 2016
 - Oral presentation about mushroom pasta (Lu *et al.*, 2016).
- ◆ 1st ICC Asia-Pacific Grains Conference, Xiamen, China
 - Poster presentation about mushroom pasta
- ◆ 67th Australian Cereal Chemistry Conference, Christchurch, 20th-22th September 2017
 - Oral presentation about mushroom bread

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Food Science.

Incorporation of mushroom powder into cereal food products

by

Xikun Lu

Convenient cereal derived foods are becoming more popular in terms of consumer interest, however they can be considered as nutritionally imbalanced. For instance, cereal products which are mainly derived from refined flour, are often low in lysine, vitamins, minerals and fibre. In addition these products may be considered as energy dense and often exhibit a high glycaemic index. As consumers demand healthier food products, the cereal food industry has endeavoured to improve the nutritional content of products by increasing their composition of bioactive phytochemicals.

On the other hand, mushrooms are the source of many nutrients, for example, the proportion of essential amino acids of most mushrooms is comparable to that of egg, especially lysine, which is an abundant essential amino acid in mushrooms, and is deficient in most cereals. Previous reports also have suggested that mushrooms are nutritionally incomplete due to the lack of essential sulphur amino acids, such as methionine and cysteine, these however are rich in cereals. Cereal products and mushrooms when consumed independently may not be able to support growth as effectively as animal foods, but the combination of cereal products and

mushrooms makes it possible, to supply an adequate level of essential amino acids and provide a good balance of nutrients for human beings.

In this study, four different kinds of mushroom powder (white button; shiitake; porcini and black ear mushroom) were incorporated into three different kinds of cereal product (fresh pasta; bread and hot extruded product), which utilised the cooking processes of cold extrusion, fermented cooking and hot extrusion respectively. The physical, chemical, textural, nutritional and microstructural properties of these products were determined. These products may introduce more edible mushroom species to western consumers' diet using a relatively simple approach.

A partial substitution of durum wheat semolina with different species of mushrooms was undertaken to increase the nutritional value of the pasta. The results showed that the addition of mushroom powder increased the cooking loss, as well as firmness of the pasta. Porcini and black ear mushroom incorporation significantly decreased the swelling index, water absorption index and moisture content values of the cooked pasta, while, for the white button and shiitake mushrooms, there was no noticeable effect on either index compared with the control sample. Furthermore, mushroom material enriched the protein (except black ear mushroom) and dietary fibre contents of the pasta samples compared with durum wheat only samples. Incorporation of mushroom powder significantly decreased the starch content, the extent of starch degradation and the area under the curve (AUC) of reducing sugars released

during the digestion of pasta, while it also increased the total phenolic content and antioxidant capacities of samples.

Powder from different mushrooms was used to replace high grade wheat flour at levels of 5 %, 10 % and 15 % to make novel breads. Bread physical and textural qualities, including height, specific volume, moisture content, hardness, springiness and gumminess were determined. The breads made with mushroom powder were smaller in specific loaf volume (except for the 5 % porcini mushroom) and exhibited reduced springiness and reduced height (except for the 5 % porcini mushroom) compared to the control samples. Additionally, starch characteristics (content, gelatinisation and digestibility), antioxidant capacities and microstructural properties (bread crust and crumb) of mushroom enriched bread samples were also investigated. The decrease of total starch content and increase of total phenolics of the breads were significant with increased mushroom powder content compared to the control. The reducing sugar released in an *in vitro* starch digestion of the control bread increased more rapidly than in the mushroom enriched breads. The area under the curve (AUC) values illustrated clearly that mushroom powder addition reduced the predicted glycaemic response of the bread. Mushroom powder incorporation also enhanced the antioxidant capacities compared to the control bread. Furthermore, mushroom powder altered the internal microstructure of the bread crust and crumb by affecting the interactions between starch and the other components of the bread. These results indicate that mushroom powder can be added to bread to deliver health benefits to consumers.

Mushroom powders were also processed with semolina to produce hot extruded snacks. Similar to the other two products, a series of analyses were conducted in order to evaluate these functional hot extruded products comprehensively, including the nutritional profile, macrostructure, colour, textural properties, microstructure, starch characteristics (content, gelatinisation and digestibility) and antioxidant activities (total phenolic content, DPPH and ORAC). The results showed that different mushroom powders had different effects on the physical properties. Products with mushroom powder had a higher fibre content and decreased degree of starch gelatinisation, which might be responsible for the decreased glycaemic response. Additionally, incorporation of mushroom powder into hot extruded products resulted in delivering more phenolic compounds and antioxidant benefits to consumers.

Keywords: white button mushroom (*Agricus bisporus*), shiitake mushroom (*Lentinula edodes*), porcini mushroom (*Boletus edulis*), black ear mushroom (*Auricularia auricula*), pasta, bread, hot extruded snack, physical property, textural property, nutrients, starch gelatinisation, glycaemic index, antioxidant capacities, microstructure.

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Chapter 1

Introduction and thesis outline

The popularity of convenience foods is increasing due to the ease of preparation, long shelf-life, attractive appearance and texture. Currently, busy lifestyles and the increasing demand from consumers for meals and snacks that are quick sources of good nutrition have prompted the food industry to develop foods that combine convenience and nutrition (Reis and Abu-Ghannam, 2014). Most cereal snacks owe much of their universal appeal to their texture, such as crunchiness, the generation of satisfying acoustic and other pleasant sensations in the mouth (Peleg, 2015), thus physical and textural properties are important parameters to determine the quality of cereal products. In terms of nutrition, many snack foods are derived from cereals which makes them low in protein and nutritionally imbalanced, for example, they are low in lysine (an essential amino acid), vitamins, minor minerals and fibre, furthermore, they are also energy dense and exhibit high glycaemic index (GI) values (Agu *et al.*, 2010; Giami, Mepba *et al.*, 2003). As consumers demand healthier food products, the cereal food industry has endeavoured to improve the nutritional content of products by increasing their composition of bioactive phytochemicals. It has been reported that grain products complement lysine-rich vegetables well in order to prepare nutritionally balanced composites of high physiological value (Pradeep *et al.*, 2014). Consumers need nutritious, convenient and tasty snacks to satisfy their hunger momentarily between meals. It has been estimated that 40 % consumers prefer snack products with health benefits than basic nutrition because of the increase in the incidence of multi-tasking while eating, resulting in upsurge in demand for

“on-the-go” handheld snacks (Sloan, 2011). Thus, it is necessary to make an effort to improve the nutritional benefits of cereal products since they are mainly made from wheat flour or starches and therefore have a low physiological value.

Mushrooms are a good source of some nutrients. Most species contain all the essential amino acids in about the same proportion as egg (Bora and Kawatra, 2014). Lysine which is deficient in most grain cereals is an abundant essential amino acid in mushroom (Bora and Kawatra, 2014; Chang and Miles, 2004). Much of the recent research on the nutritional quality of mushrooms has focused on the dietary fibre content and, in particular, the role of β -glucan components in lowering blood cholesterol and glycaemic responses (Brennan *et al.*, 2013 a). Additionally the interest in antioxidants in mushrooms is increasing (Tseng *et al.*, 2008; Wasser and Weis, 1999). Thus, mushrooms complement wheat flour well to produce nutritionally balanced high-quality cereal products. With the advent of modern food technologies and new consumer appeal factors, the interest in the use of mushrooms in the food industry, including fruiting bodies, stalks and mycelia, has increased due to their capability of being a source of bioactive components, hence they may be useful in the development of functional foods (Tseng *et al.*, 2008; Wasser and Weis, 1999). When considering convenience food, mushrooms can contribute to high value protein, vitamins, minerals, fibres and even antioxidants. Many edible mushroom species have been regarded as food for several millennium in many Asian countries, while most edible mushroom species are still unfamiliar to Western consumers. Furthermore, no special processing or treatment is generally given to mushrooms by the food

industry. However, the nutrient composition and technological properties of mushroom offer a number of opportunities for processing and value addition to cereal snacks.

This study incorporated four kinds of mushroom powder into three types of cereal food products, including fresh semolina pasta, hot extruded product and bread processing thereby utilising cold extrusion, hot extrusion and fermentation cooking methods respectively. The aim of this work was to determine the impact of such addition on the physical and textural properties as well as on the nutritional profile. This research illustrated the possibility of creating functional cereal food products with increased antioxidant effects and a reduced glycaemic impact on individuals. Additionally, the effect of food processing on the bioactive components of mushroom powder was evaluated. Such value added products will be nutritionally beneficial in the maintenance of metabolic function and produce larger economic benefit than normal cereal products.

1.1. Aims

The aim of this study was to use four different kinds of common edible mushrooms (white button mushroom, shiitake mushroom, porcini mushroom and black ear mushroom) as potential value added ingredients. The mushroom powders were incorporated into three different cereal products (extruded products, fresh pasta and bread). The effect of adding mushroom powder on the physical and chemical properties of cereal products was analysed

as well as the effects on the nutritional and functional values of these novel food products. Additionally, the bioactive components in mushrooms and in the final products were evaluated to illustrate the influence of food processing on these nutrients. The main aims of the research can be summarised into 4 key points.

1. To develop cereal products and evaluate their physico-chemical characteristics and nutritional values in order to identify the contributions that mushroom powder can make to food quality and human nutrition.
2. To determine the physical and textural characteristics of these mushroom enriched cereal products.
3. To analyse the nutrients, energy content, starch gelatinisation, glycaemic response, antioxidant properties and microstructure of these mushroom enriched cereal products
4. To compare the nutrients and bioactive ingredients in the products to identify the changes of these components after a series of food processing

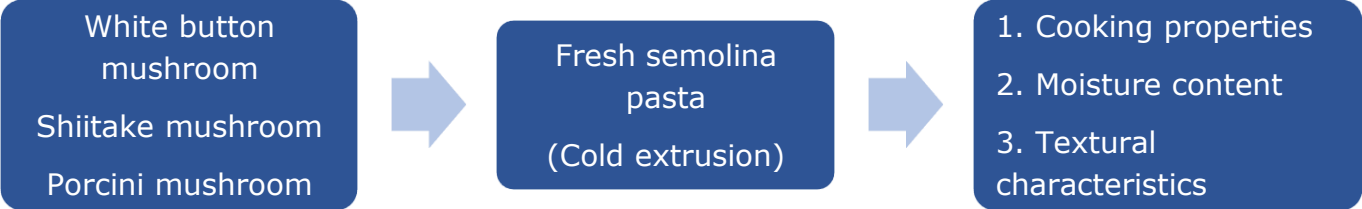
1.2. Hypothesis

1. Addition of mushroom powders will have a negative effect on the physical and textural properties.
2. Addition of mushroom powder will increase the total content of phenolics and antioxidants of the products, and will have a positive effect on the antioxidant capacities of these final products.

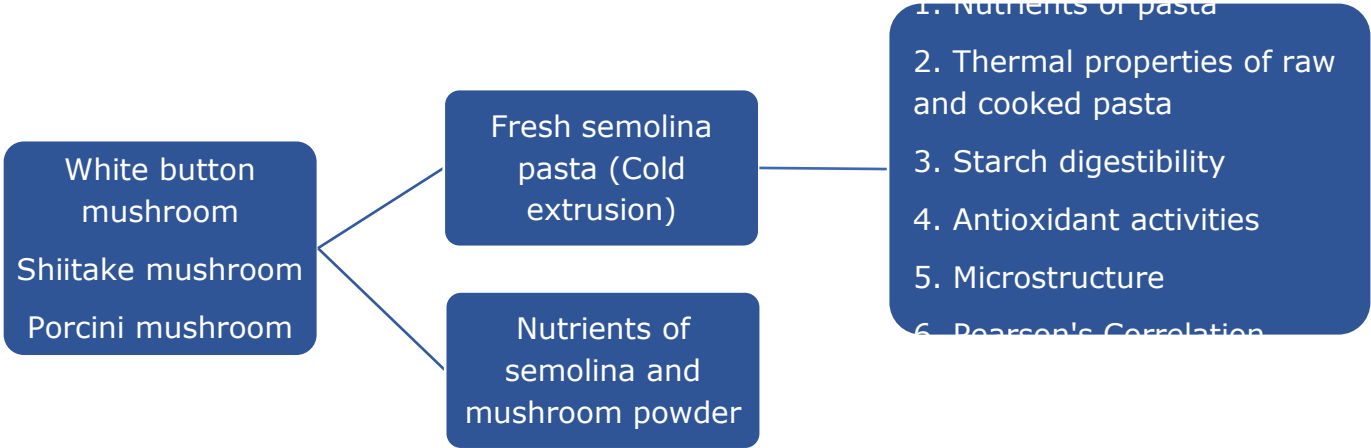
- 3. Addition of mushroom powder will increase the dietary fibre content and decrease the starch content and glycaemic impact of these enriched products.
- 4. Thermal food processing will have a negative influence on the bioactive components and functional properties of the products.

1.3. Thesis outline

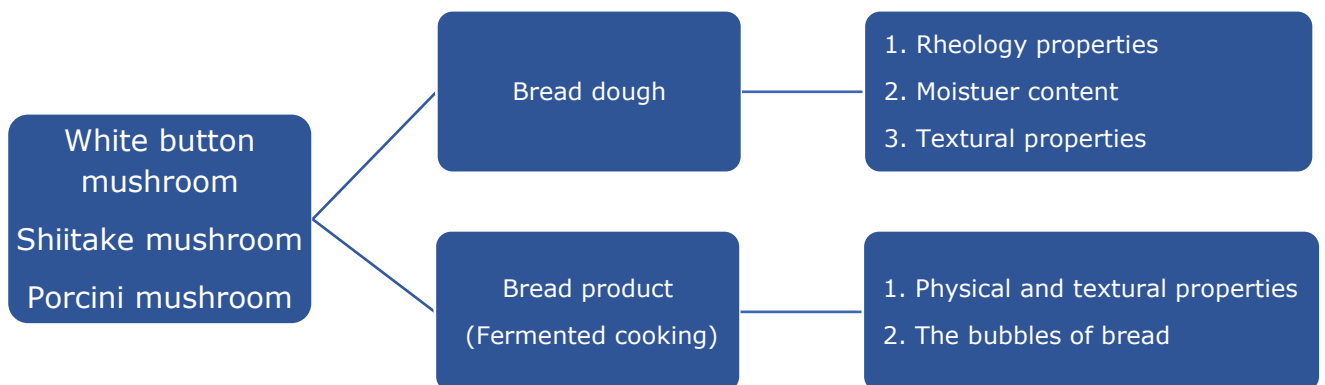
- **Project Title:** Incorporation of mushroom powder into cereal food products
- **Chapter 1:** Introduction and thesis outline
- **Chapter 2:** Literature review
- **Chapter 3:** Material and method
- **Chapter 4:** How the inclusion of mushroom powder can affect the physicochemical characteristics of pasta- Published in International Journal of Food Science and Technology



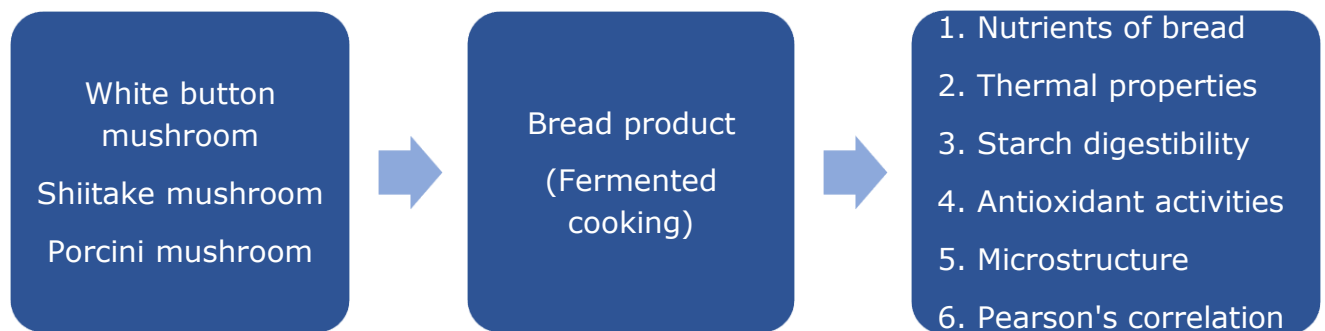
- **Chapter 5:** Mushroom material enhanced the antioxidant content and modulated the predictive glycaemic response of pasta-Submitted to Food Chemistry



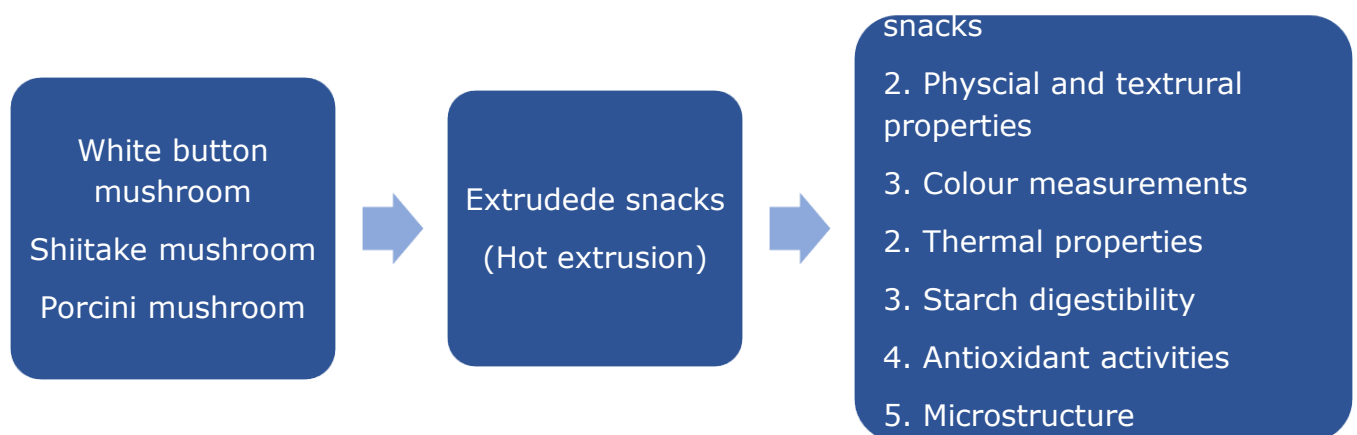
- **Chapter 6:** Effects of different mushroom powder on wheat flour dough and bread properties- Published in Cereal Chemistry



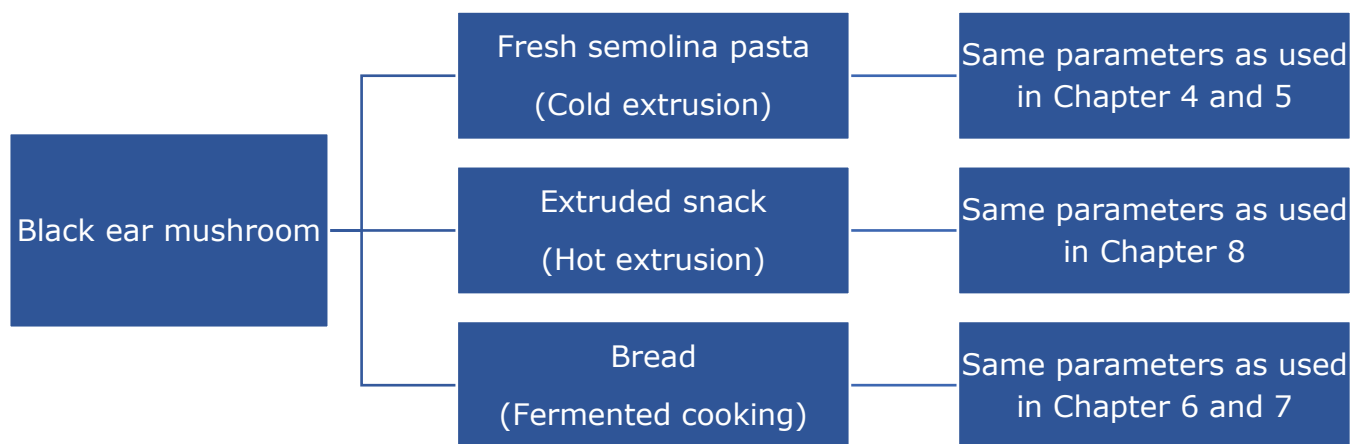
- **Chapter 7:** Manipulation of the predictive glycaemic response and the phenolic properties of mushroom enhanced bread-Submitted to Journal of Agricultural and Food Chemistry



- **Chapter 8:** Matrix transformation of fortified extruded snacks: effect of different mushroom powders on texture, macrostructure, nutrition, microstructure and digestibility-Submitted to the Journal of Cereal Science



- **Chapter 9:** Effect of cooking processing on the benefits of mushroom (*Auricularia auricula*) supplementation in cereal snack products-Submitted to Nutrients



- **Chapter 10:** General discussion and conclusions
- **Reference**

Chapter 2

Literature review

2.1. Mushrooms

Mushrooms are macroscopic fungi which have various shapes, sizes, appearance and edibility, however the typical feature of mushrooms is a fleshy sponge umbrella shaped form. Mushrooms have been used as a food since time immemorial, they are still popular and are commercially available to different societies worldwide. Mushrooms are not only appreciated for their colour, taste, aroma and textural characteristics, but also for their nutritional value. Nutritional attributes enhance food aesthetic value and increases consumption (Liu *et al.*, 2005). Mushrooms are generally and widely used as raw food, functional food and seasoning because of their complex flavour and good sources of nutrients, such as protein, vitamins, minerals and other bioactive components (Poojary *et al.*, 2017).

2.1.1. Composition and nutritional values

The relationship between mushrooms and man can be traced far back into antiquity. Feeding humans, means to supply a sufficient quantity and quality of the bodybuilding material, namely protein. As the world's population increases, feeding humans a sufficient and balanced diet is always a great difficulty (Chang and Miles, 2004). The incidence of malnutrition reported by FAO in 2010, especially protein and micronutrient deficiency, is still very high around the world and the situation in sub-Saharan Africa is quite severe. Mushrooms can be

valued as one of the most important sources of vegetable protein for people who are trying to combat the growing shortage of protein, especially hungry people and vegetarians. Nevertheless, until the last decade most types of mushroom, except medical species, were considered only as a delicacy. Additionally, their consumption in many developed countries has been marginal. As a result, there was little interest from researchers and the knowledge of their composition and nutritional value was limited compared with vegetables. Fortunately, this situation has started to change noticeable: the yearly number of original papers about mushroom is now several times higher than 10–15 years ago (Kalač, 2009).

It has been reported that, with the exception of green peas and pulses, fresh mushrooms have a higher protein content than most vegetables (Bora and Kawatra, 2014). The protein content of mushrooms can range from 10–40 % on a dry weight basis (Bora and Kawatra, 2014; Breene, 1990). Mushroom protein includes all nine essential amino acids required by man (Table 2.1), although it can be limiting in sulphur-containing amino acids, such as cystine and methionine (Breene, 1990). In terms of the essential amino acid index, amino acid score and nutritional index, mushrooms are between low grade vegetable and high grade meats with values that are close to that of milk, some species even well above milk which is an animal product (Bora and Kawatra, 2014; Chang and Miles, 2004). As a result of this, FAO has recommended mushrooms as a supplementary food item in the context of the world protein shortage for the growing populations of the developing countries.

Table 2.1 Essential amino acid profiles (mg/g protein) of edible mushrooms (Cheung, 2008)

Mushrooms	Ile	Leu	Lys	Met	Cys	Phe	Tyr	The	Val	Trp
<i>Volvariella bombycina</i>	54.1	50.1	54.1	1.22	19.1	60.2	45.8	46.5	35.8	ND
<i>Lyophyllum ulmarium</i>	58.9	101	46.1	19.1	18.2	51.1	72.1	41.2	56.1	ND
<i>Pleurotus citrinopileatus</i>	35.1	71.2	56.3	25.4	15.8	39.7	32.2	49.2	60.7	ND
<i>Tricholoma portentosum</i>	37.2	93.7	86.3	29.6	16.2	43.6	32.1	94.5	77.6	9.60
<i>Tricholoma terreum</i>	35.8	81.5	76.3	34.6	17.0	66.1	30.0	90.7	88.7	10.6
<i>Lentinus edodes</i>	33.0	63.8	49.8	21.6	34.0	38.1	26.0	55.5	38.1	19.2
<i>Pleurotus eryngii feruale</i>	41.1	65.6	67.1	16.9	15.7	40.4	34.2	50.4	45.1	12.2
<i>Pleurotus ostreatus</i>	44.5	72.8	61.1	20.1	16.8	46.9	40.6	51.6	48.8	40.6

ND, not determined.

In order to maintain good health, the other nutritional categories, carbohydrates, fats, vitamins and minerals are also essential. Scientists have increasingly appreciated the nutritional value of mushrooms due to their low calorific value, vitamins and minerals. The moisture content of fresh mushrooms is about 70–95 %, depending on the species, harvest time and environmental conditions, it falls to around 10–13 % when dried (Breene, 1990). The carbohydrates and fibre contents of fresh mushrooms are 3–21 % and 3–35 % respectively. The main classes of lipid compounds, including free fatty acids are quite low at around 2–8 % of dry weight (Breene, 1990), while the proportion of unsaturated fatty acids are relatively high and especially oleic and linoleic acids (Ng *et al.*, 1999). It has been found that the levels of polyunsaturated fatty acids in mushrooms are more than 75 % of the total fatty acids, of which palmitic (19.2 %), oleic (8.3 %), and linoleic (68.8–84.0 %) acids are the most significant (Chang and Miles, 2004). Edible mushrooms contain a significant amount of vitamins (B₁, B₂, B₁₂, C, D, E) (Heleno *et al.*, 2010). Additionally, mushrooms are regarded as a relatively good source of some other nutrients, such as phenolic compounds, as well as phosphorus, iron and

vitamins, including carotenoids, ergosterine and niacin, however the calcium content of mushrooms is low (Barros *et al.*, 2007; Ng *et al.*, 1999).

Mushrooms are extensively consumed all over the world and regarded as valuable food not just due to their abundant nutrients, but also due to the various kinds of characteristic flavour substances, which give desirable tastes to consumers (Cremades *et al.*, 2012). These kinds of components can be classified into non-volatile (taste) and volatile substances (smell). Several water soluble compounds are responsible for the particular taste of mushrooms primarily, including soluble sugars and polyols, free amino acids, 5'-nucleotides and organic acids (Tsai *et al.*, 2007). Many volatile compounds have also been investigated, for instance, 1-octen-3-ol, 1-octen-3-one, aromatic alcohols and aldehydes. Among these, 1-octen-3-ol and 1-octen-3-one play a main role in the characteristic flavour of mushrooms, thus they are regarded as "mushroom-like flavour" (Perera *et al.*, 2003). Furthermore, it has been well documented the effect of umami is flavour enhancement and appetite regulation. Umami ingredients can provide meaty flavour, create mouth fullness and make food richer in taste, thus resulting in reduction of energy and fat intake (Miyaki *et al.*, 2016; Poojary *et al.*, 2017). Food industries look for natural umami (MSG, mono sodium glutamate-like) ingredients due to the negative perceptions of consumers to the "added MSG" label (Radam *et al.*, 2010). On the other hand, it has been published that there are significant amounts of umami based ingredients in a wide variety of edible mushrooms, such as glutamic acid and aspartic acid (Zhang *et al.*, 2013 a).

2.1.2. Medicinal properties

People, who consume a cereal-based diet, or live in regions where the soil has an imbalanced mineral content for a long time, appear to be more susceptible to nutrition disequilibrium (Johns and Eyzaguirre, 2007). These people usually have health problems, such as metabolic disorder and sleep problems (McClung, 2007). Due to the wide range of side effects that may be caused by conventional medicines, consumers tend to be interested in using natural products with therapeutic properties. Among which, mushroom is one of the most potent candidates in clinical studies, because of their bioaccumulation potential of nutrients with essential elements and bioactive compounds for human health. What is more, mushrooms are readily obtained in relatively large quantities and are inexpensive (Seo *et al.*, 2003). In traditional Chinese medicine, mushroom extracts are extensively used for the dietary supplements and nutraceuticals, along with lots of combinations of other herbal preparations in order to treat various medical conditions, some of these are employed as immunomodulators in cancer therapy (Diyabalanage *et al.*, 2008).

Due to their very low calorie content, mushrooms are suited to people who would like to reduce their calorie intake, especially those who are obese. The low fat content and lack of cholesterol, means that mushrooms are also valued as an ideal diet for heart patients. Being very high in fibre and containing alkaline elements, mushrooms are beneficial to those suffering from hyperacidity and constipation, not forgetting that it is important to consume sufficient fibre for general health maintenance (Bora and Kawatra, 2014). With their very high

content of fibre, protein, microelements and low fat content, mushrooms are ideal for those people who are interested in prevention of atherosclerosis (Crison and Sands, 1978). Furthermore, mushrooms are also well known as ideal medicinal materials for the dietetic hypotensive effect (Yip *et al.*, 1987).

A lot of chemical compounds have been extracted from different parts of mushrooms, most of them have been demonstrated to have considerable pharmaceutical application. For instance, lectins extracted from edible mushrooms (Wang *et al.*, 1997) and polysaccharide-protein complex from mycelia cultures (Ng *et al.*, 1999; Wang *et al.*, 1995) have immunomodulatory and anti-tumour properties. Anti-tumour, antibiotic, antibacterial and immunomodulating agents have been isolated from *Pleurotus* species (Cohen *et al.*, 2002). Type I ribosome-inactivation protein has been isolated from *Volvariella volvacea* (Yao *et al.*, 1998). *Ganoderma lucidum* has been shown to contain medicinal substances (Chang and Miles, 2004). Polysaccharides in mushrooms, such as 1,6-branched 1,3- β -glucans, have been proven to have various physiological functionalities including inhibiting the growth of tumours (Hetland *et al.*, 2011; Standish *et al.*, 2008).

Mushrooms are also good sources of antioxidant materials. Polyphenols and related antioxidants are one of the most important bioactive nutrients in mushrooms and they effectively prevent food oxidation (Bandonienė *et al.*, 2002; Shan *et al.*, 2009). Ergothioneine content in button mushroom (*Agaricus bisporus*), shiitake (*Lentinula edodes*), oyster

mushroom (*Pleurotus ostreatus*), king oyster (*Pleurotus eryngii*) and maitake mushroom (*Grifola frondosa*) is around 0.4–2.0 mg/g dry weight (DW), while the ergothioneine content of shiitake and oyster mushroom is approximately 2.0 mg/g DW, which is the highest level of ergothioneine (Beelman *et al.*, 2007). As ergothioneine is able to scavenge and quench most reactive oxygen species (Aruoma *et al.*, 1997), to chelate various divalent metallic cations (Akanmu *et al.*, 1991; Motohashi *et al.*, 1976) and to suppress the oxidation of haemoproteins (Arduini *et al.*, 1990), it is regarded as an effective antioxidative component. It has been reported that ergothioneine has numerous antioxidant and cytoprotective abilities *in vitro* and a few *in vivo*, such as free radical scavenger activity, radio-protective properties, protection against UV radiation and inhibition of neuronal injury (Botta *et al.*, 2008; Damaghi *et al.*, 2008; Decome *et al.*, 2005; Jang *et al.*, 2004; Markova *et al.*, 2009; Song *et al.*, 2010). In addition, the ergothioneine rich extract of the fruiting body and solid cultivating media of *Flammulina velutipes* has been studied in relation to seafood preservation which showed that it prevents discolouration and lipid oxidation in fish meat, as well as controlling the development of melanosis in crustaceans during post-mortem storage (Ashida *et al.*, 2005; Bao *et al.*, 2009; Encarnacion *et al.*, 2011). Therefore, phenolic compounds and ergothioneine can be extracted from mushrooms and utilised as nutraceuticals and dietary supplements.

2.1.2.1. Mushrooms and dietary fibre

As mentioned previously, mushrooms are rich in dietary fibre. The characteristic texture of mushrooms is partly determined by the fibre in the tissue. Fibre plays an important role in the

firmness of mushrooms. More specifically, the textural properties of mushrooms depend upon three aspects, firstly, the complex attributes of the individual fibre strength; secondly, the chemical compounds (total dietary fibre and species) concerned with the physicochemical bindings of spawns; finally, the fibre arrangement and/or formation (Ogawa *et al.*, 2012).

Dietary fibre also improves the nutritional and medicinal values of mushrooms. In recent decades, the interest in studying dietary fibre as a functional food component has increased. The Dietary Fibre Definition Committee reported to AACC (2001) that “Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation”. Dietary fibre can be divided into two categories: water-soluble fibre (SDF) and water-insoluble fibre (IDF), and each category has different therapeutic effects. More specifically, SDF consists of non-starch polysaccharides (NSP), mainly β -glucan and arabinoxylan. Water-insoluble fibre (IDF) contains lignin, cellulose, hemicelluloses, and NSP such as water-unextractable arabinoxylan (Devi *et al.*, 2014).

However, a large proportion of people still continue to consume less than the recommended amount of fibrous food (Cheung and Lee, 2000; Wong *et al.*, 2003). Mushrooms and

mushroom products could be a comprehensive way to change this situation. Although there is a large variation in the total dietary fibre content of edible mushrooms, depending on their morphological form and species, in general, mushrooms are a good source of dietary fibre, with 100 g of fresh mushrooms providing between 10–40 % of the recommended dietary intake of fibre (Cheung, 2008).

2.1.2.2. Mushroom and oxidation

Oxidation is essential to the production of energy to fuel biological processes that keep all the organisms alive, such that the production of free radicals is inevitable in both normal and pathological cell metabolism (Elmastas *et al.*, 2007). The uncontrolled production of oxygen-derived free radicals is related to many diseases, such as cancer, rheumatoid arthritis, cirrhosis, arteriosclerosis, and degenerative processes associated with ageing (Elmastas *et al.*, 2007).

The antioxidant constituents (phenolic compounds) could be important protective agents to help the endogenous defence system to reduce oxidative damage (Barros *et al.*, 2007). Many research studies have set out to find natural sources of free radical scavenging molecules or antioxidants, and epidemiological studies have demonstrated that a higher intake of polyphenols, peptides or other bioactive molecules decrease the mortality of cancer and coronary heart diseases (Misharina *et al.*, 2009). Recently edible mushrooms have attracted

more interest as an antioxidant-rich foodstuff. Mushrooms accumulate a wide range of secondary metabolites, which include phenolic compounds, polyketides, terpenes and steroids (Cheung *et al.*, 2003). These antioxidants in mushrooms are able to help the human body reduce oxidative damage as protective agents without any interference. In addition to antioxidation, edible mushrooms also have other bioactive benefits, including the lowering of cholesterol levels, anti-microbial, anti-viral, anti-tumour and immunomodulating activities (Chiang *et al.*, 2006).

Studies about antioxidant properties have proven that edible mushrooms could be part of human diet directly and help to combat oxidative stress, while inedible mushroom species could be valued as a source of extractable phenolic compounds and used as food additives or the components of pharmaceutical and cosmetic formulations (Vaz *et al.*, 2011). For example ergothioneine, which is a hydrophilic antioxidant, can act as a singlet oxygen quencher and a non-radical species scavenger and its intracellularly functions include photo-oxidation prevention; DNA repair of UV-damaged cells (Hseu *et al.*, 2015); protection against lipid peroxidation and conservation of endogenous antioxidants glutathione and α -tocopherol (Pahila *et al.*, 2017). It has been reported that certain fungal species biosynthesise the hydrophilic antioxidant ergothioneine (Cheah and Halliwell, 2012), however, this component can not be synthesised by higher organisms such as vertebrates (Pahila *et al.*, 2017). Meanwhile, it has been reported that aqueous extracts of certain mushroom species are rich in ergothioneine (Liang *et al.*, 2013). Many mushroom strains have been valued as a good

source of antioxidants. For example, *Agaricus bisporus*, *Pleurotus sp.*, *Lentinula edodes* which are commonly consumed and cultivated are shown to contain the powerful antioxidant ergothioneine as well as many other active constituents (Boa, 2004). Some wild mushroom species have also been identified as having high radical scavenging activities too, such as *Boletus edulis*, *Agaricus blazei* Murill, *Agrocybe cylindracea* Gillet, *Amanita caesarea* (Caglarirmak, 2007), *Inonotus obliquus* and *Lactarius deterrimus* (Lee *et al.*, 2007).

Valuable antioxidant properties are always influenced (enhanced or reduced) by the cultivation, environmental conditions, developmental stage, species variety and storage conditions of mushrooms (Antmann *et al.*, 2008; Mattila *et al.*, 2000). Furthermore, in many publications, mushroom samples were harvested, washed and dried, for later analysis, but consumers do not usually consume edible mushrooms in a raw state. Mushrooms are processed by different culinary techniques, such as cutting and heating, which will alter, both the sensory characteristics and potentially beneficial activities (Richardson, 2001). Nutrients (including antioxidants) then have to pass through the human digestive track enduring gastric and intestinal digestions. Finally, components that survive digestive treatment will pass through the intestinal enterocyte barrier to arrive in the lymph or blood (Fang *et al.*, 2006).

2.1.2.3. Mushroom and cancer

Experience has shown that mushrooms have the ability to prevent and treat cancer. Hundreds of pharmaceutical products derived from mushrooms have been widely used in research or therapy about their anti-tumour properties, and because of this, there is an incentive to explore and identify novel anti-tumour bioactive constituents of mushrooms. To be specific, many previous studies have reported that the proteins in edible or medicinal mushrooms possess high levels of anti-tumour properties. For example, the lectins from *Agaricus bisporus* (Yu *et al.*, 1993), *Grifola frondosa* (Kawagishi *et al.*, 1990), *Pleurotus citrinopileatus* have been shown to have anti-tumour effects against human cancer cell lines and deserve study as a potential agent for cancer therapy (Li *et al.*, 2008). Other anti-tumour proteins have also been identified, such as ubiquitin-like proteases (Lam *et al.*, 2001), ribosome inactivating proteins (Lam and Ng, 2001) and many deoxyribonucleases (DNases) have also been extracted from mushrooms (Chen *et al.*, 2012; Ye *et al.*, 2004). Even though no evidence has yet been announced to confirm anti-tumour activity of DNases, they have still been recommended as an attractive candidate for cancer prevention and treatment (Linardou *et al.*, 2000). Some polysaccharides, such as polysaccharide-K (Oba *et al.*, 2007), polysaccharide peptide (Luk *et al.*, 2011) and lentinan (Guo *et al.*, 2009), have already been utilised in modern cancer therapy. It is also noted that some polysaccharide-protein complexes in mushrooms play an important role in stimulating the nonspecific immune system and anti-tumour activities (Chen *et al.*, 2012).

2.1.2.4. Mushroom and metabolic disorders

Metabolism is an essential process for the human body to get energy from food. More specifically, when food passes through human digestive system, the components of food will break down into sugars, amino acids, fatty acids, vitamins and minerals. Undernutrition or overnutrition, caused by a poor diet or overeating, can lead to metabolic disorders and abnormal chemical reactions in the human body. Metabolic disorders can cause a lot of diseases, such as obesity, diabetes, hyperlipidaemia and cardiovascular (Whelan *et al.*, 2013).

The increasing percentage of obesity is a worldwide difficult problem, especially in developed countries. For obese people, subsequent diseases, such as cardiovascular disease, type 2 diabetes, cancer, osteoarthritis, sleep apnoea, hypertension and dyslipidaemia are the biggest cause of mortality (Visscher and Seidell, 2001), therefore more effort needs to be put into obesity prevention. Previous studies have already shown that mushrooms are a good source of dietary fibre and are low in calories, which makes mushrooms a good choice for weight loss. In the past decade, more researchers have focused on the effects of mushrooms on the prevention and treatment of chronic diseases, when forming a nutritious component of the daily diet (Kalaras *et al.*, 2017). According to the Satiety Index list (Holt *et al.*, 1995), food with more fibre can provide greater satiety and help people to control their total energy intake during a meal. In this way, consuming more fibre-rich food could make a big contribution to weight loss while maintaining a balanced diet. Additionally, there are some other advantages of increasing the intake of dietary fibre, for example, blood lipids will decrease and

inflammation can be modulated (Neyrinck *et al.*, 2009). The content of chitosan in mushrooms also appears to play a role in the reduction of ectopic fat deposition in the liver and the muscles. It has been reported that chitosan supplementation (300 mg/kg, twice daily) prevented the increase of body weight and lowered liver lipids (Neyrinck *et al.*, 2009). Furthermore, low glutathione levels have been proved to be associated with increased risks for cancer, cardiovascular diseases, arthritis and diabetes (Kalaras *et al.*, 2017; Townsend *et al.*, 2003). Many reports describe the polysaccharide fraction of mushrooms, which has been highlighted as having antioxidant, anti-aging, anti-tumour and hepatoprotective properties (Gan *et al.*, 2012; Souilem *et al.*, 2017). Additionally, the antioxidant activities of mushroom protein hydrolysates and their peptide fractions also have been analysed and the results suggested that they could be potential bioactive ingredients as well as natural antioxidants (Kimatu *et al.*, 2017).

In terms of the blood lipids, controlling blood lipids, especially cholesterol, can reduce the risk of many diseases, including atherosclerosis. Dyslipidaemia is a kind of metabolic disorder and is normally caused by eating habits and lifestyle. Oyster mushrooms have been indicated to bring about significant hypocholesterolemic and anti-atherogenic effects to rabbits that were used in experiments to simulate humans (Bobek and Galbavý, 1999). Such functions of mushrooms may be attributed to the presence of lovastatin which is the major compound of the statins (Lindequist *et al.*, 2005). Similarly, *Auricularia judae* also have been demonstrated hypocholesterolemic ability, and some triterpenes from *G. lucidum* are able to inhibit the

biosynthesis of cholesterol as well (Lindequist *et al.*, 2005), the two mushrooms have been viewed as Chinese herbal medicines and valuable tonic foods for thousands of years. Diabetes is also a metabolic disorder and more than 250 million people are estimated to suffering from it worldwide. The treatment of this disease is now focused on overcoming peripheral insulin resistance. Studies have shown that some polysaccharide fractions of mushroom possess a hypoglycaemic effect, for instance, the polysaccharide fraction from *G. ludicum* was observed to lower blood glucose for a diabetic group (Gao *et al.*, 2004). Research on mice has illustrated that mushroom extracts can inhibit hyperglycaemia in mice with non-insulin-dependent diabetes and some extracts also act as an insulin sensitiser in glucose tolerance tests (Sato *et al.*, 2002). Additionally, many mushroom species have been traditionally used as medicinal foods in Asian countries, particularly in China, and are well appreciated for their therapeutic usages for tumours, diabetes, stomachaches, inflammation, pneumonia, carcinogenic activities (Liu *et al.*, 2017; Souilem *et al.*, 2017; Wu *et al.*, 2012).

The mycelia and the culture media of mushrooms have also been reported to be potential sources of bioactive compounds (Ma *et al.*, 2016). Due to the shorter incubation time and easier culture conditions of the cultured mushroom mycelia, they are becoming a promising alternative fungal sources of bioactive compounds, to be specific, their cultivation requires less space with a low probability of contamination and higher production of biomass compared to the fruiting bodies (Zhang *et al.*, 2016). Studies of mushroom mycelia have shown that they contain higher contents of ergosterol, phenolic compounds, and stronger

antioxidant, anti-inflammatory and cytotoxicity activities (sometimes similar) to their fruiting bodies (Souilem *et al.*, 2017).

2.1.3. White button mushroom (*Agricus bisporus*)

Due to their nutritional, organoleptic and medicinal characteristics, the *Agricus bisporus* known as button mushroom and whose fruiting bodies are white or cream coloured with crumbly, fragile, dry and hard flesh (Jaworska *et al.*, 2010), is widely cultivated all over the world (Chen *et al.*, 2017) and its yield account for around 32 % of total edible fungi production of the world (Chang, 1999). For example, in China, the amount of button mushrooms produced from 1997 to 2003 had increased from 180,500 to 1,330,400 tonnes (more than 7 folds), which was based on the improvements of culturing of superior strains and spawn breeding and related technologies (Chang, 2006). It has been reported that the white button mushroom has been cultivated for about 350 years (Asic *et al.*, 2015). Its natural habitat is leaf and needle litter in the forests of Western, Central, and Southern Europe, American continent, and Northern Africa, where it acts as a secondary decomposer (Asic *et al.*, 2015).

2.1.3.1. The composition of button mushroom

In terms of nutrients, button mushrooms contain high amounts of high quality proteins, several essential amino acids, carbohydrates, vitamins (B₂, C, niacin, and folate), polyphenols, mineral elements (iron, calcium, potassium, phosphorus, selenium, zinc and copper) and

dietary fibres which is attributed to the polysaccharide chitin in the mushrooms' cell walls (Chang, 1999; Manzi *et al.*, 2001) (Table 2.2). Furthermore, white button mushrooms are particularly rich in the antioxidant thiol-amino acid, ergothioneine, which is not found in high levels in most animal based food sources and is not synthesised by humans (Calvo *et al.*, 2016).

Table 2.2 Proximate composition of fresh, frozen, canned and salted *Agaricus bisporus* mushrooms in a dry weight basis (Manzi *et al.*, 2001)

Proximate composition	fm/content (%)	FM/content (%)	CM/content (%)	SM/content (%)
Dry matter	7.29 ± 0.35C	7.67 ± 0.37C	9.45 ± 0.41B	23.23 ± 0.38A
Protein	26.27 ± 0.36A	24.35 ± 0.21B	22.26 ± 0.19C	16.54 ± 0.27D
Fibre	22.45 ± 0.39C	23.12 ± 0.28C	24.63 ± 0.36B	25.48 ± 0.45A
Fat	3.22 ± 0.18A	2.11 ± 0.44B	1.94 ± 0.21C	0.92 ± 0.29D
Total carbohydrate	62.36 ± 0.22D	65.43 ± 0.37C	67.58 ± 0.29B	72.64 ± 0.47A
Ash	7.24 ± 0.68B	7.48 ± 0.51B	7.56 ± 0.47B	9.37 ± 0.49A

Mean ± standard error (n = 6). Mean in the same row with different superscript (A, B, C, D) are significantly different ($p < 0.05$). fm - fresh mushroom, FM - frozen mushrooms, CM - canned mushrooms, SM - salted mushrooms.

In addition to the abundance and valuable nutrients, button mushrooms are extensively consumed all over the world, depending on the various kinds of characteristic flavour substances, which bring the delicious taste to consumers (Cremades *et al.*, 2012). Specifically, soluble sugars and polyols, free amino acids, 5'-nucleotides and organic acids have been illustrated to be quite plentiful in button mushrooms, (Tsai *et al.*, 2007) demonstrated that 1 g of *A. bisporus* contained 48.8–64.2 mg of free amino acids and 6.59–8.14 mg of 5'-nucleotides. Furthermore, the umami taste proven by (Yamaguchi *et al.*, 1971) is an overall food flavour sensation caused or enhanced by monosodium glutamate (MSG) and it also contributes to the pleasant taste of button mushrooms.

The medicinal benefits of button mushrooms have received wide attention. Studies have shown that the white button mushrooms could take inhibitory effect on aromatase activity (an enzyme involved in oestrogen production), which has also been proven that white button mushrooms have the ability to bring benefits to the treatment and prevention of breast cancer, especially in postmenopausal women (Beelman and Edwards, 1989). White button mushrooms are also reported to be good sources of dietary components that have shown to lower lipids and stimulate release of the anti-inflammatory adipokine, adiponectin (Calvo *et al.*, 2016). Good bioavailability of ergothioneine from white button mushroom has been carried out an acute feeding study in healthy young adults, the results represented no significant physiological health changes (Weigand-Heller *et al.*, 2012). While the whole mushrooms or extracts have been demonstrated to have anti-inflammatory effects on *ex-vivo* in murine macrophages and *in vivo* in growing rats (Babu *et al.*, 2014). Additionally, white button mushrooms were fed to diabetic hypercholesterolemic rats which resulted in lower blood glucose and cholesterol concentrations (Jeong *et al.*, 2010). The extracts of mushrooms from one species of the genus *Agaricus* were fed to subjects with type 2 diabetes and showed significant improvement in the homeostasis model assessment for insulin resistance (HOMA-IR) index and increases in circulating adiponectin after 12-weeks of treatment (Hsu *et al.*, 2007).

2.1.3.2. The shelf life of button mushroom

Even though the white button mushroom is considered the most extensively cultivated edible mushroom in the world (Meng *et al.*, 2012), its postharvest life is very short, of only less than three days at room temperature, mainly due to browning, senescence, high respiration, water loss and microbial attack (Ye *et al.*, 2012). Browning especially determines the marketability and consumer acceptability of mushrooms (Qin *et al.*, 2015), which is mainly attributed to the oxidation of phenolic substances into quinones by enzymatic action, such as the polyphenol oxidase, and ultimately polymerise to produce the browning appearance (Mohapatra *et al.*, 2008). Furthermore, another reason of such surface discolouration is microbial contamination (Jahangir *et al.*, 2011).

Such a short shelf life is an impediment to the distribution and marketing of the fresh button mushrooms. Various strategies have been attempted to retard browning and prolong shelf life of fresh whole or sliced mushrooms. Conventionally, fresh button mushrooms are recommended to be packed in plastic punnets and overwrapped with perforated PVC film and refrigerated. However, this method is also problematic: both the high transpiration rate of mushrooms and poor water vapour permeability of the film create high humidity and thus cause condensation inside the package (Gao *et al.*, 2014). Therefore, much work still needs to be done to find an appropriate method to prolong post-harvest storage that will bring huge benefits to the mushroom industry as well as consumers.

The effects of 6 months storage on composition, free amino acids and 5'-nucleotides in *A. bisporus* by three different storage methods (freezing, canning and salting) has been observed and the results suggested that the most beneficial preservation is freezing (Liu *et al.*, 2014). Traditionally, button mushrooms are usually preserved by dehydration. Freeze drying (FD) is regarded as one of the most effective drying methods to preserve food. Freeze drying gives a quality end product that has good sensory properties with high nutrient retention (Babić *et al.*, 2009), however, it is a long process that has a high energy input. Therefore, freeze drying combined with microwave vacuum drying (FMVD) is a beneficial compromise. Previous studies, considering the appearance, texture, nutritional value, honeycomb ultra-structure and the rehydration capacity of dried button mushroom show that the quality of products dehydrated by FMVD was very close to that by FD (Pei *et al.*, 2013 a and b). In addition to this, FMVD is more environmental friendly (saving energy and time). What is more, the non-volatile components of dried button mushrooms by FD and FMVD have been compared. The products after FMVD had relatively low soluble sugars and polyols, significantly higher free amino acids and similar content of 5'-nucleotides, organic acids and umami concentration (Pei *et al.*, 2013 a). Both FD and FMVD had good at flavour retention. Other physical techniques that have been used to prolong the shelf life of mushrooms, include modified atmosphere packaging (Simón *et al.*, 2010), electron-beam irradiation (Koorapati *et al.*, 2004) and ultrasound treatment (Lagnika *et al.*, 2014).

Desirable results of preservation have been obtained through some chemical treatments, such as citric acid (Brennan *et al.*, 2000), hydrogen peroxide (Cliffe-Byrnes and O'Beirne, 2008), calcium chloride (Miklus and Beelman, 1996), ethylenediaminetetraacetic acid (EDTA) (Sapers *et al.*, 1994) and sorbitol (Anantheswaran *et al.*, 1996). In addition to traditional preservation, other studies have been done to find novel techniques to storage mushrooms. Thereinto, essential oils (EOs) are an interesting natural preservative as an alternative to synthetic chemicals. EOs can be defined as aromatic oily extracts obtained from plant materials (flowers, seeds, leaves, roots, fruits and other plant parts) (Burt, 2004). Many essential oils have been proven to have an anti-microbial effect and able to reduce postharvest diseases and disorders in crops (Serrano *et al.*, 2005; Valverde *et al.*, 2005). It is compelling that some essential oils have been found to have the potential to improve the antioxidant capacities of several kinds of fruit. For instance, Wang *et al.*, (2008) found that the flavonoid content and oxygen radical absorbance capacity in blueberries could be increased by carvacrol, anethole, or perillaldehyde. Methyl jasmonate, or tea tree oil, increased the antioxidant capacities and antioxidant enzyme activities in Chinese bayberries or raspberries (Chanjirakul *et al.*, 2006; Wang *et al.*, 2009). Natural EOs should also be valued as reducing agents to the peroxidase activity of leafy vegetables (Ponce *et al.*, 2004). Button mushrooms were fumigated with EOs (clove, cinnamaldehyde and thyme) and their browning and postharvest qualities were evaluated. It was found that all EOs had the ability to inhibit the mushroom senescence, while cinnamaldehyde was the most effective choice and it also promoted the accumulation of phenolics and ascorbic acid successfully (Gao *et al.*, 2014).

2.1.4. Shiitake mushroom (*Lentinula edodes*)

Shiitake mushrooms belong to a special group of macroscopic edible fungi, which is one of most common edible mushrooms all over the world and rich in a variety of nutrients with special flavour (Wang *et al.*, 2017). Their production is increasing faster than that of any other mushroom due to their flavour and nutritional benefits, especially in Asian countries where their cultivation began over 1000 years ago (Caglarirmak, 2007). Shiitake mushrooms are highly valued as both food and tonic in some eastern countries. They contain several bioactive compounds, such as polysaccharides, dietary fibre, ergosterol, vitamin B₁, B₂, B₁₂ and C, folates, niacin, minerals and antioxidants (Antmann *et al.*, 2008). Also, shiitake mushrooms are good source of vitamin D₂ and like other mushroom species, the secondary compounds with antioxidant effects (phenolics and flavonoids) in shiitake are attracting interest as potential protective agents for human health (Mattila *et al.*, 2000). Additionally, there is a compound named lentinan which play an important role in adjusting the body immune function of T cell activity and reducing or even stopping the tumour growth. One, three-beta glucan, also known as activated hexose-containing compound, is another bioactive component which can reduce tumour activity and lessen the side effects of cancer treatment (Fang *et al.*, 2006). Shiitake mushrooms also contain eritadenine which is said to lower the level of cholesterol through preventing cholesterol from being absorbed into the bloodstream (Fang *et al.*, 2006). What is more, it has been indicated that the ergosterol and fungisterol in mushrooms have an effect on disease resistance and common cold prevention as they are converted to vitamin D by ultraviolet light from the sun (Perera *et al.*, 2003).

2.1.4.1. The shelf life of shiitake mushroom

Like all the other mushroom species, fresh shiitake are very perishable and undergo loss of valuable nutrients during the whole postharvest period. During postharvest storage, shiitake mushroom is sensitive to saturated humidity and high CO₂ concentration (Wang *et al.*, 2017). Therefore, scientists have done much work to find the appropriate preservation methods in order to prolong shelf-life. Firstly, drying as a common technology has been applied to stored shiitake and the advantages and disadvantages are similar to that as mentioned previously. While it has been observed that consumers prefer dried shiitake mushroom products to fresh ones, this phenomenon is attributed to the superior umami flavour (similar to products like meat and cheese) due to the break down of proteins into amino acids during drying (García-Segovia *et al.*, 2011; Qi *et al.*, 2014). Essential oils have been reported not only to be the preserving agents for shiitake mushrooms but have also been reported to be able to enhance the antioxidant effects of shiitake mushrooms (Jiang *et al.*, 2015). Thirdly, to date, it has been reported that controlling both humidity and CO₂ concentration in the shiitake mushroom package can provide the benefit of quality preservation (Wang *et al.*, 2017). There is much research about modified atmosphere packaging (MAP) on fresh vegetables and fruits, some of it has been conducted on fresh shiitake mushroom physiology and the nutritive constituents have been found to change under active MAP with different initial gas compositions (Li *et al.*, 2014). Additionally, ultraviolet (UV) irradiation has been utilised as a preserving technique for mushrooms to prolong shelf life and improve nutritional values. A study has carried out on processing shiitake mushrooms by UV-C irradiation of 4 KJ m⁻² and

results illustrated this pre-treatment could delay softening of mushroom structures as well as increase total antioxidant capacity, total flavonoids and ascorbic acid content in mushrooms (Jiang *et al.*, 2010). The shiitake irradiated by UV-B also shown a stronger antioxidant properties compared with untreated samples (Kim *et al.*, 2014).

2.1.4.2. Vitamin B₁₂ and shiitake mushroom

Vitamin B₁₂ is a vital nutrient for human health. Medical research has discovered that the major symptoms of B₁₂ deficiency are neuropathy and megaloblastic anaemia (Scalabrino, 2009). People who are strict vegetarians are at risk of developing B₁₂ deficiency (Millet *et al.*, 1989), because vitamin B₁₂ is synthesised only by certain bacteria which are concentrated in the bodies of animal-derived foods that are higher predatory organisms in the natural food chain system. This means that meat, milk, egg, fish and shellfish are the primary dietary sources of B₁₂, not plant derived foods (Watanabe, 2007). Therefore, it is very necessary to identify plant food with a comparatively high content of vitamin B₁₂. Among European vegetarians some consume wild edible mushrooms, the fruiting bodies of black trumpet (*Craterellus cornucopioides*) and golden chanterelle (*Cantharellus cibarius*) contain a considerable amount of B₁₂, 1.09–2.65 mg/100 g dry weight, whereas the remaining fruiting bodies just have zero or trace amounts (Watanabe *et al.*, 2012). Some Asian edible wild (*Lactarius laeticolorurus*, *Suillus spectabilis*, *Ramaria botrytis*, *Cortinarius pseudosalor*, *Boletopsis leucomelas*, and *Sarcodon aspratus*) and cultivated (*Pleurotus eryngii*, *Grifola frondosa*, and *Hypsizygus marmoreus*) mushroom fruiting bodies have been documented to have trace amounts of vitamin B₁₂ about 1.0 mg/100 g dry weight (Bito *et al.*, 2014).

Shiitake mushrooms are popular in various dishes including vegetarian dishes, both fresh and dried ones. The B₁₂ content was determined in commercially available dried fruiting bodies of shiitake mushrooms, and was found to be approximately 5.61 ± 3.90 mg/100 g dry weight (Bito *et al.*, 2014). The presence of an unnatural corrinoid vitamin B₁₂ (c-lactone) which is inactive in humans is rare. The level of vitamin B₁₂ in the bed logs of shiitake was similar to that in the dried fruiting bodies (Bito *et al.*, 2014). Given the shiitake mushrooms' inability to synthesise vitamin B₁₂ *de novo*, the B₁₂ observed in fruiting bodies must come from the bed logs (Bito *et al.*, 2014). The recommended dietary allowance of vitamin B₁₂ for adults, which is 2.4 mg/day, could be reached by consuming about 50 g of dried shiitake fruiting bodies (Shibata *et al.*, 2013). However it is impossible to ingest such great amount of shiitake in one day. Notwithstanding as mentioned earlier, dried shiitake fruiting bodies are valued as a plant based source of vitamin B₁₂.

2.1.4.3. Shiitake mushroom application

Firstly, there have been so many suggestions concerning about how to use nutrients to improve the survival and viability of probiotic strains in many commercial yoghurt brands, due to the probiotic cultures in these products reported to be viable for only 3–4 weeks under 4 °C (Ibrahim and Carr, 2006). Shiitake mushroom extract contains many oligosaccharides and polysaccharides and has been applied as a prebiotic to enhance the viability of probiotics. It has been found that 4 % of shiitake extract could support the growth of several lactic acid

bacteria and bifidobacteria (Hassan *et al.*, 2011). Further study indicated that the effect of shiitake extract is strain-dependent and most effective for the viability of *L. reuteri* DSM 20016 and *B. breve* ATCC 15701 in yoghurt products during refrigerated storage. The results showed that *L. reuteri* DSM 20016 exhibited best stability and highest viability in yoghurt products with 4 % shiitake extract followed by *B. breve* ATCC 15701, and then the control samples (Hassan *et al.*, 2014). Secondly, shiitake mushrooms are the second most widely used traditional Chinese medicinal mushrooms in the world. The main reason is that phenolic compounds in shiitake mushrooms have been valued as excellent antioxidants and synergists that are not mutagenic (Cheung *et al.*, 2003). Both fruiting bodies and mycelia cultivation have received huge interest from polyphenolic compounds industrial production. For instance, it has been proven that fermented soy bean curd residue enhanced the production of total polyphenols in shiitake mushrooms considerably (Shi *et al.*, 2012). Thirdly, some researchers tried to incorporate mushrooms into beverages to create novel refreshments with both higher nutritional benefits and pleasant flavours. In this study, they fermented wort with 31 screened fungi and analysed the flavour of the products. Shiitake mushroom produced flavours such as fruity, slightly sour and plum, and was perceived as the most popular flavour (Zhang *et al.*, 2014). Furthermore, supplementation of health-promoting ingredients is an approach to make meat products more nutritious. It has been showed that the potential of shiitake powder to enhance sensorial characteristics effectively, such as taste and flavour in frankfurters on the first day of refrigerated storage, as well as retarding lipid oxidation (Pil-Nam *et al.*, 2015). Formulated chicken nuggets which are low in fat and high in dietary fibre and free from

phosphate were developed by adding various levels of a konjac flour/xanthan gum mixture and shiitake powder (Akesowan, 2016).

2.1.5. Porcini mushroom (*Boletus edulis*)

Boletus edulis and allied species are known as “porcini mushrooms” (Casale *et al.*, 2016). Porcini is a type of mushroom with a unique and elegant aroma, which is a very popular food item, particularly in Europe (Midoh *et al.*, 2013). While actually the term “porcini” does not represent a single species, but a group of several species, including *Boletus aereus*, *B. aestivalis*, *B. edulis*, and *B. pinophilus* (Hall *et al.*, 1998). There are 24 species recorded as porcini in China (Zhang, 2006). This group shares the following features the surface of the immature poroid hymenophore is covered with a layer of tangled white hyphae, stipe is more or less reticulated, and the whitish to white flesh is without colour change when cut (Halling *et al.*, 2014). The feature “stuffed pores” of porcini has been regarded as one of the key characters to distinguish porcini mushrooms from other boletes (Cui *et al.*, 2015). Porcini mushrooms are one of the most important fungal groups because of their ecological and economic importance (Sitta and Davoli, 2013). It has been affirmed that their economic value is clearly substantial since 20000–100000 metric tonnes are estimated to be consumed annually and the median wholesale price in the U.S. for fresh mushroom in 2009 was 60 USD/kg and can reach 200 USD/kg (Dentinger *et al.*, 2010). Porcini mushrooms are widely collected and consumed in their main production areas in North America, Europe and eastern Asia (Arora, 2008; Feng *et al.*, 2012). Yunnan Province in southwestern China is one of the

most important centres in the for producing, consuming and trading porcini mushrooms. The Bolete Association of Yunnan Province reported that there were 10572 tons of fresh boletes exported from Yunnan to other regions (especially to Europe) in 2010, which resulted in gross sales of 71.83 million USD (Feng *et al.*, 2012).

Porcini mushrooms have been used as traditional Chinese medicines. Porcini mushrooms are nutritionally complete, especially rivalling the protein content of meats (Dentinger *et al.*, 2010). Several researchers have conducted studies into their pharmaceutical benefits, results show that porcini mushrooms are able to improve blood flow and relieve tension in both muscles and joints (Hall *et al.*, 1998). Furthermore, porcini mushrooms have also been proved to obtain anti-tumour and antioxidative physiological effects (Bovi *et al.*, 2011; Luo *et al.*, 2012). Midoh *et al.* (2013) studied the oral administration of a hot water extract of porcini mushrooms to spontaneously hypertensive rats. The supplementation of porcini mushrooms decreased the systolic blood pressure, diastolic blood pressure, heart rate, blood urea nitrogen, creatinine, triglyceride and increased high-density lipoprotein-cholesterol in the blood.

2.1.6. Black ear mushroom (*Auricularia auricula*)

Ear mushrooms are a traditional Chinese medicine and are also commonly used as food. Generally, the cultivation methods for ear mushrooms are parallel to that of shiitake mushrooms on logs or on sterilised sawdust (Mau *et al.*, 2001). Ear mushrooms were found to be medically active in several therapeutic effects, such as anti-inflammatory, anti-tumour,

blood pressure regulation, hypercholesterolemia, hyperlipidaemia, cardiovascular disorders and chronic bronchitis (Wasser and Weis, 1999). Furthermore, the methanolic extracts from ear mushrooms have been shown to possess excellent antioxidant activities (Mau *et al.*, 2001). Black ear mushrooms, whose fruit bodies are jelly-like, occur as saprophytes on stumps or at the bases of dead or dying woody trees (Chang and Miles, 2004). Black ear mushroom, belonging to heterobasidiae of basidiomycetes and also called Jew's ear, wood ear, black tree fungus or ear fungus, and is frequently consumed as a food and a traditional medicine in the far east (Jiangwei *et al.*, 2011). Black ear mushrooms contain a range of bio-compounds and have anti-tumour, anti-viral, anti-bacterial and anti-parasitic effects (Onyango *et al.*, 2011). It has been reported that the fruiting bodies of black ear mushroom contained great amount of fibre (12.9 %), which makes its water extract highly viscous (Wu *et al.*, 2014). The water soluble polysaccharides extracted from black ear mushrooms have a hypoglycaemic effect (Cheung, 1996), which may be attributed to the high viscosity of polysaccharides without reduction of food intake. Wu *et al.* (2014) carried out further studies into the *in vitro* hypoglycaemic effect of black ear mushroom extract and results showed that the extract had a similar ability to psyllium to absorb glucose, thus decreasing the level of glucose hydrolysis, and also suppressing the activity of α -amylase to inhibit the digestion of polysaccharides.

Black ear mushrooms are able to grow on wheat straw, sugar bagasse, sawdust, maize cobs and maize stalks (Onyango *et al.*, 2011). Additionally, supplementation of substrates with other nutrients also have been utilised in order to achieve maximum yields, such as soybean

meal, rice and wheat brans, and they were reported to increase mushroom yield two-fold (Ayodele and Akpaja, 2007; Royse *et al.*, 1991). Furthermore, black ear mushrooms are peculiar mushrooms that have captured the palate of Asian mycophagists for centuries in that they always rehydrate readily from a dried state, embellish soups and sauces, and impart a unique and pleasing texture to most meals (Mau *et al.*, 1998). Small amounts of black ear mushroom and ginger are often added to fish meat gel to mask the fish odour (Makinodan and Hujita, 1990).

2.2. Mushroom cereal products

The popularity of bread, pasta and extruded foods is increasing and are now considered convenience foods, predominantly due to their ease of preparation, storage ability, attractive appearance and texture. It is reported that there was an increased tendency among children and adults to move away from traditional eating pattern of three meals a day to eating snack foods (Okafor *et al.*, 2012). Although relatively simplistic, snack foods can be regarded as a series of food products often derived from cereals. Cereal derived product are low in protein and nutritionally unbalanced, for example, they are low in lysine (an essential amino acid), vitamins, minor minerals and fibre (Agu *et al.*, 2010; Giami *et al.*, 2003). Furthermore, many of these products can be regarded as energy dense and nutrient poor exhibiting high glycaemic index (GI) values (Brennan *et al.*, 2013 b). Recently, driven by the consumer expectations of high nutritional quality convenience foods, fortification of wheat flour with high nutritional value materials from plant sources has been recognised as important (Agu *et*

al., 2010; Misra *et al.*, 1991). Over the years, many ingredients such as mushroom fruiting bodies (shiitake stipe, silver ear) have been included in cereal product formulation to increase variety, nutritional value and product appeal, (Lin *et al.*, 2008; Tseng *et al.*, 2010).

Mushrooms have been popular for centuries due to their flavour. Originally, mushrooms have been mainly used as fresh food (just mushroom fruiting bodies) and food flavouring material. However, with the advent of modern food technology and new consumer appeal factors, interest in the use of mushrooms in the food industry has recently increased. This includes use of fruiting bodies, stalks and mycelia, due to their being a source of bioactive components hence they may be useful in the development of functional foods (Tseng *et al.*, 2008; Wasser and Weis, 1999). Mushrooms can contribute to the vitamins, minerals, fibres and even antioxidant content, thus improving the nutritive value of convenience food. This research concentrates on the role of mushroom powders in bread, cookies, pasta and extrusion food.

2.2.1. Mushroom pasta (noodle)

Pasta, which is a cold extruded food, represents a fast growing segment of the food industry because of their palatable taste, cooking convenience, affordable prices, long shelf life and easy to transport (Riley, 1987). Furthermore, as wheat derived staple food, noodles are the second most consumed foods next to bread in the world. Pasta is mainly made from wheat flour in most countries. For example, in several Asian countries, noodles occupy nearly 50 % of total wheat flour consumption (Hou, 2010). Pasta, cookies, whole wheat bread among other bakery items can be easily enriched with other food sources (Giuntini *et al.*, 2003). Generally,

pasta products are always manufactured using semolina in order to get high quality due to its excellent rheological properties as well as superior cooking quality and consumer acceptance (Kim *et al.*, 2016). While pasta is an optimal vehicle for health promotion (Ciccoritti *et al.*, 2017), partial or complete replacement of semolina with other flour to produce pasta have also been investigated to improve the nutritive value and functional effects of pasta, such as lupin flour and pigeon pea (Jayasena and Nasar-Abbas, 2012; Majzoobi *et al.*, 2011; Martínez-Villaluenga *et al.*, 2010). Many studies have indicated that pasta can be formulated with mushroom powders according to the consumer demand of more balanced nutrition (Feillet *et al.*, 1996). For instance, the experiments performed by (Kim, 1998) showed a positive relationship between the levels of oyster mushroom and shiitake mushroom in wet noodles and the protein and fibre contents, in this case the noodles still had good acceptability up to 7 % of mushroom powder. Similarly, using oyster mushroom mycelia powder as a fibre-enriching agent in pasta, the protein and total dietary fibre contents increased while the lipid content decreased. In addition, this observation suggested that semolina pasta made with 5, 10 and 15 % oyster mushroom mycelia powder gave good scores in colour, flavour, mouthfeel, elasticity and overall acceptability (Salama, 2007). This new kind of pasta may help consumers to increase fibre consumption and as a result, lower their risk of coronary heart diseases and diabetes. Interestingly, it has been reported that common wheat flour with 4 % β -glucan-rich fractions from *Pleurotus eryngii* mushroom could be used to produce pasta with an improved quality similar to semolina pasta (Kim *et al.*, 2016). The properties of wet noodle with the addition of sangwhang mushroom (*Phellinus linteus*) powder and extract were investigated and the sensory properties of noodles added with sangwhang mushroom extracts were

significantly superior to the control, and the results also suggest addition of sangwhang mushroom extract is more suitable than mushroom powder for noodle processing (Kim *et al.*, 2005). Brown oak mushroom (*Lentinus edodes*) was used to substitute wheat flour to produce noodles, the results of sensory evaluation showed addition of 20 % and 30 % had higher values of acceptability, and brown oak mushroom added to a concentration of up to 30 % effectively improved the quality of noodles (Kim *et al.*, 2008 b). The influence of noodles made from brown oak mushroom (*Lentinus edodes*) of unmarketable quality, on the lipid metabolism and antioxidant system in high cholesterol fed rats was studied. The results indicated that consumption of oak mushroom noodle could lower atherosclerosis cardiovascular disease risk effectively (Kim *et al.*, 2009 b). More recent research concentrated on the effect of button mushroom powder on the dough and nutritional qualities of pasta. A positive correlation between the amount of button mushroom powder and cooking time, water absorption, volume expansion, gruel solid as well as the contents of protein and fibre was found. Simultaneously, the fat was kept at an optimum level. Furthermore, this fortified pasta appeared to be highly acceptable with respect to sensory attributes and the authors recommended the resultant pasta was suitable as a nutritious food for low income groups in developing countries (Kaur *et al.*, 2013).

Cereals other than wheat, such as rice and buckwheat, have been used due to consumer demands for variety and health. In terms of rice noodles, traditionally rice flour is partly incorporated into wheat flour based noodle formulations or different noodle-making

procedures are utilised as rice flour is not able to form a cohesive dough structure (Fu, 2008). As such, there are several preceding studies in literature to enhance the quality attributes of rice noodles (Cham and Suwannaporn, 2010; Hormdok and Noomhorm, 2007). Recent attention has focused on the potential incorporation of ingredients with health functionality to boost the beneficial health effects of rice noodles as well as their processing performance. It has been illustrated that (1-3)(1-6)- β -glucan-enriched materials which were developed from *Lentinus edodes* mushrooms seemed worthwhile to extend their use as a good source of β -glucan, high-fibre and low-calorie flour substitute for a wider variety of foods (Kim *et al.*, 2011). On this basis, the effect of mixing β -Glucan-rich fractions (BGRFs), which were prepared from *Lentinus edodes* on rice noodles has also been examined. Indeed, this supplementation could form a rigid dough structure and obtain the enhanced extensibility and firmness of rice noodles. What is more, BGRFs played a positive role in improving the cooking qualities of rice noodles by significantly reducing their swelling index and cooking loss (Heo *et al.*, 2014).

2.2.2. Mushroom hot extruded product

Hot extrusion is a high temperature and high pressure, but short time process, as well as, involves shearing forces which could damage starch granules/chains and influence the other nutrients of foods (Brennan *et al.*, 2013 b). Meanwhile, such process usually results in the complete gelatinisation of any starch granules present because of the high temperature, pressure and mechanical shearing effect of the extruder, and it is rare to find ungelatinised starch granules in a hot extruded product (Wang *et al.*, 1993). While, as we know,

ungelatinised starches are poorly digested by humans (Butterworth *et al.*, 2011), because the human body's enzymes don't work so readily raw/ungelatinised starch, as a result, extrusion always brings about high glycaemic load (GL) status. Additionally, extruded snack products tend to generally have a high calorie content (Ovaskainen *et al.*, 2006).

Mushrooms are rich in dietary fibre and β -glucan. Diets rich in DF have been linked to reductions in both the extent and rate starch digestion and subsequent control of glucose and insulin responses (Brennan *et al.*, 2012 a). Beta-glucan has been shown to enhance immune function, lower blood cholesterol and potentially attenuates the GL of foods (Charlton *et al.*, 2012). Therefore mushrooms are good choices as added-value ingredients to rebalance the nutritional profile of extruded products by increasing their composition of bioactive phytochemicals. However, mushrooms may be considered relatively under-utilised with regard to their potential use as a functional ingredient in extrusion food products. Information on incorporation of mushroom powder in extrusion foods is quite scanty. To my knowledge, only Brennan and her group have focused on the role of mushroom waste in extrusion food, especially the glycaemic response. Their work has clearly illustrated the effect chestnut mushroom hyphae and basal material have on the quality of extruded snack products. The purpose of their research was to develop chestnut mushroom hyphae and basal material as an added-value co-product rich in dietary fibre and β -glucan, as this, could lead to substantial reductions in disposal costs. The inclusion of mushroom co-product material was significantly correlated to increased product expansion and density but negatively correlated to water

absorption index and water solubility index, moreover, this novel snack products showed significantly lower final viscosity values and a reduction in the potential glycaemic response (Brennan *et al.*, 2012 b). Apart from mushroom hyphae and basal material, it has also been reported the utilisation of mushroom fractions (prepared from stems discarded during mushroom production) that were β -glucan fibre rich with barley to reduce the energy content and help modulate the overall glycaemic response of snack foods which were extruded (Brennan *et al.*, 2013 a). However, mushroom hot extruded products is an area where little research has been done.

2.2.3. Mushroom bread

Bread is a staple food, consumed all over the world, and the major ingredients of most breads on the market are wheat flour, water, salt and yeast that are then both fermentated and processed thermally. Worldwide bread consumption accounts for one of the largest consumed foodstuffs, with over 9 billion kg of bread being produced annually (Heenan *et al.*, 2008). Traditional bread does not contain enough protein for human nutrition, thus bread fortification is necessary to improve its quality in order to reduce or control nutrient deficiency (Mahamud *et al.*, 2012).

Numerous papers have discussed the role of oyster mushroom in the manufacture of breads. These authors have shown relationships between amount of oyster mushroom powder and

dough rheology and bread quality (Eissa *et al.*, 2007; Lindequist *et al.*, 2005; Okafor *et al.*, 2012; Regula and Gramza-Michalowska, 2013). For instance, extensive research has illustrated the increasing then decreasing viscosity, the gradually increasing water absorption, dough development time, mixing tolerance index and loaf weight, and decreasing dough stability and loaf volume, with the increased amount of oyster mushroom powder. The crumb texture became rough and coarse and had a dark colour with increased oyster mushroom powder. The firmness of bread crumb containing oyster mushroom powder increased during storage periods (Song *et al.*, 2005). It has been established that the mixing tolerance index values (MTI) as an indicator for staling revealed that wheat bread was better than wheat-mushroom flour bread regarding freshness (Eissa *et al.*, 2007). Some other mushroom dough rheology properties have also been investigated such that the addition of oyster mushroom powder not only brought about increased water absorption and decreased loaf volume, but also decreased specific volume, crumb grain and loaf quality (Song *et al.*, 2005).

In terms of the oyster mushroom bread nutritional quality, the utilisation of oyster mushroom powders in bread enrichment generally resulted in increased crude protein, ash and crude fibre contents (Okafor *et al.*, 2012). The results also showed a significant contribution of amino acids and increased the biological value and digestibility coefficient. Hence the developed supplementary foods are recommended in the diet of vulnerable groups to overcome protein malnutrition (Mane *et al.*, 2000). Based on the experiments conducted, it has been determined that oyster mushroom breads have higher contents of insoluble and soluble

dietary fibre and cellulose and such breads were characterised by a lower glycaemic index compared to the products without this additive (Regula and Gramza-Michalowska, 2013). Some researchers have focused on the acceptability of oyster mushroom breads. Indeed, the most interesting observation is that the results of their acceptability trials and the recommended oyster mushroom powder level were quite different. For instance, according to sensory tests, it was reported that the oyster mushroom powder could be added to wheat flour up to 10 % without any observed detrimental effect on bread sensory properties (Okafor *et al.*, 2012). While another report conducted in 2005 estimated that the addition of only 1 % oyster mushroom powder could be supplemented to make an acceptable quality of bread (Song *et al.*, 2005). More detailed study has also been made, the colour, texture, mouthfeel, taste and overall acceptability of unleavened and leavened breads made with whole and refined wheat flour, respectively, and added with powdered oyster mushroom at 5 and 10 % levels were determined (Mane *et al.*, 2000). They found that all the experimental breads were acceptable and they are recommended daily consumption of bread with 5 % powdered oyster mushroom for better leavened and unleavened breads. Similar results were also reported by (Mahamud *et al.*, 2012), considering both nutritional composition and consumer acceptability, bread with 5 % oyster mushroom powder had better quality than control, however, addition of more than 10 % mushroom powder affected the baking quality and acceptability of bread negatively.

Some other research attention has focused on distinctive breads produced by the incorporation of oyster mushroom powder. For example, it is indicated that 15 % of wheat flour could be replaced with oyster mushroom powder to make Egyptian balady bread and still provide good quality in terms of baking properties, colour and sensory evaluation tests (Eissa *et al.*, 2007). In addition, paratha flat bread also has been formulated with oyster mushroom powder which enhanced its essential nutritional components (the percentages of all nutrients were higher than control except for fat) and well accepted by consumers (Aishah and Wan Rosli, 2013).

Over the years, there has also been research examining other mushrooms added into breads (Hong *et al.*, 2003; Regula and Gramza-Michalowska, 2010). For instance, it has been reported that, with the increasing content of mushroom powder, the viscosity increased then decreased, water absorption, dough development time, mixing tolerance index, loaf weight increased, and dough stability and loaf volume decreased. Rough and coarse crumb texture with dark colour was observed with the increase of shiitake mushroom powder (Hong *et al.*, 2003). The firmness of bread crumb containing shiitake mushroom powder increased during storage periods (0–3 days). Sensory evaluation revealed that an acceptable quality of bread could be obtained by the addition of 1 % shiitake mushroom powder. It is interesting to note that all these results are similar to those obtained using oyster mushroom bread previously mentioned (Song *et al.*, 2005). Under pH conditions similar to those found in the human digestive tract, the availability of minerals in shiitake mushroom breads has been assessed,

and the percentage of dried shiitake mushrooms added to the products has a significant effect on contents of insoluble fibre, Fe, Cu, Zn, K and Na (the highest amounts of Fe, Cu and Zn were recorded in bread with a 20 % addition of dried mushrooms) and the products could be recommended as a dietary supplement (Regula and Gramza-Michalowska, 2010). The role of *Lentinus Tuber-Regium* mushroom on bread making also receives interest from some authors and it was found that *Lentinus Tuber-Regium* mushroom powder could be used to replace up to 4 % wheat flour to make breads (Lee *et al.*, 2004). Maitake mushroom has also been incorporated into wheat bread and resulted in decreased dough strength, as well as deteriorating bread quality parameters, such as bread height and specific volume. These negative effects may be attributed to a metal protease. This study also found that the high-molecular-weight protein/total protein in mushroom bread dough correlated well with bread height, however, no clear effect on bread specific volume was noted (Seghchi *et al.*, 2001). According to the study of Okamura-Matsui *et al.* (2003), addition of *Grifola frondosa* to white bread could supply carbohydrates to the baker's yeast and promote alcohol fermentation under anaerobic conditions, which led to increased CO₂ production.

Another widely researched aspect of mushroom breads making is using mushroom waste as a new food material (Lin *et al.*, 2008). There is a need to determine new value-added uses for off-grade and/or overmature culinary-medicinal mushrooms in food products (Corey *et al.*, 2009). Shiitake mushroom stipe, which is a potential source of fungal chitin, may be used to substitute wheat flour to make bread and the influence of shiitake stipe flour on bread quality

has been evaluated (including specific volume, colour, the taste components and sensory evaluation). Overall it was found that shiitake stipe could be incorporated into bread to provide beneficial health effects (Lin *et al.*, 2008). Further research has illustrated the use of mushrooms (*Agaricus bisporus*) waste in the manufacture of breads (Corey *et al.*, 2009). The waste was freeze dried and then incorporated into the bread which was evaluated using physical and chemical properties. The results showed that loaf size decreased, whereas bread firmness increased, with increasing levels of mushroom powder substitution. During storage, amylopectin recrystallisation was negligible in breads prepared with mushroom powder. This study also demonstrated that adding mushroom waste powder could supply valuable micronutrients such as selenium and vitamin D₂, along with the novel antioxidant, ergothioneine (Corey *et al.*, 2009). Additionally, food scientists tend to study the utilisation of mycelia because the cultivation of mushrooms requires a long time to produce fruiting bodies, whereas the submerged culture only requires a short time to mass-produce mycelia (Mau *et al.*, 2004). Mycelia is not used directly as food, but could be used as a food-flavouring material and a food ingredient. Therefore, the incorporation of mycelium into the formulation of different conventional foods to provide beneficial effects is of great interest (Ulziijargal *et al.*, 2013). It is of important to emphasise that 15 % mushroom products are based on extracts from mycelia. Therefore, mycelia products are the “wave of the future” because they ensure standardised quality and year around production (Lindequist *et al.*, 2005). Mushroom mycelia of *Agaricus blazei*, *Hericium erinaceus*, *Antrodia camphorate* and *Phellinus linteus* have been used to substitute 5 % of wheat flour to make bread. In this case, it was found that mycelium-supplemented bread was smaller in loaf volume and coloured, and had lower lightness and

white index value. Incorporating 5 % mushroom mycelia did not adversely affect the texture profile of the bread, however, it did lower consumer acceptability (Ulziiargal *et al.*, 2013). Work has been done with milled mushroom micelles grown on sorghum and wheat grains as a partial substitute for wheat flour in pan bread production, rheological properties of flour dough and baking quality properties, such as physical, chemical, protein content, colour and sensory characteristics were evaluated (Abou-Zaid *et al.*, 2012). Water absorption, dough development time and dough weakening increased, but mixing tolerance index and dough stability decrease by milled mushroom micelles at the level of 15 and 30 % was relatively marginal. Baking properties, colour and sensory evaluation tastes showed that 15 % of wheat flour could be replaced with milled mushroom micelles and still provided a good quality of pan bread (Abou-Zaid *et al.*, 2012). A recent publication found that mushroom bread had good appearance and internal quality, the aroma differed to the control bread due to the presence of the mushrooms and the nutritional value was greater than that of the control bread (Zhu and Wu, 2013).

In this project, four edible mushroom species were incorporated into three different cereal products to investigate the possibility of utilising whole mushroom fruiting body in cereal food structure, in order to evaluate the effects of different mushroom species on the physical, textural and nutritional properties of cereal products. Furthermore, three different cereal products processing to mimic different cooking processing (hot extrusion, cold extrusion and fermented cooking) were selected to determine the cooking processing treatments on the

bioactive benefits of mushroom powder, then representing the possibility of utilisation in food industry. This project was able to establish a comprehensive comparison based on addition of different mushroom powder into different cereal products.

Chapter 3

Material and methods

3.1. Materials

3.1.1. Mushroom powder

Dried shiitake mushroom and porcini mushroom slices (Jade Phoenix, China) were obtained from the local New World supermarket (Foodstuffs, New Zealand), dried black ear mushroom was obtained from the local Chinese supermarket (Sunson, New Zealand), and fresh white button mushroom was obtained from Meadow Mushrooms (Christchurch, New Zealand).

White button mushrooms were cleaned and sliced, before being put into the oven and dried at 55 °C for 24 h. Dried white button mushroom, shiitake mushroom, porcini mushroom and black ear mushroom slices were ground into powder by a mill (model: BCG200; Coffee Grinder, Breville, Sydney, Australia), and the powder was put in sealed bag and stored at room temperature until required.

3.1.2. Other materials

Semolina (Sun Valley Foods, New Zealand), high grade white flour (Champion, Auckland, New Zealand), white sugar (Chelsea, Auckland, New Zealand), iodised table salt (Cerebos, Auckland,

New Zealand), yeast (Edmonds, Auckland, New Zealand) and butter (Pams, New Zealand) were obtained from local New World supermarket (Christchurch, New Zealand).

3.2. Sample preparation

3.2.1. Pasta preparation

Fresh pasta was produced using a machine fitted with a spaghetti die (2.25 mm diameter of die hole; model: MPF15N235M; Firmar, Villa Verucchio, Ravenna, Italy). Each blend, 500 g dry ingredients and 32.5 g per 100 g water (tap water, 41 °C), was mixed for 20 min according to the manufacturer's guidelines. Extruded fresh pasta samples were cooked and analysed immediately.

Each of the four mushroom powders (white button, shiitake, porcini and black ear mushroom) were used to replace durum wheat at three different levels, namely 5 g per 100 g, 10 g per 100 g and 15 g per 100 g, and a control sample was prepared using 100 % durum wheat semolina.

3.2.2. Dough and Bread preparation

The straight dough method (AACC, 2000) was adopted in bread making. The recipe consisted of wheat flour (500 g), granulated sugar (30 g), salt (7.5 g), yeast (7.5 g), fat (25 g).

The dough was formed by using a commercial food mixer (Brabantia, Eindhoven, Netherlands) to mix wheat flour, mushroom powder, yeast and water at speed 1 for 5 min. The speed of the mixer was increased to 3 for another 10 min until an elastic dough has formed. The dough pieces was divided into two portions, one was used for the analysis of dough texture properties, and the other was used to make bread: the dough was rested for 30 min, hand-kneaded for 2 min and left for 15 min. Dough pieces were divided (100 g), manually rounded, rolled and put into baking pans greased with margarine and placed in a prover (Sanyo, Osaka, Japan) at 40 °C for 60 min. The bread was baked in an electric oven at 200 °C for 15 min. The baked bread loaves were allowed to cool for 2 h prior to subsequent analysis (Chinma *et al.*, 2015).

Samples supplemented with mushroom powder were produced substituting flour with white button mushroom, shiitake mushroom, porcini mushroom and black ear mushroom powder respectively. Mushroom powder enriched formulations replacing 5 g/100 g, 10 g/100 g and 15 g/100 g (w/w) flour respectively. The control reference sample was prepared using exclusively flour.

3.2.3. Hot extruded products preparation

Snack products were produced using a single screw extruder (screw length: 30 cm, diameter: 30 mm), and the barrel length was 300 mm, the inside shaft diameter was 30 mm, the outside shaft diameter was 40 mm and the die diameter was 3 mm (Northern Finance Ltd, Auckland,

New Zealand). The water speed was kept in 1 drop/second. The expanded snack products were collected, cooled and then stored in sealed polyethylene bags, and stored at room temperature until further analysis.

Different samples supplemented with mushroom powder were produced substituting durum wheat semolina with white button mushroom, shiitake mushroom, porcini mushroom and black ear mushroom powder respectively. Mushroom powder enriched formulations replacing 5 g/100 g, 10 g/100 g and 15 g/100 g (w/w) semolina respectively. The control reference sample was prepared using exclusively durum wheat semolina.

3.3. Physical analysis

3.3.1. Cooking properties of pasta

3.3.1.1. Cooking procedure

Fresh pasta (100 g) was cooked in 600 mL boiling tap water for a standard period of 6 min and strained for 30 s. The cooking time was standardised to 6 min following previous work to reduce variability associated with optimal cooking time on the potential glycaemic impact of the pasta. Cooked pasta was then analysed for cooking loss (CL), swelling index, water absorption and textural properties.

3.3.1.2. Cooking loss (CL)

The CL, the amount of solid substance lost in the cooking water, was determined according to Approved Method 66–50 (AACC, 2000). The cooking water was collected in an aluminium vessel, placed in an air oven at 105 °C and evaporated until constant weight was reached. The residue was weighed and reported as a percentage of starting material.

3.3.1.3. Swelling index and water absorption index

The swelling index (SI) of cooked pasta (g water per g dry pasta) was determined according to the procedure described by Cleary and Brennan (2006). Pasta (100 g) was weighed after cooking and dried at 105 °C until constant weight was reached. The results were expressed as:

$$SI = \frac{W_c - W_d}{W_d}$$

where W_c is weight of cooked pasta (g) and W_d is weight of pasta after drying (g).

The water absorption index (WAI) (g per 100 g) was determined as:

$$WAI = \frac{W_c - W_r}{W_r} \times 100$$

where W_c is weight of cooked pasta (g) and W_r is weight of uncooked pasta (g).

3.3.2. Physical properties of bread

3.3.2.1. Specific volume of bread

The specific volume of bread samples was measured by AACC (10-05) standard method: using the rapeseed displacement method to obtain the volume values, and then dividing loaf volume by loaf weight. The height was determined using Vernier callipers.

3.3.2.2. Bubbles in the bread

The method used was that carried out according to the work reported by (Martínez-Cervera *et al.*, 2011). Fresh bread samples were cut into slices of 25 mm thickness before analysis. The images of the freshly cut surface of the crumb (placed a ruler next to the sample) were taken by a camera (Canon, Japan). Image Pro-Plus 6.0 software (Media Cybernetics, Bethesda MD, USA) was used to perform the image processing. The images were converted to an 8-bit greyscale and segmented separately using a histogram-based segmentation. The ruler in photo was used to calibrate the scale in software. The measurement of each sample was done in duplicate (two bread slices from each batch were measured).

3.3.3. Physical properties of hot extruded products

3.3.3.1. The radial expansion ratio (ER) and specific length (l_{sp})

The length (l_e), diameter (D_e) and mass (m_e) were measured for 20 pieces of hot extruded from each treatment. ER and l_{sp} were obtained by these formulas below (Karkle *et al.*, 2012):

$$ER = \frac{D_e^2}{D_d^2}$$

$$l_{sp} = \frac{l_e}{m_e} \text{ (mm/g)}$$

Where D_d = die diameter (3 mm).

3.3.3.2. Density and percentage expansion of extrudate

The product density and the percentage expansion of samples (using the formula below) were calculated following the method of (Brennan *et al.*, 2008 a):

$$\% \text{ Expansion} = \frac{D_e}{D_d} \times 100$$

3.3.3.3. Extrudate WAI, water solubility index (WSI) and moisture content

The WAI and water solubility index (WSI) were determined by the method described by Anderson *et al.* (1970): 1 g sample was weighed into a tared centrifuge tube and was mixed with 10 mL distilled water. The resulting slurry was vortexed for 1 min and rested for 30 min, then centrifuged at 2000 g for 30 min. The supernatant was moved into a pre-weighed evaporating dish and then evaporated to a constant weight at 65 °C overnight. WSI is the mass of dry solids in the supernatant expressed as a percentage of the original mass of sample. WAI is the mass of gel obtained after removal of the supernatant per unit mass of original dry solids. The determinations of hot extruded samples' moisture content were analysed according to the air-oven method (AACC, 2010).

3.3.3.4. Colour of hot extruded products

The colour of all hot extruded samples was measured using a colorimeter CR-210 (Minolta, Japan). The results were expressed using the L*, a* and b* parameters. The total colour difference ΔE^* between the control and mushroom enriched samples was determined by the

following formula (Martínez-Cervera *et al.*, 2012): $\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$

3.4. Textural characteristics

3.4.1. Textural analysis of pasta

Cooked pasta textural properties were determined using a Texture Analyser (TA.XT2; Stable Micro System, Godalming, UK) equipped with a 50 kg load cell. Samples were rested for 10 min after cooking before testing. Firmness and resistance to uniaxial extension of the cooked pasta were determined according to the method described by (Foschia *et al.*, 2015 a). Four measurements were recorded from each of three different cooking replications (mean of twelve measurements). Firmness was analysed according to Approved Method 66-50 (AACC, 2000). Resistance to uniaxial extension of the cooked pasta was analysed by tension test using the A/SPR spaghetti/noodle rig (settings: pre-test speed was 3 mm/s; test speed was 3 mm/s; post-test speed was 5 mm/s; initial distance was 10 mm; final distance was 100 mm).

3.4.2. Textural analysis of bread dough

Dough texture properties were determined using the TA-XT2 Texture Analyser (Stable Micro

Systems, Surrey, UK). The extensibility (the distance to extension limit, mm) and resistance to extension (the peak force, g) of the dough were measured using a Kieffer rig (Smewing, 1995). Firstly dough was rested for 20 min at 28 °C, then was pressed into a Teflon mould for 20 min at 25 ± 1 °C before being measured. The pre-test speed was 2.0 mm/s; test speed was 3.3 mm/s; post-test speed was 10.0 mm/s; distance was 75 mm and trigger force was 5 g (Liu *et al.*, 2016). Dough stickiness (positive peak force) and cohesiveness (the distance of the sample extended on probe return) was performed by (Liu *et al.*, 2016) and performed with 12 subsamples. The test settings were as follows: pre-test and test speed was 0.5 mm/s; post-test speed was 10.0 mm/s; distance was 4 mm; time was 0.1 s and trigger force was 5 g.

3.4.3. Textural analysis of bread

In order to determine hardness (the peak force of the first compression), springiness (the distance of the detected height during the second compression divided by the original compression distance), cohesiveness (the area of the second compression divided by the area of the first compression), gumminess (hardness \times cohesiveness), chewiness (hardness \times cohesiveness \times springiness) and resilience (by dividing the upstroke energy of the first compression by the downstroke energy of the first compression) of bread, the texture profile analysis (TPA) was executed using TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) with a 25-mm-diameter cylinder probe. Bread samples were cut into slices of 25 mm thickness before analysis. The exact test settings were as mentioned by (Liu *et al.*, 2016): pre-test speed was 1.0 mm/s; test speed was 1.7 mm/s; post-test speed was 10.0 mm/s; strain was 40 % and

trigger force was 5 g.

3.4.4. Textural analysis of hot extruded products

The hardness and crunchiness of all hot extruded products were analysed by a TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK). An aluminium cylinder probe of 25 mm diameter was used with a test speed of 1 mm/s at a trigger force of 5 g. The extruded samples were compressed to 50 % of their original height, and the peak force obtained during the compression represented the hardness of the product, whereas the number of fracture peaks exhibited on compression was used to represent the crunchiness. All measurements were performed on a minimum of 12 samples (Brennan *et al.*, 2012 b).

3.5. Nutritional analysis

3.5.1. Total starch content

Total starch was determined according to AOAC Official Method 996.11, using the amyloglucosidase- α -amylase method as of the Megazyme starch analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Sample (0.1 g of food) and 5.0 mL of 80 % EtOH) was placed in a centrifuge tube it was then incubated at 80–85 °C for 5 min. The contents were mixed on a vortex stirrer and 5.0 mL of 80 % EtOH was added. The tube was centrifuged (ROTINA 380, Hettich LAB TECHNOLOGY, Tuttlingen, Germany) for 10 min at 3000 rpm on a bench centrifuge and then the supernatant was discarded. The pellet was resuspended in 10

mL of 80 EtOH and stirred on a vortex mixer, centrifuged as above and the supernatant discarded. Thermostable α -amylase diluted 1:30 with 100mM sodium acetate buffer, pH 5 (3mL) was added, then the tube was placed in a boiling water bath for 12 min (the tube was vigorously stirred after 4, 8 and 12 min). Amyloglucosidase (0.1 mL) was added mixed on a vortex mixer, then incubated at 50 °C for 30 min. The contents of the test tube were quantitatively transferred to a 100 mL volumetric flask, made to volume with distilled water and mixed thoroughly. An aliquot was transferred to a centrifuge tube and then centrifuged (ROTINA 380, Hettich LAB TECHNOLOGY, Tuttlingen, Germany) at 3000 rpm for 10 min. Triplicate aliquots (0.1 mL) of the supernatant were transferred to the bottom of glass test tubes. Then 3.0 mL of GOPOD reagent was added to each tube and incubated 50 °C for 20 min, including the D-glucose controls and reagent blanks. The absorbance was read at 510 nm, against the reagent blank.

3.5.2. Fibre content

Total dietary fibre (TDF) content was determined in duplicate using a Total Dietary Fibre assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland) and measurements were recorded for soluble (SDF) and insoluble fibre (IDF) composition as described by (Brennan *et al.*, 2008 b). The sample (1g) was weighed into a 400 mL tall-form beaker and 400 mL MES-TRIS buffer (0.05 M, pH 8.2) added, the sample was evenly dispersed on a magnetic stirrer. Heat-stable α -amylase (50 μ L) was added and incubated in shaking water bath at 98–100 °C for 30 min. The beaker was scraped down with a spatula and if necessary rinsed with 10 mL water. Protease

(100 µL) was added and incubated in a water bath at 60 °C for 30 min. Then 5 mL 0.56 N HCl and 200 µL amyloglucosidase were added, incubation in the water bath at 60 °C continued for another 30 min. The resulting solution was filtered through a sintered glass crucible prepared with celite, and washed twice with 10 mL water at 70 °C. The filtrate and water washings were transferred to a 600 mL tall form beaker for SDF determination. The remaining residue was washed twice with 10 mL of 95 % EtOH and twice with 10 mL acetone, then crucible was used to determine IDF.

The saved filtrate and water washings for determination of SDF had 4 volumes 95 % EtOH at 60 °C added and precipitate was allowed to form for 1h. The precipitate was filtered through crucible as described above. The precipitate was washed with two 15mL portions of each of 78% EtOH, 95 % EtOH and acetone. Both IDF and SDF crucibles were dried overnight in an oven at 103 °C and weighed before being analysed for protein and ash.

3.5.3. Protein content

The protein content was determined using a Rapid Max N exceed (Elementar, Langenselbold, Germany) using the conversion factors of 5.7 (AOAC 992.23) and 4.4 (Mariotti *et al.*, 2008) for semolina and mushroom powder respectively.

3.5.4. Fat content

The total fat content was determined by Soxhlet extraction method (Luque de Castro and

García-Ayuso, 1998).

3.5.5. The moisture content

The moisture of the sample was determined by oven-drying method, (Nollet and Toldra, 2015): samples were weighed using an analytical balance (ARC120; OHAUS Corp., Parsippany, NJ, USA) into a pre-weighed dish. The dish was placed in an oven at 105 ± 2 °C overnight. The dish was placed in the desiccator for 1 h to allow it to cool to room temperature before reweighing.

$$\text{Moisture (\%)} = \frac{\text{Loss of weight}}{\text{Sample weight}} \times 100$$

3.6. Rheological properties of bread dough

The dough rheological properties were determined using a DoughLAB (Perten Instruments Australia, Macquarie Park, Australia) according to the method reported by (Liu *et al.*, 2016). All the indexes were measured using DoughLab software (version 1.3.0.185).

3.7. Near infrared spectroscopy

Near infrared spectra of ground samples were determined using Calorie Answer™ (CA-HM, JWP, Japan) according to the method reported by Lau *et al.* (2016): the wavelength ranged from $(1100-2200) \times 10^{-9}$ m, the resolution was 7.5×10^{-9} m, and the data interval was 2.0×10^{-9} m. The reflectance mode was used for all samples and the reference reflectance was analysed by a calcium carbonate filled cell. Samples were scanned in cylindrical sample cell and the

cereals-powdered cereals and dried noodles mode was selected as the analysis setting. Each triplicate portion was scanned 10 times and then the averaged mean spectrum was obtained in order to improve accuracy by the computer software (CA-HM Measurement Application Software, JWP, Japan). The primary constituents of foods are C-H, O-H and N-H groups. The protein, fat, carbohydrate and water content of samples could be estimated based on the different energy absorbed at specific wavelength regions of those functional groups, which in turn to obtain the metabolisable energy of the samples. The data were transformed into $\log 1/R$ and the calorie content was calculated according to the regression expressions pre-programmed by this software, which was in accordance with the selected setting for analysis.

3.8. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (TA Q20, TA Instruments, Newcastle, DE) was used to measure the starch gelatinisation characteristics of samples using the method reported by Aravind *et al.* (2012 b) with some modifications. Ground samples (4–6 mg) were weighed into a hermetic DSC pan with 10 μL of water, the sample was hermetically sealed and allowed to equilibrate overnight at room temperature. The reference was an empty pan and lid which were pressed together. The processing was performed from 10 $^{\circ}\text{C}$ to 110 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C}/\text{min}$. A DSC heating curve was generated in this process and measurements were conducted in duplicate. Temperatures of onset (T_{onset}), gelatinisation (peak), endset (T_{endset}) and the enthalpy of the transition (ΔH) were obtained in this process.

3.9. *In vitro* starch digestion

An *in vitro* digestion process was used to evaluate the sugar release over a period of 120 min as described previously (Foschia *et al.*, 2015 b). In brief: digestions were carried out in 60 mL plastic biopsy pots placed on a pre-heated 15 place magnetic heated stirring block (IKAMAG® RT15, IKA®-WERKE GmbH & Co., Staufen, Germany). Standardised the amount of starch in all samples for *in vitro* digestion according to the results of total starch analysis. The weighed sample was mixed with 30 mL of distilled water and was held at 37 °C for 10 min, 1 mL 10 % pepsin (Acros Organics, New Jersey, USA CAS:901-75-6) in 0.05 M HCl was added to replicate gastric digestion. The sample was stirred at 130 rpm and 37 °C for 30 min. A 1 mL aliquot of the digest was taken (0 min) and added to 4 mL ethanol. Amyloglucosidase (0.1 mL) and 5 mL pancreatin (EC: 232-468-9, CAS: 8049-47-6, activity: 42362 FIP-U/g, Applichem GmbH, Darmstadt, Germany) in 0.1 M pH 6 maleate buffer to represent ileal digestion. Aliquots of 1 mL were taken at 20, 60 and 120 min and added to 4 mL ethanol. Samples were stored at 4 °C until analysis of reducing sugar content by the 3,5-dinitrosalicylic acid (DNS) method. Glucose release was plotted against time and areas under the glucose release curves were calculated using the trapezoid rule.

3.10. Antioxidant analysis

One gram of ground sample was mixed with 40 mL of 70 % methanol (20 mL only for ORAC) and stirred overnight on a magnetic agitator. Then sample extractions were used for total phenolic content (TPC), DPPH and ORAC analysis.

TPC of all samples was measured using 0.2 N Folin-Ciocalteu reagent (Sigma, St Louis, USA) according to the method reported by (Singleton and Rossi, 1965) with some modifications. Sample extract (0.5 mL) was mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent and 2 mL of 7.5 % sodium carbonate. The sample were incubated at room temperature for 2 hours in a dark place and then absorbance was read at 76 nm (V-1200 spectrophotometer, Global Science). Gallic acid (25–200 µg) was used for standard curve (Sigma-Aldrich, Steinheim, Germany). The results were expressed as gallic equivalent per gram dry weight.

The ability of all the samples to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was determined by the method adapted by (Floegel *et al.*, 2011) with some modifications. A 0.1 mM of DPPH· stock solution was prepared with 100 % methanol. To 0.5 mL of sample extract, 1 mL of DPPH· and 1.5 mL of 100 % methanol were added and the mixture was incubated for 30 min in the dark. The absorbance was read at 517 nm (V-1200 spectrophotometer, Global Science). A standard calibration curve was prepared using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (0–100 µmol). The results were expressed as micromoles of Trolox per gram dry weight.

The oxygen radical absorbance capacity-fluorescence assay was carried out using the method described by (Thaipong *et al.*, 2006) and conducted using FLUOstar OPTIMA plate reader (BMG Labtech GmbH, Offenburg, Germany). The results were expressed as micromoles of Trolox per gram dry weight.

3.11. Microstructure

The microstructure analysis of samples was evaluated by scanning electron microscope (SEM) on samples. Samples were freeze-dried, then coated with gold using a MCI000 Ion Sputter Coater. Samples were viewed using a SU8010 SEM at different magnification levels.

3.12. Statistical analysis

Unless stated otherwise, experiments were performed in triplicate. Statistical differences were determined by one-way analysis of variance (ANOVA) and Tukey's comparison test ($p < 0.05$). Pearson's correlations were also carried out to analyse the significant correlations at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Chapter 4

How the inclusion of mushroom powder can affect the physicochemical characteristics of pasta

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Abstract

In this study, a partial substitution of durum wheat semolina with three different species of mushrooms (white button, shitake and porcini) was undertaken to increase the nutritional value of the pasta. The cooking properties and textural characteristics of the pasta produced were also determined. The results showed that the addition of mushroom powder increased the cooking loss, as well as firmness and resistance of the uniaxial tension of the pasta. Porcini mushroom incorporation significantly decreased the swelling index, water absorption index and moisture content values of the cooked pasta, while, for the white button and shiitake mushrooms, there was no noticeable effect on either index compared with the control sample (containing exclusively durum wheat semolina). The addition of shiitake mushroom powder resulted in pasta with the highest firmness and tensile strength.

4.1. Introduction

Pasta is one of the most important foods worldwide, usually made of semolina from durum wheat and water (Marti and Pagani, 2013). During processing, a firm but elastic protein-starch network that withstands cooking is established (Sissons, 2004). The overall quality of pasta is determined by its cooking properties, textural characteristics and nutritional value. Specifically, high cooking quality is defined by low cooking losses and minimal increase in volume during cooking. The nutritional and functional properties of pasta play a significant role in its overall acceptability while the texture characteristics are possibly more important due to the impact they have on consumer acceptance (D'Amico *et al.*, 2015; Ficco *et al.*, 2016; Sobota *et al.*, 2015). In order to produce novel cereal-based foods, the main challenge for large-scale industrial application is to provide optimal product characteristics and ease of production without affecting structure and quality (Struck *et al.*, 2014).

Previous studies investigated the effect of incorporating novel ingredients such as non-durum wheat varieties, inulin, psyllium and oats in pasta as a means enhancing its nutritional and functional properties (Bustos *et al.*, 2013; Foschia *et al.*, 2013; Foschia *et al.*, 2015 a; Foschia *et al.*, 2015 b). Mushrooms are a popular food due to their taste, texture, medicinal properties and use as tonics (Tian *et al.*, 2012). *Agaricus bisporus*, commonly known as the white button mushroom, constitutes the majority of the total edible mushrooms consumed in New Zealand (Tian *et al.*, 2012). These mushrooms are a good source of high-quality proteins, acidic polysaccharides, dietary fibre and antioxidants (Chang, 2008; Dubost *et al.*, 2007). These

bioactive components are involved in immune regulation and also have anti-tumour, anti-microbial, anti-inflammatory, hypoglycaemic, hypocholesterolaemic and antioxidant effects (Chen *et al.*, 2006; Jeong *et al.*, 2010; Kozarski *et al.*, 2011).

Shiitake (*Lentinula edodes*) mushrooms are the second most cultivated edible mushroom in the world, representing about 25 % of production; over recent years, their production has increased faster than any other mushroom species (Jiang *et al.*, 2015). Shiitake mushrooms have also been reported as having a wide range of health benefits, such as their antioxidative, anti-fungal, anti-viral, anti-tumour properties and hypocholesterolaemic effects (Kim *et al.*, 2014). Shiitake mushrooms have high nutritional values and contain several bioactive compounds, such as polysaccharides, antioxidants, dietary fibre, ergosterol, minerals, vitamins B₁, B₂ and C, folates and niacin (Jiang *et al.*, 2015; Li *et al.*, 2014). Shiitake mushrooms are usually sold in the dry form and require rehydration before use. Many people prefer dried shiitake mushrooms to fresh ones because of their superior umami flavours (similar to meat and cheese flavours), which arise from the breakdown of proteins into amino acids during drying (Qi *et al.*, 2014). Therefore, dried mushroom powder was used in this study to provide more flavour and enhance acceptability of the mushroom supplemented pasta.

Porcini mushrooms (*Boletus edulis*) are a very popular food item with a unique and elegant aroma and have also been used as a major ingredient in traditional Chinese medicines (Midoh *et al.*, 2013). Previous studies have reported that these mushrooms have beneficial effects on

health by improving blood flow and relieving tension in muscles and joints, as well as having anti-hypertensive, anti-hypercholesterolaemia, anti-cancer, anti-tumour and antioxidative effects (Midoh *et al.*, 2013; Wuilloud *et al.*, 2004).

Pasta derived from cereal is naturally typically unbalanced as it is low in vitamins, minerals and fibre (Onipe *et al.*, 2015; Sandhu *et al.*, 2015). It has been pointed out that new sources of dietary fibre may confer significant health benefits to cereal products; for example, fibre extracts from fruits such as apples and kiwi may also cause beneficial co-extraction of bioactive compounds namely flavonoids and carotenoids (Grigor *et al.*, 2016). In this study, the whole mushroom fruit body rather than extracts were used to enrich the pasta. Such incorporation of mushroom powder into pasta can take full advantage of mushroom bioactives and could provide a good source of nutrients for humans; such a novel pasta would be beneficial for human metabolic functions and could result in larger economic benefits than traditional pasta.

In this study, pasta was made using semolina and different mushroom powders (white button, shiitake and porcini). The aim of this study was to investigate the effect of the incorporation of the individual mushroom powders on the cooking properties and textural characteristics in order to determine the possibility of producing high-quality functional foods

4.2. Materials and methods

4.2.1. Materials

As described in 3.1.1.

4.2.2. Sample preparation

Samples were prepared as described in 3.2.1.

4.2.3. Cooking properties of pasta

Cooking properties were determined as described in 3.3.1.

4.2.4. Moisture content of pasta

Moisture content was determined as described in 3.5.5.

4.2.5. Textural characteristics

Textural characteristics were determined as described in 3.4.1.

4.2.6. Statistical analysis

Statistical analysis was carried out as described in 3.12.

4.3. Results and discussion

4.3.1. Effect of mushroom powder on the cooking property of pasta

Cooking loss, SI and WAI were used as quality parameters to represent the cooking properties of pasta. It has been agreed by several authors that the protein content, quality and the formation of a continuous protein network are of great importance in the entrapment of starch and to produce pasta with good cooking quality (Cleary and Brennan, 2006; Pagani *et al.*, 1986). Adding fibre-rich fractions into pasta decreased protein contents (Cleary and Brennan, 2006) and negatively affected the integrity and tenacity of protein-starch network and hence pasta quality (Foschia *et al.*, 2015 b). Therefore, fibre and protein-rich food such as mushroom powders were incorporated in pasta (Fig. 4.1) to face up to this challenge.



Figure 4.1 The photo of pasta samples

From top to bottom (cooked pasta): control pasta, white button mushroom pasta, shiitake mushroom pasta and porcini mushroom pasta. From left to right (except first row): 5 %, 10 % and 15 % mushroom powder substitution levels.

Mushroom-supplemented pasta showed a significant increase in CL compared with the control

pasta, with the increase in CL as the mushroom powder content increases (Table 4.1). The CL values of the supplemented pasta (5.14 g per 100 g) were higher than those observed for the control sample (2.51 g per 100 g). All the CL samples were below 8 g per 100 g, the value above which pasta quality was considered unacceptable (Foschia *et al.*, 2015 a). The higher CL in pasta with added mushroom could be attributed to a loss of continuity of the pasta protein-starch matrix, as a consequence of the competitive hydration tendency of the fibre which leads to uneven distribution of water within the matrix (Tudorică *et al.*, 2002). Similarly, (Kaur *et al.*, 2013) studied the effect of button mushrooms on the leaching of solids from pasta and reported that the solids that leached into the cooking water increased as the level of mushroom powder was increased in the blend. These results were in agreement with those from Bahnassey and Khan (1986) and Zhao *et al.* (2005), who reported an increase in CL of spaghetti containing 5–30 % pea and bean inclusion. In contrast to this study, El-Shatanovi *et al.* (2000) and Rasmay *et al.* (2000) reported that macaroni containing chickpeas (5–15 %) reduced the CL of the pasta samples. Aravind *et al.* (2012 b) also obtained different results: they compared β -glucan-enriched spaghetti with a control sample and found such incorporation had no, or minimal, effect on CL of pasta. Similarly, pasta containing guar gum showed lower or similar cooking losses compared with the control (Cleary and Brennan, 2006). Both β -glucan and guar gum are soluble non-starch polysaccharides (NSPs; dietary fibres) and have been shown to form hydrated polysaccharide networks which then encapsulate the protein-starch matrix (Brennan and Tudorică, 2007). The structural integrity of the pasta may be strengthened by the encasing action, thus preventing further material losses. As shown in Table 4.1, pasta with white button mushroom powder always had a higher CL value than the

shiitake or porcini mushroom powders at the equivalent replacement value (although this was not significant at the 5 % level). This result may be due to the different ratios of soluble and insoluble dietary fibres in the different mushroom species. Additional research is being conducted to establish this hypothesis.

Table 4.1 Cooking properties and moisture content of three species mushroom pasta White button mushroom pasta (WBP); shiitake mushroom pasta (SP) and porcini mushroom pasta (PP)

Sample	Cooking loss (g/100 g)	Swelling index (g water/g dry pasta)	Water absorption index (g/100 g)	Moisture %
Control	2.51 ± 0.00077 ^f	1.83 ± 0.054 ^{ab}	82.46 ± 3.71 ^a	64.68 ± 0.0067 ^a
5 % WBP	3.13 ± 0.0016 ^{de}	1.85 ± 0.084 ^a	81.49 ± 3.18 ^a	64.91 ± 0.010 ^a
10 % WBP	3.76 ± 0.0015 ^{bc}	1.85 ± 0.031 ^a	78.76 ± 0.94 ^a	64.89 ± 0.0039 ^a
15 % WBP	5.14 ± 0.0015 ^a	1.86 ± 0.059 ^a	77.13 ± 1.88 ^a	65.02 ± 0.0072 ^a
5 % SP	2.74 ± 0.00012 ^{ef}	1.80 ± 0.033 ^{ab}	80.47 ± 1.57 ^a	64.24 ± 0.0042 ^a
10 % SP	3.33 ± 0.0019 ^{cd}	1.83 ± 0.034 ^{ab}	80.56 ± 2.29 ^a	64.60 ± 0.0042 ^a
15 % SP	4.12 ± 0.0019 ^b	1.87 ± 0.058 ^a	81.54 ± 3.22 ^a	65.17 ± 0.0070 ^a
5 % PP	2.7 ± 0.0027 ^{ef}	1.65 ± 0.0063 ^c	68.76 ± 1.48 ^b	59.30 ± 0.0037 ^c
10 % PP	3.13 ± 0.0019 ^{de}	1.62 ± 0.0023 ^c	66.41 ± 0.40 ^b	61.88 ± 0.00034 ^b
15 % PP	3.99 ± 0.0013 ^b	1.7 ± 0.034 ^{bc}	66.29 ± 1.70 ^b	63.75 ± 0.010 ^{ab}

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$, $n = 3$)

As shown in Table 4.1, white button mushroom and shiitake mushroom pastas were not significantly different to the control for either SI or WAI, although there was a trend for lower WAI in the mushroom enriched samples compared with the control. The SI values ranged from 1.80 g water per gram dry pasta to 1.87 g water per gram dry pasta for pasta containing 5 % and 15 % shiitake mushroom, respectively. Values for WAI ranged from 77.13 g per 100 g to 82.46 g per 100 g for pasta with 15 % white button mushroom and control, respectively. Similarly, as reported by other authors, when button mushrooms were added into unsteamed and steamed pasta, there were no significant differences at either a 6 % or 8 % substitution with respect to WAI (Kaur *et al.*, 2013). Psyllium-derived mucilloids were added

to wheat flour to manufacture pasta and such incorporation did not differ from the control sample for WAI (Czuchajowska *et al.*, 1992). More recently, Aravind *et al.* (2012 d) found that pasta water absorption was unaffected by FH-D inulin (Degree of polymerisation, 12–13; crystallinity, 48 %). It has been shown that 7.5 % β -glucan had no, or minimal, effect on pasta water absorption compared with the control (Aravind *et al.*, 2012 a, b). Furthermore, in terms of SI, similar results were reported by Tudorică *et al.* (2002), showing that the SI values of all cooked pasta with added fibres (pea, inulin and guar) were not significantly different from the control except for guar at 10 % inclusion.

Foschia *et al.* (2015 a) reported that the substitution of semolina flour with dietary fibre caused a significant increase in WAI. The potential of using cereal brans (wheat, rice, barley and oats) in the preparation of fibre-enriched pasta has also been explored by Kaur *et al.* (2012) who illustrated that beyond a 5 % bran substituent level, there was a significant increase in water absorption by all supplemented pastas on cooking compared with the control. It has been shown previously that WAI is related to the ability of starch to disperse excess moisture, and the WAI increases due to the increased degree of starch gelatinisation and fragmentation of the protein starch matrix within the product (Brennan *et al.*, 2004; Brennan *et al.*, 2012 b). As such, these disruptions in the protein matrix by fibre could promote water absorption and expose the starch granules to potential swelling and rupture (Manthey *et al.*, 2004; Sosulski and Wu, 1988). It was reported that the SI of pasta with a barley β -glucan fibre fraction addition was significantly higher than that of the control (Cleary and Brennan, 2006). Taken together, the higher WAI and SI values obtained for the

pasta containing certain fibre fractions may be explained by the higher capacity of the enriched fibre to absorb and retain water within a very well developed starch-protein-polysaccharide network in comparison with the white button and shiitake mushroom fibres used in this study. In addition, one can speculate that the results of the WAI suggested that the starch in the white button and shiitake mushroom-supplemented pasta may be less gelatinised, or fragmented, during pasta cooking compared with the starch in these products mentioned above.

Interestingly, this study also found out that both the SI and WAI values for porcini mushroom pasta decreased compared with the control pasta (Table 4.1). It is likely that fibre was competing with starch for water during pasta formation, thus reducing starch swelling and, consequently, water absorption (Foschia *et al.*, 2015 a). One study that incorporated mushroom coproduct materials into extruded products showed a negative correlation existed between the amount of total dietary fibre and the WAI values (Brennan *et al.*, 2012 b). The differences in WAI values between the different mushroom powders utilised in this research could be due to the structural difference or to the different particle size of fibres (Foschia *et al.*, 2015 a).

In terms of moisture content, both white button and shiitake mushroom cooked pasta (from 5 % to 15 % substitution level) showed no significant difference with the control sample, while for porcini mushroom cooked samples, all of them represent a significant decrease compared with the control. The moisture values increased with increasing porcini mushroom

powder content (Table 4.1). These results are consistent with their SI and WAI values.

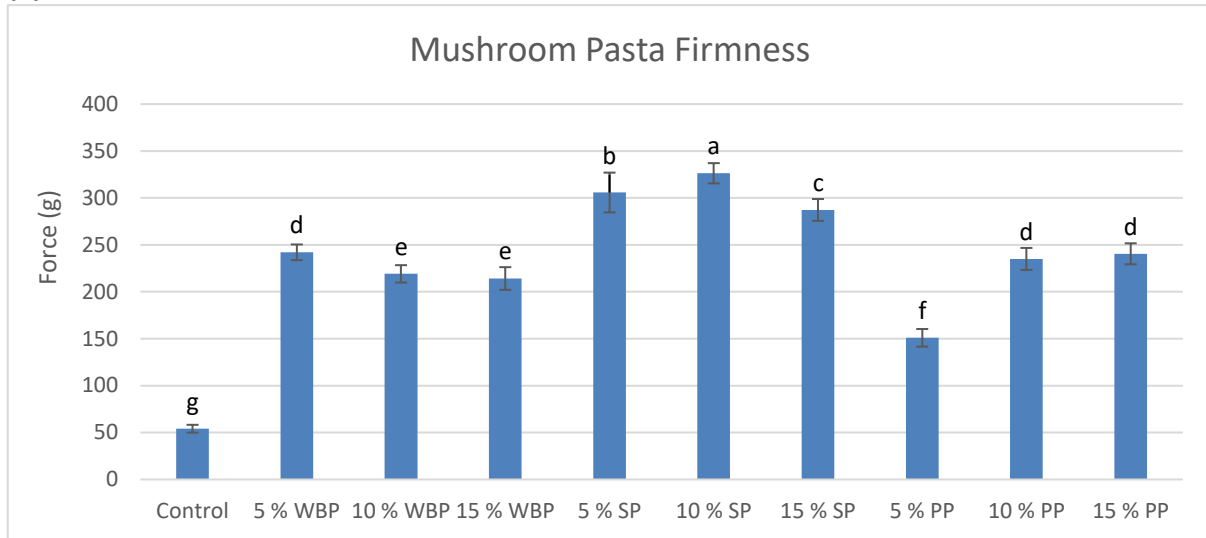
4.3.2. Effect of mushroom powder on the textural properties of pasta

The textural properties of cooked pasta, as evaluated by the texture analyser, are summarised in Fig. 4.2a and 4.2b, which show the firmness and tension properties of the three species of mushroom-enriched pasta, respectively. Substitution of semolina with mushroom powder at all levels (5 %, 10 % and 15 %) led to significant increases in firmness compared with the control. For white button mushroom, the firmness peaked at 5 % inclusion and then declined. For shiitake mushroom, the highest firmness was recorded at the 10 % inclusion level. For porcini mushroom, the firmness value increased gradually with the increasing porcini mushroom powder content. (Corey *et al.*, 2009) reported that bread firmness increased after a mushroom powder substitution. In addition, (Ulziijargal *et al.*, 2013) demonstrated that the addition of mushroom mycelia did not show consistent results on the hardness of fresh bread. Previous studies have indicated that an addition of fibre may increase the hardness of extruded snacks due to increased water retention, leading to denser, heavier and harder products (Brennan *et al.*, 2013 a). Furthermore, the significantly increased firmness might be related to nonpolar lipids (originating from the added ingredients) interacting with starch and lowering starch granule disruption as the lipids become bound to the granules; in this way, pasta could achieve a firm starch gel and hence a firmer product (Aravind *et al.*, 2012 b). However, some studies came to the opposite conclusion. For example, the enrichment of extruded snack products with coproducts from

chestnut mushrooms also resulted in a decrease in hardness in the products with increasing mushroom inclusion rate (Brennan *et al.*, 2012 b); similarly, the integration of β -glucan fibre-rich fractions from barley and mushrooms showed a decrease in hardness in the extruded snacks compared with the control sample (Brennan *et al.*, 2013 a). Reduction in pasta firmness may also be associated with the role that insoluble fibre plays by interfering with the continuity of the gluten matrix within the pasta microstructure as previously suggested by Aravind *et al.* (2012 b). Generally, pasta firmness is determined by the physical competition between protein coagulation in a continuous network and starch swelling with exudate losses during cooking. In this study, the former prevailed, so starch particles were encased in the network alveoli, and thus, cooked pastas obtained increased firmness (Foschia *et al.*, 2015 a).

After the addition of white button or porcini mushroom powders, the tensile strength (except for the 5 % level) of the pasta decreased significantly compared with the control ($p < 0.05$) and values decreased gradually with increasing substituent levels. For the shiitake mushroom pasta, values showed no significant differences to the control. Studies of previous literature revealed variable results. According to the study by Foschia *et al.* (2015 a), the maximum breaking strength and distance values of pasta samples decreased significantly due to the addition of dietary fibres. As a NSP, locust bean gum addition yielded pasta with varying elastic properties, while most of the other NSPs used caused a slight decrease in pasta elasticity. However, xanthan gum was able to increase the elasticity values in

(a)



(b)

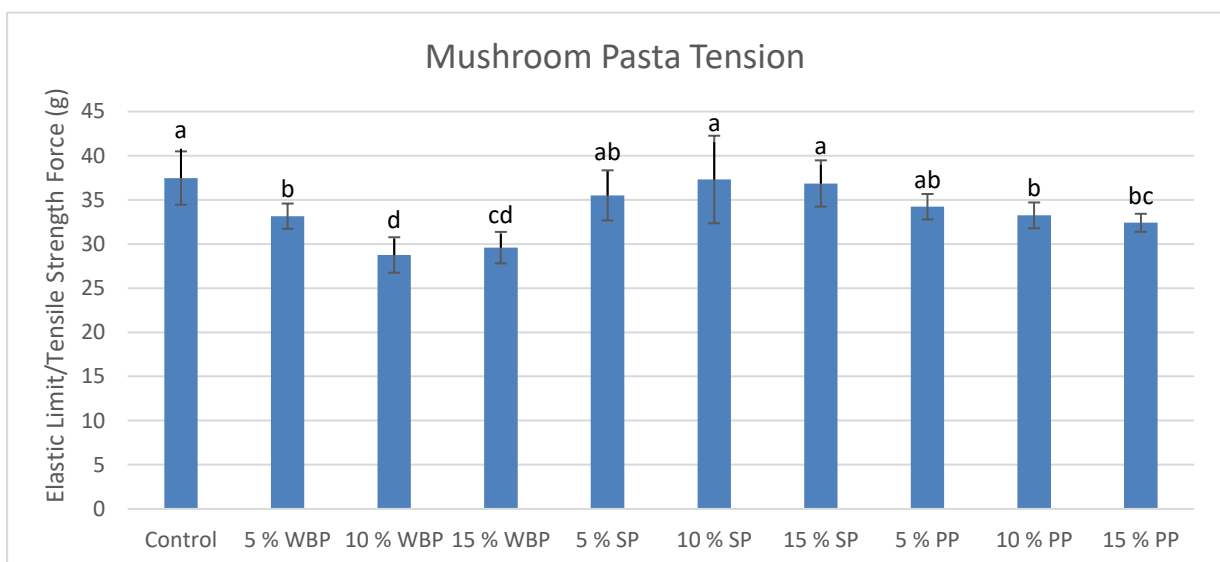


Figure 4.2 Texture properties of the three species of mushroom-enriched pasta White button mushroom pasta (WBP); shiitake mushroom pasta (SP) and porcini mushroom pasta (PP). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$, $n = 12$).

comparison with the control by strengthening the structure of pasta (Brennan and Tudorică, 2007).

From the above, it can be seen that the textural parameters of cooked pasta are affected by the specific composition of mushroom powder (both the type and level), as well as the

different types of fibre, proteins, lipids, gums and even their molecular weight. For these supplementary ingredients, some small differences can lead to very different textures in the products.

4.4. Conclusions

The results of this study illustrated that mushroom powder can be incorporated into pasta in order to obtain a product enriched with dietary fibre, protein and other bioactive ingredients. However, supplementation of mushroom powder in pasta has some negative effects on cooking and textural properties, namely increased CL and firmness.

Further work is required to identify the main composition of different mushroom powders, and what role each of these components (especially soluble and insoluble fibre) played in pasta quality. In addition, nutrition and sensory analysis of this novel functional pasta should be conducted, in order to analyse the contribution of mushroom to the nutritional values and to determine the relationship between instrumental textural analysis and sensory perception.

Chapter 5

Mushroom material enhanced the antioxidant content and modulated the predictive glycaemic response of pasta

(Submitted to Food Chemistry)

Abstract

The use of bioactive ingredients to improve the physical and nutritional quality of pasta. This study reported the effect of the addition of mushroom powder on the nutritional properties, the predictive *in vitro* glycaemic response and antioxidant potential of durum wheat pasta. Mushroom material enriched the source of protein, soluble and insoluble dietary fibre compared with durum wheat semolina. Incorporation of mushroom powder significantly decreased the extent of starch degradation and the area under the curve (AUC) of reducing sugars released during the digestion of pasta, while increased the total phenolic content and antioxidant capacities of samples. What was interesting was that a mutual inhibition system existed between the starch gelatinisation degree and antioxidant capacity of pasta samples. The results suggested that mushroom powder could be incorporated into fresh semolina pasta for conferring healthier characteristics, namely lowering the potential glycaemic response and improving the antioxidant capabilities of pasta.

5.1. Introduction

While durum wheat semolina is the traditional ingredient used to produce pasta, recent

research has focussed on using bioactive ingredients to improve the physical and nutritional quality of pasta (Foschia *et al.*, 2015 b). Much of this interest has been related to the manipulation of the glycaemic index of food materials. The glycaemic index (GI) of food is a measure of the rate at which carbohydrates in food are converted into sugar components, and how these foods affect the postprandial blood glucose responses (Foschia *et al.*, 2015 b). Clinical research has shown a correlation between low GI diets in diabetes, and the manipulation of hyperlipidaemia and cardiovascular diseases (Dona *et al.*, 2010). Researchers have proposed that highly digestible starchy foods affect the satiety levels of individuals and can increase the tendency to snack between meals (Dona *et al.*, 2010). Conversely, foods which are considered to be low-GI have been shown to have an effect in prolonging the feeling of satiety and improving insulin sensitivity (Chillo *et al.*, 2011 a). Pasta is an important starchy, staple food widely consumed around the world and is considered to be a low GI food due to a slow rate of starch degradation following ingestion (Foschia *et al.*, 2015 b).

The main component of pasta is starch and many studies have used dietary fibre and protein materials to enhance the nutritional quality of pasta. These ingredients include wheat bran (Sobota *et al.*, 2015), inulin, β -glucan, guar gum or bamboo fibre (Chillo *et al.*, 2011 b; Foschia *et al.*, 2015 b). However, to the author's knowledge, little work has been undertaken about the substitution of semolina with mushroom powders to produce pasta. Generally, mushroom powder is a rich source of protein and dietary fibre compared with semolina and, while mushrooms may contain more fat than semolina, 75 % of this is in the form of polyunsaturated fatty acids. Additionally, mushrooms contain bioactive components that have been reported

to be effective antioxidants, especially the phenolic compounds and polysaccharides (Cheung, 2008).

Many attempts have been made to mimic digestion using *in vitro* methods in an effort to model its complexities, these have included studies on the evaluation of the glycaemic response of pasta. While little work has been undertaken using mushroom pasta, in this study, the potential GI of pasta with the addition of three different substitution levels mushroom powder (0, 5 %, 10 % and 15 %) were evaluated using an *in vitro* model system, as described by Gao *et al.*, 2016).

5.2. Materials and methods

5.2.1. Materials

As described in 3.1.1.

5.2.2. Pasta preparation

Pasta samples were prepared as described in 3.2.1.

5.2.3. Proximal analysis

The total starch content was determined as described in 3.5.1.; the fibre content was determined as described in 3.5.2.; the protein content was determined as described in 3.5.3. and the fat content was determined as described in 3.5.4.

5.2.4. Near infrared spectroscopy

The analysis of near infrared spectroscopy was determined as described in 3.7.

5.2.5. Differential scanning calorimetry (DSC)

The DSC analysis was determined as described in 3.8.

5.2.6. *In vitro* starch digestion analysis

Frozen pasta (20 g) was defrosted for 10 min at room temperature and cooked for 6 min in boiling tap water (600 mL). The pasta was then drained and cut with a knife in order to obtain a 2–5 mm size (Foschia *et al.*, 2015 b).

The digestion was carried out as described in 3.9.

5.2.7. Antioxidant analysis

The antioxidant analysis was determined as described in 3.10.

5.2.8. Microstructure

The microstructure analysis of the both longitudinal surface and transverse cross section of raw pasta samples was evaluated by scanning electron microscope (SEM) using 500 times magnification level. The analysis of microstructure was determined as described in 3.11.

5.2.9. Statistical analysis

Statistical analysis was carried out as described in 3.12.

5.3. Results and discussion

5.3.1. Nutritional composition

Previously we investigated the role of mushroom material on the physical characteristics of pastas (Lu *et al.*, 2016); the current research extends this to evaluate the nutritional enhancement of pasta from mushroom additions. Table 5.1 shows the nutritional composition of uncooked semolina and mushroom powder, as well as the cooked pasta samples. The dietary fibre (soluble, insoluble and total dietary fibre), protein and fat contents of all the three

kinds of mushroom powder (white button, shiitake and porcini mushroom) were significantly higher than that of durum wheat semolina (Table 5.1a). Table 5.1b shows that incorporation of mushroom powder significantly increased the soluble, insoluble and total dietary fibre contents of the pasta samples. These results are in agreement with those of Cheung (2008), who reported the values at between 4.5–54.5 % dietary fibre, 19–35 % protein and less than 10 % fat by dry weight in mushroom. Table 5.1a illustrates that even though the total fibre content of semolina was much lower than mushroom powder, the ratio of soluble dietary fibre to total dietary fibre was much higher than that of mushroom powder. During cooking, part of soluble dietary fibre may be dissolved in the boiling water, thus the soluble dietary fibre ratio to total dietary fibre in control pasta decreased compared to raw semolina (Table 5.1b).

Compared to the semolina the three kinds of mushroom powder had significantly lower starch contents (Table 5.1a). All mushroom-supplemented pasta showed a decrease in total starch content (except for the 5 % porcini mushroom), with no significant differences between the control sample and the 5 % white button, 5 % shiitake and 10 % porcini mushroom-supplemented pasta (Table 5.1b). The total starch content of pasta generally decreased with increasing mushroom powder content. Furthermore, supplementary Table 5.1 shows strong negative correlations between the starch content and IDF, SDF and TDF ($p \leq 0.001$). Significant positive correlations (Table 5.2) were observed between fibre content (IDF, SDF and TDF) and both SI and WAI, except SDF and WAI. This may be because the high fibre content enhanced the water-holding capacity of the pasta, which was consistent with the strong positive correlations between moisture content (oven method) with both SI and WAI. Interestingly,

Table 5.1 The nutrient components of durum wheat semolina, mushroom powder and cooked pasta samples

(a)

Sample	Starch g/100 g (Dry basis)	IDF g/100 g (Dry basis)	SDF g/100 g (Dry basis)	TDF g/100 g (Dry basis)	Protein g/100 g (Dry basis)	Fat g/100 g (Dry basis)	Moisture g/100 g
S	73.11 ± 1.17 ^a	2.36 ± 0.12 ^c	1.59 ± 0.32 ^b	3.95 ± 0.44 ^c	11.32 ± 0.28 ^d	1.17 ± 0.09 ^d	13.95 ± 0.17 ^a
WBM	1.90 ± 0.02 ^b	21.58 ± 2.47 ^b	3.02 ± 0.13 ^a	24.61 ± 2.34 ^b	30.52 ± 0.13 ^a	2.42 ± 0.04 ^b	11.05 ± 0.16 ^b
SM	2.45 ± 0.14 ^b	38.85 ± 1.09 ^a	3.12 ± 0.01 ^a	41.97 ± 1.08 ^a	15.04 ± 0.04 ^c	1.48 ± 0.02 ^c	10.31 ± 1.10 ^b
PM	2.62 ± 0.08 ^b	23.12 ± 1.15 ^b	3.14 ± 0.02 ^a	26.26 ± 1.13 ^b	23.83 ± 0.19 ^b	3.03 ± 0.03 ^a	11.70 ± 0.58 ^b

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$, $n = 3$). Abbreviations: S=semolina; WBM=white button mushroom; SM=shiitake mushroom; PM=porcini mushroom; IDF=insoluble dietary fibre; SDF=soluble dietary fibre; TDF=total dietary fibre.

(b)

Sample	Total starch g/100 g (dry basis)	IDF g/100 g (dry basis)	SDF g/100 g (dry basis)	TDF g/100 g (dry basis)
CP	69.95 ± 1.58 ^{ab}	2.10 ± 0.02 ^d	1.07 ± 0.04 ^e	3.17 ± 0.02 ^e
5 % WBP	69.11 ± 0.50 ^{abc}	4.02 ± 0.18 ^{bcd}	2.03 ± 0.08 ^{abc}	6.04 ± 0.11 ^{abcd}
10 % WBP	65.10 ± 1.07 ^{def}	4.75 ± 0.27 ^{abc}	2.14 ± 0.05 ^{ab}	6.89 ± 0.32 ^{abc}
15 % WBP	63.40 ± 1.18 ^{ef}	5.49 ± 0.79 ^{ab}	2.63 ± 0.32 ^a	8.13 ± 1.11 ^a
5 % SP	67.90 ± 1.56 ^{bcd}	3.61 ± 0.44 ^{bcd}	1.55 ± 0.094 ^{bcde}	5.16 ± 0.54 ^{bcde}
10 % SP	64.05 ± 1.16 ^{ef}	5.39 ± 0.63 ^{ab}	1.77 ± 0.073 ^{bcde}	7.16 ± 0.56 ^{ab}
15 % SP	62.65 ± 0.26 ^f	6.24 ± 0.088 ^a	1.81 ± 0.072 ^{bcd}	8.04 ± 0.017 ^a
5 % PP	71.61 ± 0.43 ^a	2.95 ± 0.89 ^{cd}	1.23 ± 0.14 ^{de}	4.18 ± 1.03 ^{de}
10 % PP	68.68 ± 0.25 ^{bc}	3.17 ± 0.75 ^{cd}	1.41 ± 0.10 ^{cde}	4.58 ± 0.85 ^{cde}
15 % PP	66.26 ± 0.94 ^{cde}	4.05 ± 0.19 ^{bcd}	1.98 ± 0.42 ^{abc}	6.03 ± 0.23 ^{abcd}

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$, $n = 3$). Abbreviations: CP=control pasta; WBP=white button pasta; SP=shiitake pasta; PP=porcini pasta; IDF=insoluble dietary fibre; SDF=soluble dietary fibre; TDF=total dietary fibre.

there were significant negative correlations existing between water content (NIR method) and both SI and WAI, thus suggesting these two techniques, including oven and NIR method, indeed measured water with different characteristics within the cooked pasta matrix.

5.3.2. Near-infrared spectroscopy (NIR)

Table 5.3 shows the results of NIR analysis, including the calorie, protein, fat, carbohydrate and water contents of all cooked pasta samples. In terms of white button and shiitake mushroom pasta, both 10 % and 15 % substituent levels increased the calorie contents significantly. For porcini mushroom pasta, both 5 % and 10 % substituent levels reduced the calorie content significantly compared with the control. Significant positive correlations were observed between the energy content and fat content ($r = 0.902$; $p \leq 0.001$), IDF ($r = 0.755$; $p \leq 0.001$), SDF ($r = 0.695$; $p \leq 0.001$), and TDF ($r = 0.784$; $p \leq 0.001$). Table 5.2 also illustrates negative correlations between energy content and carbohydrate, water content (NIR method) and starch content ($r = -0.611$, -0.721 and -0.811 , respectively; $p \leq 0.001$). Interestingly there were positive correlations between energy content and ORAC ($r = 0.363$, $p \leq 0.05$). Even though inclusion of some mushroom powder increased the calorie content of pasta products to some extent, such an increase may be associated with some other health properties of final products positively, including the dietary fibre supplementation and antioxidant activity.

Table 5.2 Pearson's correlation coefficient (r) of physicochemical and nutritional properties of pasta

	SI	WAI	MS	FN	ES	TPC	ORAC	DPPH	IDF	SDF	TDF	ST	AUC	EN	PT	FT	CH	WT	HR	HC	
CL	0.488**	0.216	0.492**	0.343	-0.424*	0.345	0.754***	0.647***	0.702***	0.811***	0.775***	-0.772***	-0.593***	0.708***	0.375*	0.660***	-0.584***	-0.28	0.620***	0.362*	
SI		0.945***	0.999***	0.211	0.044	-0.309	-0.024	-0.051	0.507**	0.487**	0.533**	-0.599***	0.2	0.701***	0.253	0.488**	-0.261	-0.661***	0.022	-0.241	
WAI			0.944***	0.157	0.239	-0.519**	-0.313	-0.326	0.373*	0.267	0.366*	-0.451*	0.444*	0.592***	0.144	0.405*	-0.151	-0.716***	-0.167	-0.393*	
MS				0.223	0.044	-0.289	-0.025	-0.032	0.501**	0.488**	0.528**	-0.603***	0.204	0.7***	0.264	0.481**	-0.262	-0.660***	0.027	-0.233	
FN					0.229	0.068	0.19	0.135	0.658***	0.348	0.611***	-0.612***	-0.207	0.402*	0.222	0.478**	-0.468**	0.051	0.459*	0.195	
ES						-0.411*	-0.691***	-0.554**	-0.017	-0.514**	-0.158	0.007	0.532**	0.039	-0.279	0.027	0.216	-0.209	-0.026	-0.03	
TPC							0.536**	0.862***	-0.117	0.134	-0.053	0.037	-0.684***	-0.296	0.172	-0.352	0.04	0.581***	0.436*	0.734***	
ORAC								0.742***	0.419*	0.730***	0.532**	-0.433*	-0.742***	0.363*	0.367*	0.384*	-0.495**	0.119	0.476**	0.386*	
DPPH									0.143	0.407*	0.226	-0.292	-0.717***	0.043	0.291	-0.007	-0.211	0.309	0.573***	0.539**	
IDF										0.713***	0.980***	-0.85***	-0.393*	0.755***	0.119	0.814***	-0.529**	-0.259	0.514**	0.121	
SDF											0.837***	-0.66***	-0.428*	0.695***	0.45*	0.619***	-0.592***	-0.251	0.255	0.034	
TDF												-0.849***	-0.427*	0.784***	0.219	0.809***	-0.579***	-0.273	0.472**	0.104	
ST													0.317	-0.811***	-0.174	-0.819***	0.524**	0.419*	-0.569***	-0.147	
AUC														-0.024	-0.106	-0.124	0.246	-0.448*	-0.587***	-0.576***	
EN															0.328	0.902***	-0.611***	-0.721***	0.302	-0.071	
PT																0.171	-0.773***	-0.261	-0.041	-0.176	
FT																	-0.677***	-0.621***	0.430*	-0.025	
CH																				0.091	
WT																				0.104	0.36
HR																					0.645***

CL, Cooking Loss (g/100 g); SI, Swelling Index (g water/g dry pasta); WAI, Water Absorption Index (g/100 g); MS, Moisture % (oven method); FN, Firmness (force g); ES, Elasticity (Elastic Limit/Tensile Strength Force g); TPC, mg GAE/g DM pasta; ORAC, $\mu\text{M TE/g DM}$ pasta; DPPH, $\mu\text{M TE/g DM}$; IDF, Insoluble dietary fibre %; SDF, Soluble dietary fibre %; TDF, Total dietary fibre %; ST, Starch %; AUC, In vitro area under the curve value; EN, Energy (kcal); PT, Protein %; FT, Fat %; CH, Carbohydrate %; WT, Water % (NIR method); HR, $\Delta\text{H J/g raw pasta}$; HC, $\Delta\text{H J/g cooked pasta}$. *, significant at $p \leq 0.05$. **, significant at $p \leq 0.01$. ***, significant at $p \leq 0.001$.

Table 5.3 The nutritional and energy contents of the control and three species of mushroom-enriched cooked pasta

Sample	Energy kcal	Protein g/100 g	Fat g/100 g	Carbohydrate g/100 g	Water g/100 g
CP	377.33 ± 0.58 ^c	6.23 ± 0.23 ^{bc}	2.93 ± 0.12 ^{ef}	81.47 ± 0.31 ^a	3.5 ± 0.17 ^e
5 % WBP	377.67 ± 0.58 ^c	7.37 ± 0.40 ^{ab}	2.93 ± 0.058 ^{ef}	80.33 ± 0.31 ^{ab}	4.33 ± 0.058 ^{bcd}
10 % WBP	380 ± 0 ^b	6.9 ± 0.56 ^{abc}	3.53 ± 0.29 ^{bc}	80.13 ± 1.14 ^{abc}	4.17 ± 0.47 ^{cde}
15 % WBP	383.67 ± 1.15 ^a	7.83 ± 0.57 ^a	4.23 ± 0.15 ^a	78.5 ± 0.44 ^c	3.53 ± 0.31 ^e
5 % SP	378 ± 0 ^c	7.13 ± 0.32 ^{ab}	3.4 ± 0 ^{cd}	79.73 ± 0.38 ^{bc}	4.03 ± 0.15 ^{cde}
10 % SP	382.33 ± 0.58 ^a	7.13 ± 0.32 ^{ab}	3.87 ± 0.12 ^{ab}	79.63 ± 0.23 ^{bc}	3.73 ± 0.21 ^{de}
15 % SP	380.33 ± 0.58 ^b	5.77 ± 0.12 ^c	3.93 ± 0.12 ^a	80.47 ± 0.15 ^{ab}	4.2 ± 0.1 ^{bcd}
5 % PP	374.33 ± 0.58 ^d	6.13 ± 0.32 ^{bc}	3.03 ± 0.058 ^{de}	80.67 ± 0.51 ^{ab}	4.9 ± 0.1 ^{ab}
10 % PP	374 ± 0 ^d	7.13 ± 0.31 ^{ab}	2.57 ± 0.058 ^f	80.5 ± 0.53 ^{ab}	5.2 ± 0.26 ^a
15 % PP	378.67 ± 1.15 ^{bc}	6.9 ± 0.87 ^{abc}	3.23 ± 0.15 ^{cde}	80.37 ± 1.02 ^{ab}	4.5 ± 0.35 ^{abc}

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$, $n = 3$). Abbreviations: CP=control pasta; WBP=white button pasta; SP=shiitake pasta; PP=porcini pasta.

5.3.3. Thermal properties

Table 5.4 represents the melting enthalpy (ΔH) in all raw (Table 5.4a) and cooked (Table 5.4b) pasta samples and indicates that addition of mushroom powder increased the ΔH values slightly and incrementally with the increased substituent level compared to the control. This finding meant such inclusion reduced the gelatinised or dextrinised degree of starch granules during the both cold extrusion and cooking process of pasta (Parada *et al.*, 2011). Table 5.2 demonstrates strong positive correlations between ΔH of raw pasta and both IDF, TDF and fat content, while negative correlation existed between ΔH of raw pasta and total starch content. However, no significant correlation was observed between ΔH of raw pasta and SDF, or between ΔH of cooked pasta and all IDF, SDF, TDF, fat and total starch content. What is of added interest is that there were positive correlations between ΔH of both raw and cooked pasta and TPC ($r = 0.436$ and 0.734 , respectively; $p \leq 0.05$ and 0.001 , respectively), the results suggest

that the mushroom fibre (especially IDF) and fat played protective roles in reducing the amount of starch gelatinisation, and the TPC of mushroom exhibited same effect on both raw and cooked pasta. This in turn inhibited the enzyme accessibility to starch granules within the pasta matrix, thus reduced the release of reducing sugars during starch digestion. Certainly, Table 5.2 further illustrates strong negative correlations between ΔH of both raw and cooked pasta and AUC.

Table 5.4 Thermal properties (DSC measurements) for raw and cooked pasta

(a)

Raw pasta	T onset °C	T gelatinisation °C	T endset °C	ΔH J/g	ΔTr °C
CP	58.52 ± 1.69	65.02 ± 1.36	68.36 ± 0.79	3.31 ± 0.77	9.85 ± 0.90
5 % WBP	56.02 ± 1.24	63.16 ± 2.42	68.35 ± 1.22	2.96 ± 0.40	12.33 ± 0.01
10 % WBP	54.29 ± 0.62	62.91 ± 0.62	69.03 ± 0.23	3.97 ± 0.36	14.75 ± 0.84
15 % WBP	55.07 ± 0.73	63.8 ± 0.66	71.81 ± 1.39	4.83 ± 0.16	16.75 ± 2.11
5 % SP	55.72 ± 0.64	63.39 ± 0.75	69.26 ± 1.09	3.66 ± 0.59	13.55 ± 0.45
10 % SP	54.2 ± 1.94	62.8 ± 0.23	69.68 ± 1.43	4.46 ± 0.21	15.48 ± 3.37
15 % SP	55.32 ± 0.47	62.04 ± 0.51	70.76 ± 1.32	5.51 ± 1.65	15.44 ± 1.79
5 % PP	57.19 ± 0.93	65.57 ± 0.33	69.13 ± 1.03	3.94 ± 0.16	11.94 ± 1.97
10 % PP	57.23 ± 2.18	63.59 ± 2.23	69.27 ± 1.58	4.74 ± 0.56	12.04 ± 3.75
15 % PP	54.22 ± 1.12	64.58 ± 1.87	70.97 ± 1.39	5.22 ± 0.87	16.76 ± 2.51

Abbreviations: CP=control pasta; WBP=white button pasta; SP=shiitake pasta; PP=porcini pasta.

(b)

Cooked pasta	T onset °C	T gelatinisation °C	T endset °C	ΔH J/g	ΔTr °C
CP	57.87 ± 0.71	67.9 ± 0.47	72.07 ± 0.50	0.71 ± 0.09	14.2 ± 1.22
5 % WBP	56.96 ± 0.57	67.2 ± 0.84	70.91 ± 0.20	0.72 ± 0.02	13.95 ± 0.76
10 % WBP	58.15 ± 1.02	65.09 ± 2.14	71.2 ± 0.25	0.83 ± 0.09	13.05 ± 1.27
15 % WBP	57.39 ± 0.26	63.01 ± 0.05	67.81 ± 0.99	0.84 ± 0.13	10.43 ± 1.25
5 % SP	55.68 ± 0.74	63.31 ± 0.21	70.13 ± 2.19	0.63 ± 0.10	14.45 ± 2.93
10 % SP	55.69 ± 0.11	62.37 ± 0.12	69.16 ± 0.40	1.14 ± 0.03	13.48 ± 0.29
15 % SP	55.82 ± 0.73	63.68 ± 0.96	71.47 ± 0.71	1.49 ± 0.33	15.66 ± 0.02
5 % PP	58.21 ± 0.83	66.83 ± 1.07	70.6 ± 2.00	1.33 ± 0.06	12.40 ± 2.84
10 % PP	56.97 ± 1.15	66.23 ± 0.35	71.04 ± 0.38	1.24 ± 0.0016	14.07 ± 0.76
15 % PP	56.95 ± 0.69	66.08 ± 0.69	72.01 ± 0.08	2.62 ± 0.25	15.07 ± 0.60

Abbreviations: CP=control pasta; WBP=white button pasta; SP=shiitake pasta; PP=porcini pasta.

5.3.4. *In vitro* digestion of cooked pasta

An *in vitro* enzymatic digestion was performed in order to mimic the behaviour of pasta when eaten. *In vitro* digestibility values for the pasta samples are shown in Fig. 5.1a; b and c, representing an interpretation of the amount of reducing sugars released over a 120 min *in vitro* digestion of each pasta sample. It was noticeable that there was a significant difference between the control sample and the mushroom-supplemented samples. In all samples, the values of reducing sugars increased dramatically in the first 20 min and the peak values were reached between 20 min and 60 min. There were significantly more reducing sugars released from the control pasta than from all the mushroom powder supplemented pasta samples (Fig. 5.1d). The effects of the incorporation of white button, shiitake and porcini mushroom powders into durum wheat semolina pasta on standardised AUC values are shown in Fig. 5.1d. The addition of white button and porcini mushroom into pasta led to a decrease in the AUC reducing sugars levels. What was of interest was that the different substitution levels did not show any significant differences between the AUC for the same mushroom species. For shiitake mushroom pasta with the increased mushroom powder content, the AUC values decreased gradually, while there were no significant differences between the 5 % substitution level and the control. In particular, the substitution of semolina with 15 % shiitake mushroom powder in the pasta caused a major decrease in the standardised values. Furthermore, at the same addition levels, all three kinds of mushroom pasta showed no significant differences in their standardised values (except for the 5 % and 10 % shiitake mushroom pasta). Previous work has indicated that when co-products from chestnut mushroom were added to extruded snack products they restricted the amount of readily digestible carbohydrates in the extruded

samples compared with a control sample (Brennan *et al.*, 2012 b). The rate of digestion of carbohydrates present in the food controls the glycaemic impact of foods, thus high GI foods, in which the carbohydrate fractions are digested and absorbed rapidly, result in marked fluctuations in blood glucose levels (Foschia *et al.*, 2015 b). The rate, and extent, of carbohydrate digestion are governed by factors such as the structure and composition of starch, as well as the amount of fibre, protein and fat within a product (Dona *et al.*, 2010). For instance, previous research has indicated that a reduction in starch digestibility may be observed by enriching snack products with barley β -glucan (BBG) or mushroom β -glucan (Brennan *et al.*, 2013 a).

Starch can be classified into three categories: rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) (Englyst *et al.*, 1992). As shown in Fig. 5.1, the largest increase in reducing sugars released for all pasta samples occurred in the first 20 min (the RDS fraction). The SDS fraction is digested after the RDS (Englyst *et al.*, 1992). From Fig. 5.1, it is clear that there was a transition in the smoothness of the digestion curves, showing the change in reducing sugar production from RDS to SDS. For white button and shiitake mushroom pasta, the curves between 20 to 120 min were nearly horizontal, while porcini mushroom pasta reflected an upward trend between 20 to 60 min, the growth trend was much slower than that of the first 20 min. This indicates the possibility of different SDS contents in the three mushroom species, although further work needs to be conducted to establish the exact relationship of RDS and SDS to sugar release in these samples.

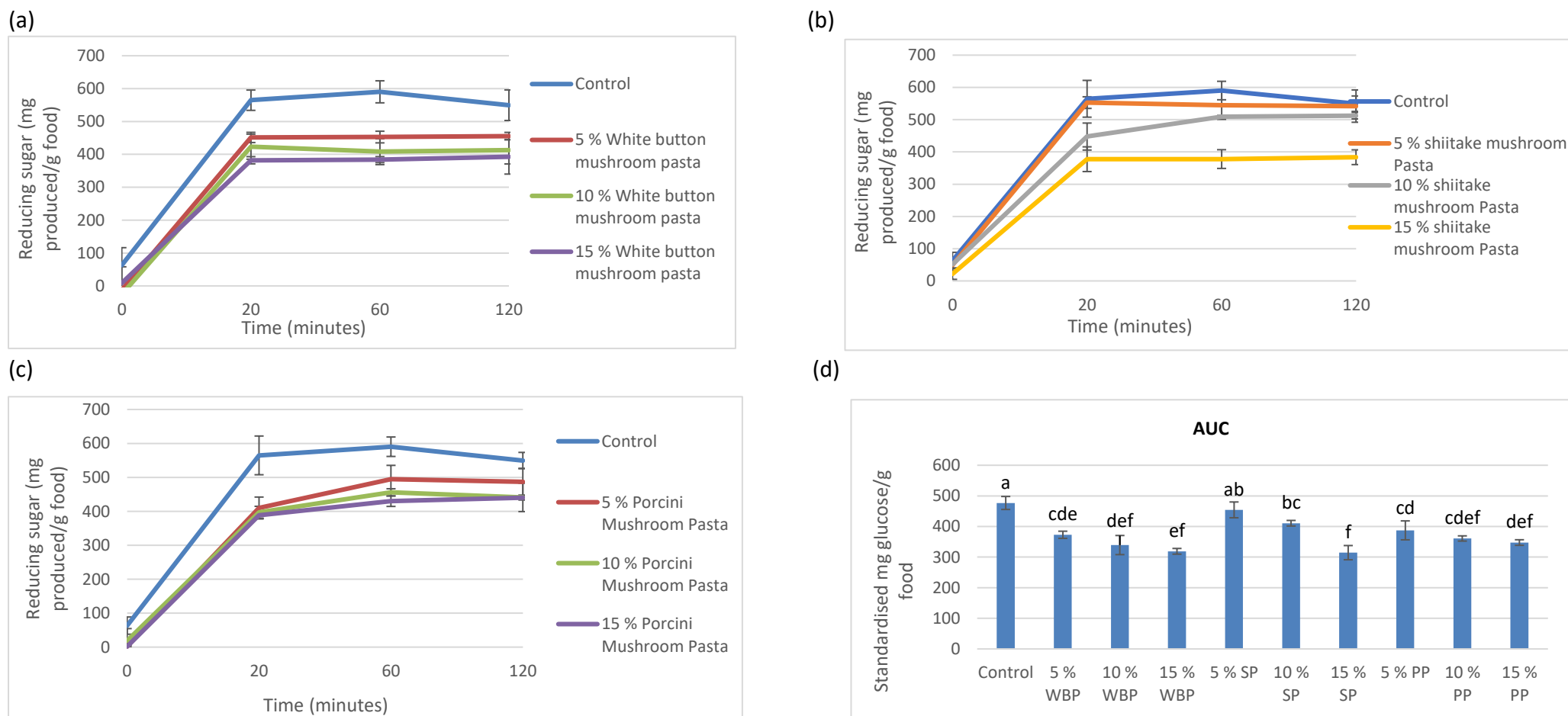


Figure 5.1 Levels of reducing sugars released during *in vitro* digestion

Comparing the control to 5 %, 10 %, 15 % white button mushroom pasta (a); shiitake mushroom pasta (b); and porcini mushroom pasta (c). Values for area under the curve (AUC) (d). Comparing the control to all mushroom powder enriched pasta samples: white button mushroom pasta (WBP); shiitake mushroom pasta (SP) and porcini mushroom pasta (PP). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$, $n = 3$).

Mushroom powder provides more dietary fibre to pasta than semolina. Previous research has illustrated the potential to lower the glycaemic response of foods by the incorporation of different dietary fibre fractions (Foschia *et al.*, 2015 b). Table 5.2 further shows the negative correlations between AUC and IDF ($r = -0.393$; $p \leq 0.05$), SDF ($r = -0.428$; $p \leq 0.05$) and TDF ($r = -0.427$; $p \leq 0.05$). Cleary and Brennan (2006) illustrated that the addition of a β -glucan fibre fraction from barley into durum wheat pasta resulted in an attenuation of reducing sugar release following an *in vitro* starch digestion. Such observations may be brought about by changes in the starch-protein matrix of pasta and the high water binding capacity of dietary fibres; both of which affect the physico-chemical properties and digestibility of the pasta. Brennan *et al.* (2013 a) integrated the β -glucan fibre-rich fractions from mushrooms to form healthy extruded snacks illustrating that the inclusion of mushroom β -glucan rich fractions reduced the potential glycaemic response of the samples compared to the control sample. The viscosity modifying effect of dietary fibres have been shown to affect the rate of gastric emptying, transition time and the intestinal absorption of nutrients, which in turn may lead to a decreased glycaemic response (Chillo *et al.*, 2011 b). Vitaglione *et al.* (2009) found a significant decrease in hunger and an increased fullness and satiety after the incorporation of β -glucan into bread. While the effect of β -glucan on the food viscosity depended on its concentration and molecular weight (Dikeman and Fahey, 2006; Wood *et al.*, 2000), the latter can also influence its digestion and physiological properties (Kerckhoffs *et al.*, 2003; Tester and Sommerville, 2003).

Even though the addition of fibre can provide many positive effects in reducing the glycaemic

response of pasta in a variety of ways, processing and cooking have been shown to significantly influence these properties and the resultant functional benefits (Chillo *et al.*, 2011 a). The structure of foods have an important part to play in the digestibility of nutrients (Dona *et al.*, 2010). Starch encapsulation by proteins, together with the complexation of starch with lipids and porosity of food structure are known to limit the extent of starch degradation (Fardet *et al.*, 1999). Cleary and Brennan (2006) proposed that structural modifications made by β -glucan to the protein-starch matrix of pasta were responsible for the lower rates of sugar released during *in vitro* digestion. The protein-starch matrix is therefore of considerable importance in terms of pasta structure and function. In this study, mushroom powder contained more protein than semolina, which may make a contribution to both the nutritional quality and the integrity of the protein network in pasta. Therefore, the higher level of protein in mushroom pasta samples may be another explanation for the result that the proportion of starch digested at different time points was significantly lower in mushroom-enriched pasta compared to semolina pasta (except for the 5 % shiitake mushroom pasta). It has previously been reported that the presence of egg white powder in pasta influenced the starch digestibility, the higher amounts of protein probably created a stronger network and, thus, reduced the availability of starch granules to digest enzymes (Hager *et al.*, 2013). Similarly, Kim *et al.* (2008 a) reported that the presence of starch-protein interactions in pasta dough may be important for reducing the digestibility of starch in pasta and that protein enrichment at a 20 % level significantly delayed the rate of dextrin release. Part of this effect of protein on starch digestion in pasta could be due to alterations of the three-dimensional structure of the protein network from protein-enriched pasta, and potential encapsulation of starch by protein

fractions reducing enzyme hydrolysis (Fardet *et al.*, 1999; Fardet *et al.*, 1998). Aravind *et al.* (2011) studied durum wheat pasta enriched with purified gluten, gluten, glutenin, gliadin, and low molecular (LMW-GS) or high molecular weight glutenin subunits (HMW-GS) isolated from durum gluten from durum wheat varieties, respectively. Inclusion of these protein fractions weakened the dough structure and the authors proposed that this in turn enabled the starch granules to become more accessible to starch degrading enzymes, hence an observation of increased GI values in the protein enriched pastas. Further research is under way with the mushroom enriched pasta samples to fully understand these mechanisms.

As mentioned above, all the three kinds of mushroom contain more fat than durum wheat semolina. While it has been proved that there was approximately 75 % of polyunsaturated fatty acids in the total mushroom fatty acids and that included 19.2 % palmitic acid, 8.3 % oleic acid and 68.8–84 % linoleic acid (Cheung, 2008). It has been illustrated that amylose-lipid complex formation resulted decreased solubility of amylose, increased gelatinisation temperature, reduced stickiness and freeze-thaw stability, retarded retrogradation and prolonged storage time (Eliasson *et al.*, 1981; Holm *et al.*, 1983; Krog, 1971). Holm *et al.* (1983) mixed amylose from potatoes with lysolecithin (palmitic acid) and oleic acid in order to study the digestibility of amylose-lipid complexes *in vitro*. The study illustrated that complexed amylose affected the rate of starch degradation due to the formation of a lysolecithin (palmitic)-complex, which could be attributed to a structural disturbance due to the double bond that rendered the oleic-acid complex more susceptible to α -amylase (Holm *et al.*, 1983). It is possible that, in our research, mushroom powder provides more fat to pasta than semolina,

and also palmitic acid and oleic acid, which could form such a complex with amylose. This effect will play a role in starch *in vitro* digestibility of mushroom pasta, and the nature of the lipids in mushroom powder can have an effect on the glycaemic response.

Finally, some components in mushroom powder (such as mushroom fibre) may regulate free and bound water and, hence, the water mobility during digestion of pasta. It has been pointed out that water enzyme mobility and concentration plays a role in the effectiveness of starch granules hydrolysis during *in vitro* digestion (Brennan *et al.*, 2012 a). As reported in our previous work (Lu *et al.*, 2016), porcini mushroom pasta had a reduced swelling index than the control. At the molecular level, the reduced degree of starch swelling due to restricted water diffusion during cooking is also believed to be responsible for the slow degradation of starch (Colonna *et al.*, 1990). A negative correlation between water content (NIR method) and AUC was found (Table 5.2). This showed that the characteristics of water in mushroom-supplemented pastas can also play a role in the glycaemic response of the samples. Additionally, in this study, even though there were more protein, fat and less starch contents in mushroom powder than semolina, no significant correlations were observed between AUC and protein, fat and starch contents. This phenomenon might attribute to some factors, including the novel NIR method; not high enough substituent level, or the cold extrusion and cooking process limited the effects of these components on the starch digestion to some extent.

5.3.5. The antioxidant capacities of semolina, mushroom and pasta samples

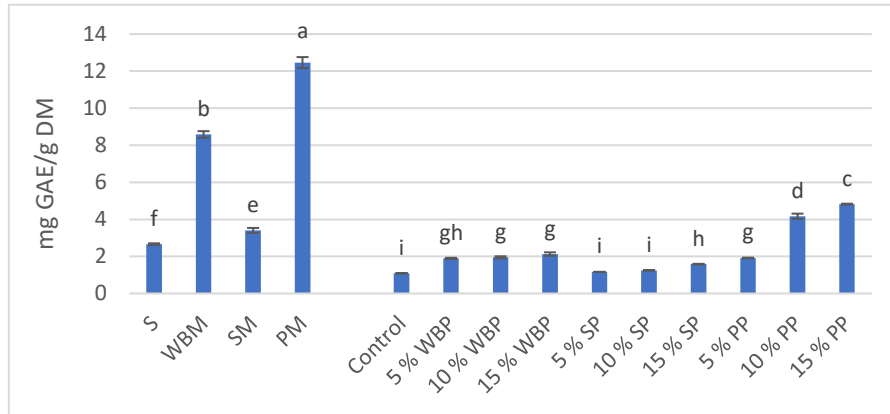
Antioxidants can quench reactive free radicals, which have health-promoting effects in the prevention from oxidative stress (Teixeira *et al.*, 2016). The antioxidant properties of food vary depending on their content of phenolic components, vitamins C and E, carotenoids and flavonoids (Saura-Calixto and Goñi, 2006). The TPC values of all the three mushrooms are significantly higher than semolina, the order is: porcini mushroom > white button mushroom > shiitake mushroom > semolina. All the mushroom pasta samples have significantly higher TPC than control pasta (except 5 % and 10 % shiitake mushroom pasta). For instance the shiitake and porcini mushroom, with the increased mushroom content showed TPC values which increased gradually (Fig. 5.2a). Fig. 5.2b and 5.2c show that the total antioxidant activity values of semolina, mushroom powder and all pasta samples followed a similar trend to the TPC values. Table 5.2 indicates that strong positive correlations existed between TPC and both DPPH and ORAC ($r = 0.862$ and 0.536 ; $p \leq 0.001$ and 0.01). ORAC values of pasta samples enriched with mushroom powder were significantly higher than the control sample. Such observations illustrate the potential of improving the oxidative radical scavenging activities of pasta with the incorporation of mushroom powder. In this study, all the pasta samples were cooked in boiling water for 6 min. Previous researchers have reported that boiling water could degrade the sensitive polyphenols or enhance the extraction of some bound polyphenols from the pasta matrices (Fares *et al.*, 2008). Interestingly Table 5.2 illustrates significant correlations between ORAC and many other components except TPC, including IDF ($r = 0.419$; $p \leq 0.05$), SDF ($r = 0.730$; $p \leq 0.001$), TDF ($r = 0.532$; $p \leq 0.01$), protein ($r = 0.367$; $p \leq 0.05$), fat ($r = 0.384$; $p \leq 0.05$) and carbohydrate ($r = -0.495$; $p \leq 0.01$). These findings pointed out that many

components (especially the SDF) mushroom powder provided to pasta could promote the absorption of oxygen radical directly or indirectly, thus enhanced the antioxidant benefits of final pasta. Additionally, *in vitro* studies and animal models have shown that antioxidants improve insulin secretion and sensitivity, and take effect on the improvements of glucose tolerance and diabetic control (Ihara *et al.*, 2000). The TPC and ORAC values indicate that pasta could be a good medium to add bioactive antioxidants to enhance human nutrition. Table 5.2 confirms the significant negative correlations between AUC and TPC ($r = -0.684$; $p \leq 0.001$), DPPH ($r = -0.717$; $p \leq 0.001$) and ORAC ($r = -0.742$; $p \leq 0.001$), suggesting that there was a mutual inhibition system between the antioxidant capacity and starch digestion of pasta products, which could be also supported by the negative correlation between ORAC and starch content ($r = -0.433$; $p \leq 0.05$).

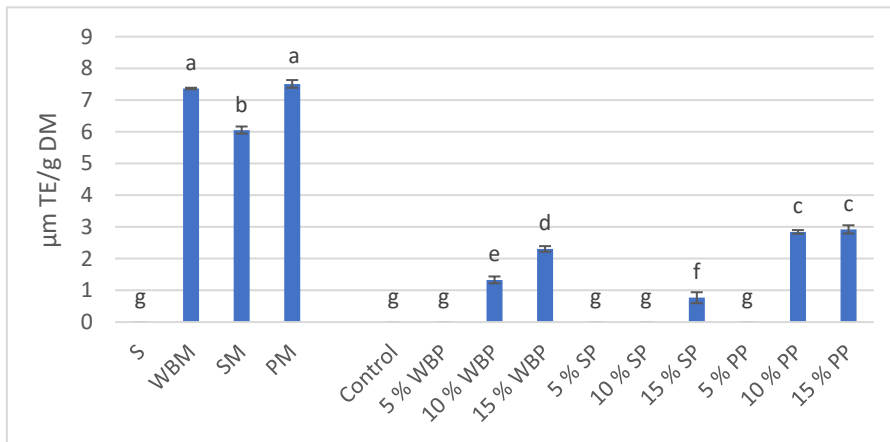
5.3.6. Microstructure

Scanning electron microscopy (SEM) was used to investigate the structure of both longitudinal surface (Fig. 5.3a) and transverse cross sections (Fig. 5.3b) of all raw pasta samples. The longitudinal surface micrographs of mushroom enriched pasta showed minimal differences with control. While there are minor visible differences between the porcini mushroom pasta and control, there appears to be an amorphous substance covering the starch granules within porcini mushroom pasta matrix with fewer spaces (especially the 5 % substituent level). Furthermore, less distinct starch granules could be observed in the porcini mushroom pasta. This might suggest a denser protein network existed in the longitudinal surface of porcini

(a)



(b)



(c)

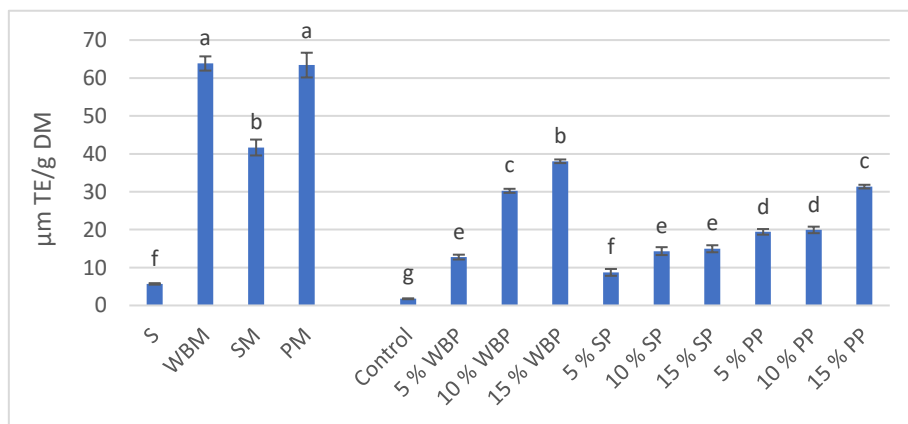


Figure 5.2 Values for total phenolic component and antioxidant capacities: Total phenolic component (TPC) (a) and antioxidant capacities: the DPPH scavenging activities (b) and the ORAC assay results (c). Comparing semolina (S), whole mushroom powder (WBM=white button mushroom; SM=shiitake mushroom; PM=porcini mushroom) and the control sample; white button mushroom pasta (WBP); shiitake mushroom pasta (SP) and porcini mushroom pasta (PP). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$, $n = 3$).

mushroom pasta and more starch granules were encapsulated by protein. The fibre provided by porcini mushroom might play a role in such microstructure, 5 % inulin F-HD enriched spaghetti was reported to obtain a more thickened protein matrix compared to the control sample (Aravind *et al.*, 2012 d).

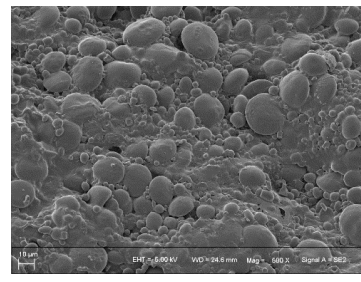
The SEM images of mushroom enriched pasta transverse cross sections appeared to be a bit more irregular and uneven compared to the control sample. While the protein-starch formation within mushroom enriched pasta matrix remained reasonably uniform. Due to the higher fibre content, filament-like structures were visible in samples with 15 % mushroom powder, especially the 5 % white button mushroom pasta. Even though fibre disturbed the gluten matrix, not much apparent disruption to the pasta matrix with mushroom powder could be identified in this study. Similarly the SEM from a study about pasta incorporating pollard, showed that samples below 40 % replacement ratio indicated minimal disruption to the raw pasta formation (Aravind *et al.*, 2012 b).

5.4. Conclusion

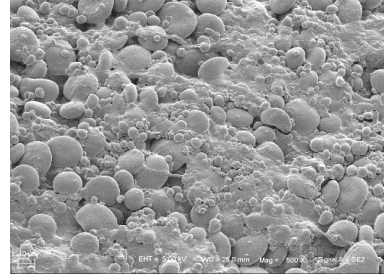
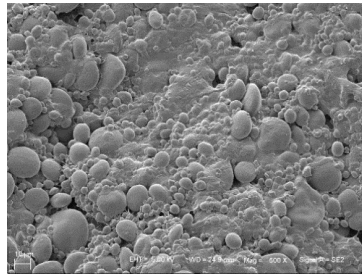
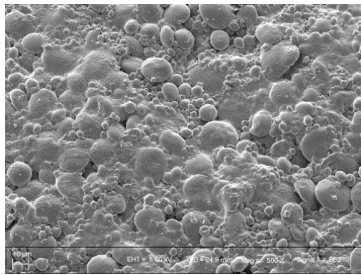
Fresh semolina pasta is a type of starchy food and considered to be nutritional imbalanced. Our research proposes to make up for the deficiency and even enable pasta to be functional diet by adding different edible mushrooms. Results are encouraging, as have revealed that the mushroom-enhanced pastas appear beneficial for delivering their health-promoting bioactive compounds to consumers. The *in vitro* digestion analysis conducted in this study has

highlighted that the addition of mushroom powder (white button, shiitake and porcini mushrooms) into durum wheat semolina can reduce the predicted glycaemic response of the resulting pasta. This work illustrated the possibility of using mushroom powder to modulate the overall GI and increase the antioxidant capacity of the pasta. At this stage, it would be interesting to undertake a further study of mushroom pasta by *in vivo* starch digestion analysis and to develop functional pasta utilising bioactive substances derived from mushrooms to provide more high-quality protein, dietary fibre and polyunsaturated fatty acids to modern consumers.

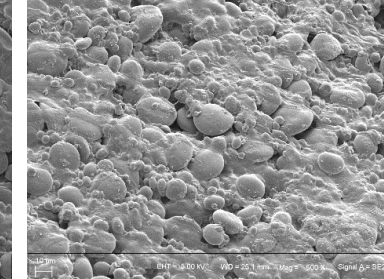
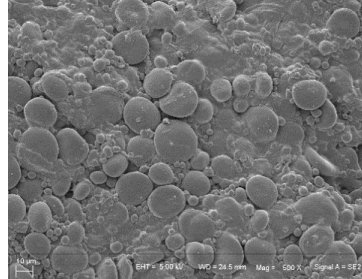
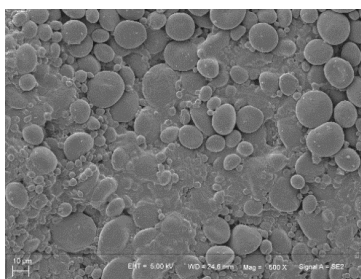
(a)



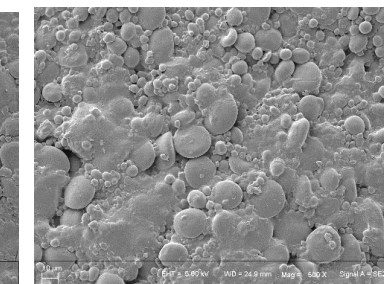
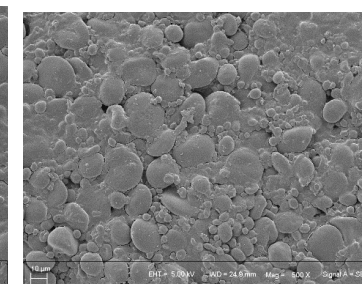
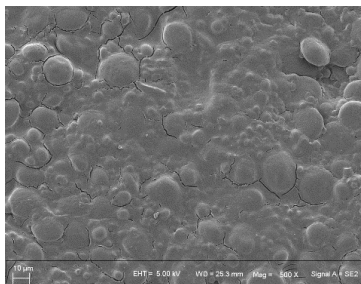
CP



WBP



SP



PP

(b)

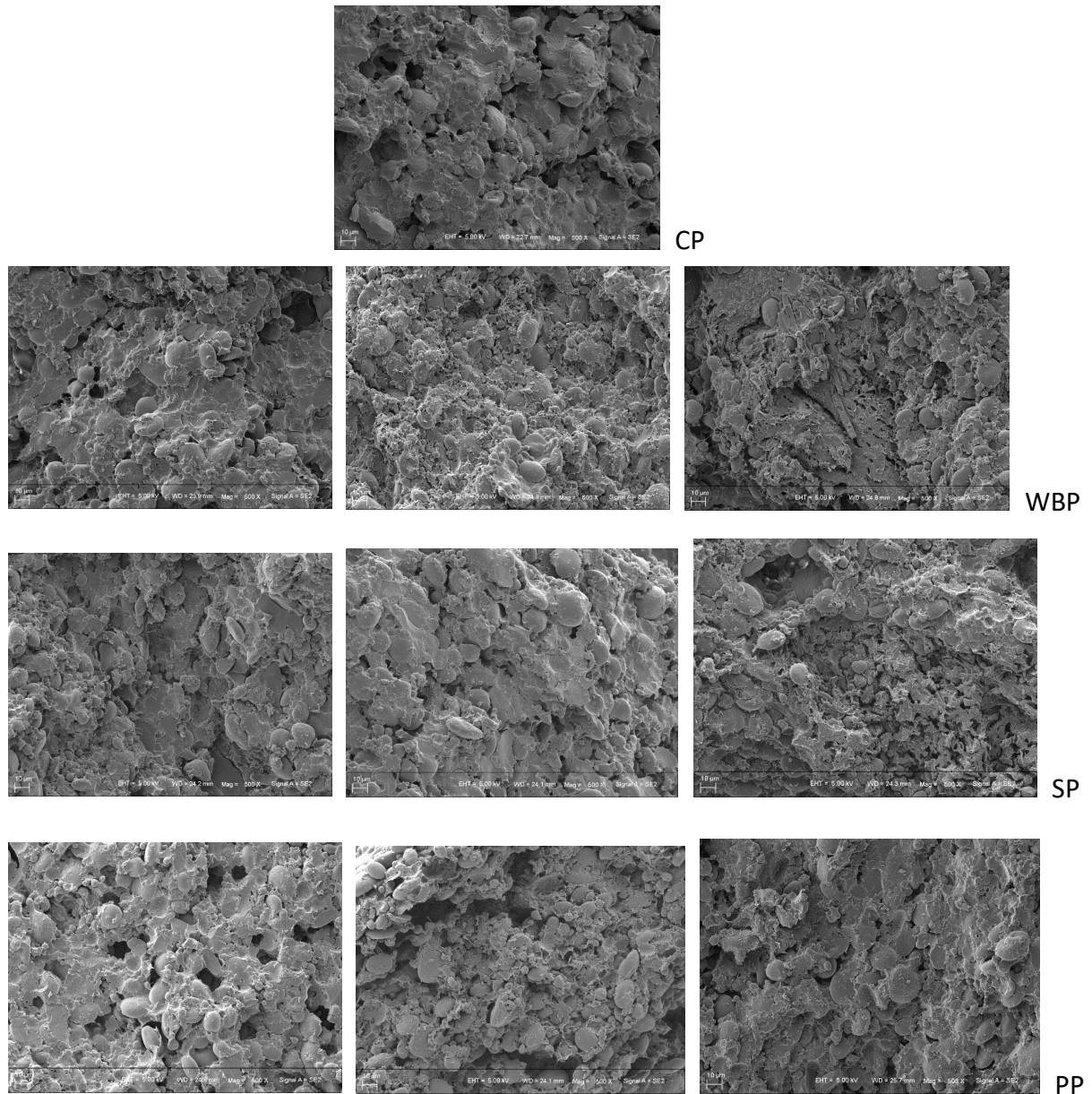


Figure 5.3 Scanning electron micrographs of raw pasta

Scanning electron micrographs of raw pasta longitudinal surface (a) and transverse cross section (b) at 500× magnification: the control pasta (CP); white button mushroom pasta (WBP); shiitake mushroom pasta (SP) and porcini mushroom pasta (PP). From left to right: 5 %; 10 % and 15 % substituent level.

Chapter 6

Effects of different mushroom powder on wheat flour dough and bread properties

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Abstract

Mushroom powder from white button, shiitake and porcini mushrooms were used to replace high grade wheat flour at levels of 5 %, 10 % and 15 % to make novel breads. Dough quality, including rheology properties, stickiness, adhesion, extension and moisture content, were analysed. Bread quality, including height, specific volume, moisture content, hardness, springiness, gumminess and bubbles, were determined. Mushroom powder-substituted dough showed significantly less stability, higher water absorption capability, less peak energy and less extensibility than the control dough. The breads with mushroom powder were smaller in specific loaf volume (except for the 5 % porcini mushroom), exhibited reduced springiness and reduced height (except for the 5 % porcini mushroom) compared to the control samples. Moreover, the addition of mushroom powder altered the porosity properties of the breads.

6.1. Introduction

Edible mushrooms are not only appreciated for their colour, aroma, texture and subtle taste and flavour, but also their nutritional value as food; they have been used as food and food

flavouring materials for centuries (Cheung *et al.*, 2003; Ulziijargal *et al.*, 2013). Edible mushrooms are a good source of bioactive compounds which have been reported to have medicinal value (Cheung *et al.*, 2003; Tseng *et al.*, 2008). Fresh mushrooms contain a higher protein content than most vegetables (Bora and Kawatra, 2014) and the essential amino acid index, amino acid score and nutritional index of mushrooms lies between low grade vegetables and high grade meats (Bora and Kawatra, 2014; Chang and Miles, 2004). Their values are close to that of milk (an animal product) and some species have values which are much higher than milk (Bora and Kawatra, 2014; Chang and Miles, 2004). Furthermore, mushrooms are also relatively good sources of dietary fibre, unsaturated fatty acids and various vitamins and minerals (Breene, 1990; Heleno *et al.*, 2010). In addition, edible mushrooms contain a wide range of functional components, such as polysaccharides; phenolic compounds; terpenes; polyketides and steroids (Cheung *et al.*, 2003; Mizuno *et al.*, 2014). Mushrooms are recognised to have anti-diabetic activity from their various bioactive ingredients, and are able to decrease water consumption, urine volume and blood reducing sugars and, thus, control glycosuria (Seghchi *et al.*, 2001). Edible mushrooms are also known to ameliorate the symptoms of cancer due to their β -D-glucan content (Mizuno *et al.*, 2014), modulate the immune system, lower blood pressure and lipid contents, and inhibit microbial activity (Chang, 1996; Wasser and Weis, 1999).

Bread is an important staple food, especially in western countries. Recently, convenience foods, such as bread, have become increasingly popular in cities, and there is an increased tendency among customers to move away from traditional eating patterns of three meals a day to

consume convenience foods, especially young people and children (Okafor *et al.*, 2012; Ulzijargal *et al.*, 2013). Bread is generally made of wheat flour, water, sugar, salt, yeast and fat (Okafor *et al.*, 2012). These products, mainly derived from cereals, are naturally low in protein, vitamins, minor minerals and dietary fibre and, therefore, are not a balanced food (Agu *et al.*, 2010; Giarni *et al.*, 2003; Okafor *et al.*, 2012). However, bread is potentially an excellent food matrix for fortifying nutrients in order to improve consumers' nutritional health. Many nutritional ingredients, such as pumpkin seeds (Agu *et al.*, 2010), alhydwan seeds (Ammar *et al.*, 2016), arabinoxylans (Buksa *et al.*, 2016), dietary fibre (Gómez *et al.*, 2003) and protein concentrates (Chinma *et al.*, 2015) have been incorporated into bread to improve its nutritional value and product quality. In addition, mushroom materials have also previously been incorporated into bread, including mushroom mycelia (Ulzijargal *et al.*, 2013), oyster mushroom powder (Okafor *et al.*, 2012) and maitake mushroom powder (Seghchi *et al.*, 2001). Generally, mushrooms are able to complement wheat flour well and produce a more nutritionally-balanced bread; for example, mushroom is rich in lysine (an essential amino acid), which is deficient in most cereals (Friedman, 1975).

In this study, the objective was to substitute 5 %, 10 % and 15 % of high grade wheat flour with mushroom powder from white button, shiitake and porcini mushrooms. The final bread samples were evaluated and compared with the control to determine the effect of different mushroom powders on bread quality, including dough rheology and texture properties; the physical and textural characteristics of bread, its thermal properties of bread and the air bubbles present in bread.

6.2. Materials and methods

6.2.1. Materials

As described in 3.1.

6.2.2. Dough and bread preparation

Dough and bread samples were prepared as described in 3.2.2.

6.2.3. Rheological properties of dough

Rheological properties of dough samples were determined as described in 3.6.

6.2.4. Moisture and texture analysis of dough

The method used for moisture content analysis was mentioned in 3.5.5.

The method used for dough textural analysis was mentioned in 3.4.2.

6.2.5. Physical properties of bread

Physical properties of bread were determined as described in 3.3.2.

6.2.6. Bubbles in the breads

Bubbles in the bread samples were determined as described in 3.3.3.

6.2.7. Statistical analysis

Statistical analysis was carried out as described in 3.12.

6.3. Results and discussion

6.3.1. Effect of mushroom powder on the rheology characteristics of bread dough

The doughLAB parameters indicate how dough responds to mechanical mixing during the blending and hydrating of flour and ingredients, and the development of the structure of gluten. This method was carried out to mimic the shearing process when dough was mixed in bakeries (Shittu *et al.*, 2014). Peak torque was measured to represent the optimum dough consistency for product production. In this study, the index of mushroom powder-substituted dough samples showed no significant differences from the control without mushroom powder (Table 6.1). Energy at peak torque is a parameter that indicates the amount of accumulated mechanical energy needed to add to the flour during the mixing process. All the mushroom dough samples showed a significant decrease in their peak energy compared to the control sample and these values decreased gradually as the mushroom content was increased (Table 6.1). Bandwidth at peak calculated the torque differences between the top and bottom curves at the time of development. In this study, 10 % and 15 % white button mushroom powder-

enriched dough had significantly increased values compared with the control (69.67 FU, 69.33 FU and 64.43 FU, respectively), while the 15 % porcini-substituted bread decreased significantly (59.5 FU).

The water absorption ability of mushroom powder enriched dough increased significantly, and incrementally, with increasing mushroom powder content compared with the control (Table 6.1). Such increases could be due to the higher dietary fibre content the mushroom powder contributed to the final bread products (Ammar *et al.*, 2016). All three mushroom powders contained higher dietary fibre contents than in wheat flour (3.5 % as labelled on the package). Among the mushrooms, the total dietary fibre content of shiitake mushroom was 41.97 %, which was significantly higher than that of the white button and porcini mushrooms. This finding was in agreement with the highest water absorption capability of shiitake mushroom dough at the same substitution levels, when compared with the other two species.

The development time indicates when the dough reaches its peak resistance to deformation, which means the time taken for dough to reach its optimum viscous and elastic properties for the retention of the gas essential for bread making (Ammar *et al.*, 2016; Sanchez *et al.*, 2014). For the white button mushroom powder-enriched dough, the development time showed no significant difference compared with the control; while, for the other two mushroom powder-enriched dough samples, the values were significantly lower than for the control (Table 6.1). These results are in agreement with previous research where the dough development time decreased gradually with increasing quantities of wheat germ (dietary fibre) in cereal products

(Cheung, 2008; Marti *et al.*, 2014). Resistant starch has been shown to have a similar physiological property as dietary fibre, and the addition of maize resistant starch was also reported to be able to bring a significant decrease in dough development time (Sanchez *et al.*, 2014).

Dough stability time was used to determine the flour's tolerance to mixing and flour strength. A lower stability value is believed to indicate a softer dough (Rosell *et al.*, 2007). The stability time of dough with increased mushroom powder content decreased significantly and gradually compared with the control (Table 6.1), and this had an adverse effect on dough quality. These results are in agreement with previous research where dough formulated with maize resistant starch had lower dough stability than the control (Sanchez *et al.*, 2014). The stability of dough is mainly related to two factors: the dilution of gluten, and the altered water behaviour as observed by many researchers (Sun *et al.*, 2015). Generally, the stability of dough is correlated with protein matrix formation, which is easily damaged by the additional ingredients (Marti *et al.*, 2014). It has been reported that some edible mushroom species are good sources of glutathione (Dogan *et al.*, 2016), a reducing agent. As such it is possible that glutathione (present in mushroom material) can break disulphide bonds then disrupt the gluten network (Every *et al.*, 2006). Therefore, the incorporation of mushroom powder not only dilutes the gluten and alters the water behaviour of the final dough, but it also a source of reducing agents and, as a result, this weakens the protein matrix. In addition, the higher dietary fibre content of mushroom dough samples may be another reason for the weakening of the dough, as the fibre will compete for water with the components of wheat flour. The proteins and dietary

fibre resulting from the mushroom powder incorporation may have a negative effect on the well-formed protein-starch complex within the bread matrix (Sun *et al.*, 2015). A specific ratio of gliadin to glutenin fractions is necessary for optimal gluten network formation (Mohammed *et al.*, 2012). So the difference between such ratios of wheat flour and mushroom powder may play a role in the decreased extensibility.

6.3.2. Effect of mushroom powder on the texture properties of bread dough

During dough preparation in this study, the same amount of water was added to every sample so as to have consistency between samples in moisture levels. To that end, the addition of mushroom powder did not show significant differences in the dough moisture content, as shown in Table 6.2. In terms of stickiness, both the white button and porcini mushroom powder incorporation did not show significant differences from the control bread dough, while shiitake mushroom addition resulted in a gradually decreasing trend for stickiness, especially the 15 % substitution level showed significantly lower stickiness than the control (Table 6.2). When it came to the adhesion values, all the shiitake and porcini mushroom dough samples showed no differences compared with the control, while the 10 % and 15 % white button mushroom substitution levels significantly increased the adhesion values. Generally, wheat protein network development during the mixing and fermentation process was the main factor that determined the dough structure. The addition of mushroom resulted in changes in the protein, fat, dietary fibre and other components, which affected the dough texture. The dough

Table 6.1 The effect of different mushroom powder on the rheology properties of bread dough

Sample	Peak (FU)	WA at target corrected (%)	stability (min)	Bandwidth at peak (FU)	Development time (min)	Peak energy (Wh/kg)
Control bread	503.77±9.1 ^{ab}	58.2±0.1 ^h	8.57±0.4 ^a	64.43±0.68 ^{bc}	18.6±0.35 ^a	19.17±0.21 ^a
5 % white button mushroom bread	498.43±3.66 ^{ab}	63.97±0.12 ^f	5.4±0.36 ^b	66.9±0.85 ^{ab}	18.8±0.35 ^a	16.13±0.06 ^b
10 % white button mushroom bread	511.53±3.35 ^a	65.27±0.12 ^e	5.33±0.67 ^b	69.67±1.91 ^a	18.6±0.35 ^a	15.67±0.12 ^b
15 % white button mushroom bread	500.37±5.23 ^{ab}	68.8±0.53 ^c	3.87±0.21 ^{cd}	69.33±0.76 ^a	18.63±0.35 ^a	13.7±0.35 ^c
5 % shiitake mushroom bread	510.33±7.36 ^a	66.93±0.47 ^d	3.7±0.1 ^d	64.87±0.67 ^b	10.97±0.12 ^c	9.13±0.25 ^{ef}
10 % shiitake mushroom bread	505.5±7.3 ^{ab}	70.77±0.49 ^b	3.2±0 ^{de}	64.33±1.88 ^{bc}	11.17±0.25 ^{bc}	8.73±0.12 ^f
15 % shiitake mushroom bread	492.33±2.58 ^b	78.07±0.21 ^a	2.77±0.06 ^e	61.6±0.82 ^{cd}	11.4±0.2 ^{bc}	8.03±0.21 ^g
5 % porcini mushroom bread	503.63±4.07 ^{ab}	62.6±0.08 ^g	4.63±0.35 ^{bc}	65.97±0.76 ^b	11.8±0.1 ^b	10.3±0.1 ^d
10 % porcini mushroom bread	505.53±7.97 ^{ab}	65.47±0.21 ^e	3.73±0.12 ^d	63.9±1.01 ^{bc}	10.7±0.1 ^c	8.97±0.12 ^{ef}
15 % porcini mushroom bread	489.9±2.1 ^b	66.77±0.06 ^d	4.07±0.06 ^{cd}	59.5±0.92 ^d	11.73±0.12 ^b	9.47±0.12 ^e

Mean ± standard deviation (n=3). Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$).

stickiness properties were strongly influenced by the relationship between the additional components and gluten, and their interactions with water (Totosaus *et al.*, 2013).

According to Table 6.2, except for the 5 % shiitake and porcini mushroom substitution levels, the addition of mushroom powder resulted in lower extensibility values than the control, which indicated that such substitution decreased the dough strength. As mentioned above, mushroom is good source of dietary fibre, and such decreased extensibility was supported by Ahmed *et al.* (2013), who reported that wheat flour dough enriched with insoluble dietary fibre had a decreased extensibility value. The protein content in wheat flour was 11.5 % (according to manufactures specifications) while the material from all three mushroom species contained higher protein contents than wheat flour: 30.52 %, 15.04 % and 23.83 % in white button, shiitake and porcini mushrooms, respectively. Previous research has reported that incorporation of lupin protein concentrate into wheat flour dough could lead to the dilution of gluten because of the replacement of gluten proteins by non-gluten vegetable proteins (Paraskevopoulou *et al.*, 2010). The fat content of wheat flour was 1.4 %, and mushroom powders had slightly higher fat contents than wheat flour (2.42 %, 1.48 % and 3.03 % for white button, shiitake and porcini mushroom, respectively). On the one hand, it has been reported that large quantities of fat could have a negative influence on dough system quality, specifically, a decrease in its elastic properties, which can be attributed to the inhibition of fat on gluten matrix development (Gómez *et al.*, 2008). However, more research should be undertaken to determine the effect of mushroom fat on dough extensibility.

Table 6.2 The effect of different mushroom powder on the moisture content and texture properties of bread dough

Sample	Stickiness (Force) (g)	Work of Adhesion (Area F-T 1:2) (g.sec)	Resistance to Extension (Force) (g)	Dough Moisture %
Control bread	33.31±3.52 ^{bc}	1.94±0.32 ^{bcd}	94.51±6.02 ^a	40.32±0.13 ^{bc}
5 % White button bread	34.46±0.46 ^{abc}	2.21±0.18 ^{bc}	80.88±1.9 ^{bc}	41.30±0.13 ^{ab}
10 % White button bread	38.91±2.86 ^a	3.37±0.06 ^a	64.86±5.48 ^e	40.78±0.82 ^{ab}
15 % White button bread	35.84±1.17 ^{ab}	3.02±0.27 ^a	63.46±5.60 ^e	41.52±0.58 ^a
5 % Shiitake mushroom bread	34.20±0.72 ^{abc}	2.03±0.08 ^{bcd}	85.26±2.39 ^{abc}	40.35±0.48 ^{bc}
10 % Shiitake mushroom bread	30.60±0.41 ^{cd}	1.61±0.031 ^{cd}	66.59±0.48 ^{de}	39.48±0.27 ^c
15 % Shiitake mushroom bread	27.30±1.052 ^d	1.52±0.12 ^d	77.76±1.37 ^{bc}	40.99±0.13 ^{ab}
5 % Porcini mushroom bread	35.11±0.72 ^{abc}	2.08±0.11 ^{bcd}	86.39±1.59 ^{ab}	40.53±0.12 ^{abc}
10 % Porcini mushroom bread	34.59±2.03 ^{abc}	2.28±0.50 ^b	66.73±3.53 ^{de}	41.04±0.22 ^{ab}
15 % Porcini mushroom bread	33.70±0.89 ^{bc}	2.07±0.11 ^{bcd}	75.57±2.19 ^{cd}	41.20±0.077 ^{ab}

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$).

6.3.3. Effect of mushroom powder on the texture properties of bread

The effect of mushroom powder on the height, specific volume, moisture content and textural properties of bread are shown in Table 6.3. All white button mushroom breads had similar heights as the control sample. Shiitake mushroom bread had a significantly lower height than the control, except for the 5 % shiitake mushroom bread, and the 5 % porcini mushroom bread was much higher than the control sample. The addition of white button and shiitake mushroom powder could result in bread with a higher moisture content, especially at the 15 % substitution level, which had a significantly increased the moisture content. These observations are in agreement with research from Ulziijargal *et al.* (2013), who reported that the bread supplemented with *Agaricus blazei* and *Hericium erinaceus* mycelia had higher moisture values. Although in this study, all the porcini mushroom substitutions did not significantly alter the moisture of the bread, for the white button and shiitake mushrooms, the specific volume decreased with the increased mushroom content. For the porcini mushroom bread, a 5 % substitution level increased the specific volume significantly; the 10 % level showed no significant difference from the control; and the 15 % level significantly decreased the value. Similar results were found by Seghchi *et al.* (2001), where the bread specific volume increased up to a certain level with mushroom substitution, then decreased gradually with the increased percentage of mushroom content. Generally speaking, the incorporation of porcini mushroom also resulted in a gradual decreasing trend. The findings of Ulziijargal *et al.* (2013), illustrated that the specific volume of bread substituted with mushroom mycelium was much lower than the control bread, and resulted in a smaller sized loaf of bread. A similar

observation was recorded with the addition of 15–20 % barley flour in breads (Lin *et al.*, 2012). Among the three mushroom species with the same substitution levels, the volumes of the mushroom breads were also significantly different. This observation may be attributed to their different compositions of fibre, protein and fat. For instance it has been reported that incorporation of carob and pea fibre into breads decreased specific volume (Wang *et al.*, 2002), and the incorporation of β -glucan into wheat flour was accompanied by a gradual decrease in bread volume with increased substitution level (Skendi *et al.*, 2010). In addition, the inclusion of protein may from the substituted protein concentrates could affect water hydration and distribution during gluten network formation by competing for water (Totosaus *et al.*, 2013). The effect of lupin protein concentrate on bread volume has been studied and was found to progressively decrease the loaf volume as the level of lupin protein increased (Paraskevopoulou *et al.*, 2010; Totosaus *et al.*, 2013). The lipid content of bread has been reported to contribute to the differences in loaf volume (especially the polar lipid fraction). To be specific, it is able to develop lipid monolayers at the gas or liquid interphase of the gas cells and, as a result, increases the ability of the dough to retain gas (Maktouf *et al.*, 2016). In addition, the different nutrient compositions for the doughs had different influences on the proliferation of yeast growth and gas production during the fermentation process, thus, influencing loaf volume (Ammar *et al.*, 2016).

Hardness, springiness and gumminess were used as parameters to represent the texture properties of the bread samples in this study (Table 6.3). Hardness is regarded as one of the most important indices of bread quality, and was evaluated by the peak force of the first

compression of the samples by the Texture Analyser (Sun *et al.*, 2015). Generally, white button mushroom powder substitution resulted in higher hardness values for the bread samples. Addition of shiitake mushroom powder illustrated such effect more clearly; as the hardness of the bread increased significantly (except for the 5 % substitution) and gradually with the increased mushroom level. Whole wheat flour has also been used to make steamed bread in order to improve the protein and dietary fibre contents, as well as the substitution of mushroom powder as in this study. Whole wheat flour steamed bread was reported to have significantly higher hardness than the wheat flour bread (Liu *et al.*, 2015). While the inclusion of porcini mushroom powder had a positive effect on the bread by decreasing the hardness of the crumb, at the same time, the hardness increased gradually with the increased porcini mushroom powder amounts up until the 15 % level addition, which showed no significant differences compared with the control. This result was consistent with the work of Buksa *et al.* (2016) who illustrated that crumb hardness decreased as the growing level of cross-linked or hydrolysed water extractable arabinoxylan. In general, the hardness of bread was inversely correlated with the specific volume found in this study; this relationship has been illustrated in previous studies (Martínez and Gómez, 2017).

The gumminess of the bread samples showed a similar trend as the hardness, for both the white button and shiitake mushroom breads; with the increased mushroom contents the gumminess values increased gradually and significantly compared with the control (except for the 5 % and 10 % white button and 5 % shiitake mushroom breads). For the porcini mushroom bread, the gumminess was significantly lower than control (except at the 15 % level), while still

representing an increasing trend when the mushroom level increased. This result was supported by the study of bread substituted by mushroom mycelia, there was a decrease in gumminess by such incorporation (Ulziijargal *et al.*, 2013). Springiness is a parameter used to determine the recovery extent between the first and second compressions in order to indicate the elasticity of the sample (Ulziijargal *et al.*, 2013). Table 6.3 shows that the springiness of bread decreased significantly compared with the control. Similar results have been recorded by Ulziijargal *et al.* (2013), who reported that addition of *Agaricus blazei* mycelium and *Hericium erinaceus* mycelium led to lower fresh bread springiness values. Both the wheat germ and alhydwan seed flour are rich in dietary fibre, protein and fat (and especially high in unsaturated fatty acids) (Ammar *et al.*, 2016; Sun *et al.*, 2015), similar to the mushroom powder. The springiness of steamed Chinese bread decreased by the addition of wheat germ flour (Sun *et al.*, 2015).

6.3.4. Effect of mushroom powder on the bubbles of bread

Most of the white button and shiitake mushroom bread samples exhibited similar average bubble sizes as the control, except at the 5 % substitution level (Table 6.4). However, the porcini mushroom inclusion resulted in larger average sizes for the bread crumb bubbles, and this appeared to follow the same trend as the values for volume of porcini mushroom bubbles. There was a positive relationship between the cell expansion and bread volume during fermentation and baking. The decrease of gas cell expansion by gas pressure has been shown

Table 6.3 The effect of different mushroom powder on the physical and texture properties of bread

Sample	Hardness (Force) (g)	Springiness (mm)	Gumminess (g)	Height (mm)	Specific volume (mL/g)	Bread Moisture %
Control bread	303.04±6.65 ^d	0.96±0.0087 ^a	253.81±10.52 ^d	60.33±0.98 ^{bc}	3.08±0.035 ^{bc}	27.84±2.34 ^c
5 % White button bread	386.53±2.69 ^c	0.91±0.017 ^{cd}	237.89±8.11 ^d	58.98±2.00 ^{bc}	2.60±0.062 ^e	28.96±2.30 ^{bc}
10 % White button bread	322.17±14.36 ^d	0.91±0.017 ^{bcd}	253.31±10.88 ^d	57.18±1.44 ^{bc}	2.70±0.043 ^{de}	30.00±0.33 ^{bc}
15 % White button bread	380.85±5.46 ^c	0.91±0.0013 ^{bcd}	291.08±7.63 ^c	54.16±1.75 ^{cd}	2.40±0.047 ^f	33.35±1.57 ^{ab}
5 % Shiitake mushroom bread	224.93±1.40 ^e	0.95±0.0040 ^{ab}	185.50±3.30 ^e	61.63±0.43 ^{ab}	2.89±0.013 ^{cd}	31.78±2.59 ^{abc}
10 % Shiitake mushroom bread	458.60±14.64 ^b	0.88±0.0068 ^d	362.75±12.58 ^b	48.83±2.35 ^d	2.18±0.052 ^g	30.29±1.30 ^{bc}
15 % Shiitake mushroom bread	787.78±11.46 ^a	0.90±0.028 ^{cd}	628.24±17.37 ^a	49.16±3.97 ^d	1.58±0.093 ^h	36.11±2.09 ^a
5 % Porcini mushroom bread	176.28±0.71 ^f	0.95±0.012 ^{abc}	145.06±3.12 ^f	67.93±0.79 ^a	3.61±0.025 ^a	31.41±2.20 ^{abc}
10 % Porcini mushroom bread	243.34±6.11 ^e	0.95±0.014 ^{ab}	198.30±5.44 ^e	63.26±3.59 ^{ab}	3.20±0.090 ^b	31.11±1.35 ^{abc}
15 % Porcini mushroom bread	297.41±18.80 ^d	0.87±0.02 ^d	230.77±8.21 ^d	60.01±3.30 ^{bc}	2.80±0.13 ^d	29.26±1.74 ^{bc}

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$).

to lead to a more compact crumb structure, thus, creating a lower bread volume (Totosaus *et al.*, 2013). In addition, the 5 % porcini mushroom bread had the largest bubbles ($1-15 \text{ m}^2 \times 10^{-5}$) with the largest mean diameters, and the length and width of the crumb bubbles, implying that the addition of porcini mushroom powder at relatively low levels could help to incorporate and retain air during bread making. The presence of soluble dietary fibre in porcini mushroom may be a reason for these results. For instance, it has been observed that xanthan gum inclusion created larger air bubbles than in the control batter (Martínez-Cervera *et al.*, 2011). The fat content in porcini mushroom may also be responsible for this observation as lipids (especially the polar fraction) have been shown to contribute to the formation of the gas cells, and the increasing dough gas retention, by favouring the development of lipid monolayers at the gas and liquid interphase (Gan *et al.*, 1990).

Another interesting finding was that the highest mushroom-supplemented bread had similar total bubble numbers than the control, however the control bread possessed many more small sized ($0-0.099 \text{ m}^2 \times 10^{-5}$) and fewer medium sized bubbles ($0.1-0.99 \text{ m}^2 \times 10^{-5}$) than the mushroom samples. The control crumb, which possessed small air bubbles, conferred the appearance of springiness (Martínez-Cervera *et al.*, 2011). The large bubble numbers of most of the mushroom bread crumbs showed no significant differences from the control. It has been shown that the large bubbles could form tunnels from the base to the surface of the bread; thus, some air was lost during the crumb structure development (Martínez-Cervera *et al.*, 2011). These results were consistent with the bread moisture content. In general, the control bread illustrated more uniform gas cells in its crumbs. All the shiitake mushroom bread

Table 6.4 The effect of different mushroom powder on the bubbles of bread

Sample	Area distribution ($m^2 \cdot 10^{-5}$)			Average Size ($m^2 \cdot 10^{-5}$)	total bubbles	Diameter mean cm	Size length cm	Size width cm
	1~15	0.1- .99	0-0.099					
Control bread	10.5±0.71 ^{bcd}	97±2.83 ^f	164.5±13.44 ^a	0.24±0.0042 ^e	272±15.56 ^{cd}	0.14±0.0040 ^c	0.19±0.0071 ^{cd}	0.089±0.0071 ^b
5 % White button bread	13±1.42 ^{bc}	92.5±3.54 ^f	44±5.66 ^{fg}	0.44±0.049 ^{ab}	149.5±10.61 ^g	0.20±0.0071 ^{ab}	0.25±0.02 ^{abc}	0.12±0.0074 ^{ab}
10 % White button bread	14.5±2.12 ^{ab}	209.5±6.36 ^b	108±4.24 ^b	0.29±0.012 ^{cde}	332±12.73 ^{ab}	0.18±0.0039 ^{bc}	0.19±0.0059 ^{bcd}	0.11±0.0071 ^{ab}
15 % White button bread	12±1.41 ^{bc}	186±5.66 ^{bc}	96.5±4.95 ^b	0.29±0.0048 ^{cde}	294.5±12.02 ^{bc}	0.18±0.0053 ^{bc}	0.18±0.033 ^d	0.11±0.0018 ^{ab}
5 % Shiitake mushroom bread	5±1.41 ^d	124.5±6.36 ^e	34±2.83 ^g	0.34±0.033 ^{cd}	163.5±10.61 ^g	0.22±0.021 ^a	0.27±0.019 ^a	0.11±0.0055 ^{ab}
10 % Shiitake mushroom bread	8±1.41 ^{cd}	114±5.66 ^{ef}	50±2.83 ^{efg}	0.28±0.022 ^{cde}	172±9.90 ^{fg}	0.18±0.0032 ^b	0.21±0.0036 ^{abcd}	0.12±0.028 ^{ab}
15 % Shiitake mushroom bread	11.5±2.12 ^{bc}	127±2.83 ^e	70.5±3.54 ^{de}	0.26±0.019 ^{de}	209±2.83 ^{ef}	0.17±0.0071 ^{bc}	0.19±0.021 ^{cd}	0.11±0.012 ^{ab}
5 % Porcini mushroom bread	20±1.41 ^a	181±8.49 ^{cd}	64.5±3.54 ^{def}	0.51±0.016 ^a	265.5±10.61 ^{cd}	0.22±0.014 ^a	0.25±0.0058 ^{ab}	0.15±0.014 ^a
10 % Porcini mushroom bread	15±1.41 ^{ab}	161±7.07 ^d	73.5±3.54 ^{cd}	0.35±0.016 ^{bc}	249.5±12.02 ^{de}	0.19±0.0026 ^{ab}	0.22±0.0066 ^{abcd}	0.12±0.012 ^{ab}
15 % Porcini mushroom bread	11±1.41 ^{bcd}	260.5±7.78 ^a	94.5±2.12 ^{bc}	0.28±0.022 ^{cde}	366±11.31 ^a	0.18±0.0040 ^{bc}	0.21±0.0030 ^{abcd}	0.10±0.0075 ^{ab}

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$).

samples had significantly fewer total bubbles than the control. This phenomenon may be due to the highest total dietary fibre content being observed in the shiitake mushroom powder, as it has been revealed that fibre addition could reduce the gas retention capacity from interactions between the gluten and fibre (Gómez *et al.*, 2003). Bran particles have been reported to have an adverse effect on the starch-gluten matrix, by preventing gas cell expansion of the dough (Gan *et al.*, 1989).

6.4. Conclusion

This study has shown that mushroom powder inclusion had negative impacts on bread dough stability, development time (except white button mushroom) and extension property. While the influence on the dough moisture and stickiness was relatively slight. The three different bread products. By comparison, the control bread illustrated more uniform gas cells in its crumbs. However, these mushroom supplemented breads still have a place in the market as a kind of functional novel bread product, by delivering more dietary fibre, high quality protein and other bioactive components to consumers. So further research about the nutritional properties of these final bread products should be done in the future.

Chapter 7

Manipulation of the predictive glycaemic response and the phenolic properties of mushroom enhanced bread

(Submitted to Journal of Agricultural and Food Chemistry)

Abstract

Wheat bread supplemented with mushroom powder from 3 different species of mushrooms were investigated in terms of starch characteristics (content, gelatinisation and digestibility) and antioxidant capacities. The decrease of total starch contents and increase of phenolic contents of the breads were significant with increased mushroom powder content compared to the control. The reducing sugar released over 120 min in an *in vitro* digestion of the control increased more rapidly than in the mushroom enriched breads. According to area under the curve (AUC) values, the inclusion of mushroom powder lowered the predicted glycaemic response of the bread, which correlated to the lower degree of starch gelatinisation. Mushroom powder incorporation also enhanced the DPPH radical scavenging assay and oxygen radical absorbance capacity (ORAC) compared to control bread. The action of the addition of different mushroom powders on the bread crust and crumb microstructure properties was also studied. Mushroom powder altered the internal microstructure of the bread crust and crumb by affecting the interactions between starch and the other components of the bread. Overall, this shows that mushroom powder could be added to bread to deliver health benefits to consumers.

7.1. Introduction

There is a wide range of bakery products available to consumers, among which bread is regarded as an important dietary food in the majority of countries worldwide. Bread is mainly consumed as a staple food, especially in Western countries (Ulziijargal *et al.*, 2013). The main ingredient used for bread making is wheat flour, which is mostly composed of starch (contributing 84–88 % of the dry mass of the flour) (Ho *et al.*, 2015). Bread makes an important contribution to the carbohydrate intake in human diets. Commercial wheat bread is carbohydrate rich, which causes a quite rapid rise in blood glucose (Ronda *et al.*, 2012). Recently, however, the desire of consumers for healthier food products has meant that composite flours, partially substituted with other natural nutritional ingredients (including vitamins, minerals, dietary fibre and antioxidants), have attracted the interest of producers and consumers for distributing more health benefits.

Mushroom fruiting bodies have been used for food, in food flavouring materials and, even, in traditional Chinese medicine, for centuries. Mushrooms are a good source of high quality digestible protein (10–40 % dry mass), B-vitamins, vitamin C and essential minerals (Okafor *et al.*, 2012). Most mushroom species are rich in lysine, which is deficient in wheat flour (Okafor *et al.*, 2012). Edible mushrooms have several medical properties, for instance, anti-tumour activity related to their β -D-glucan content (Seghchi *et al.*, 2001); anti-diabetic from decreasing blood glucose related to their bioactive components (Ohtsuru *et al.*, 1999); and anti-cardiovascular and anti-atherosclerotic effects due to their range of antioxidant components (including phenolics and ergothioneine) (Lo and Cheung, 2005; Weigand-Heller *et al.*, 2012).

Thus, combinations of mushroom powder and wheat flour may possibly optimise bread formulations by improving their nutritional benefits, taste, flavour and product appeal. Previous researchers have studied the effect of shiitake stipe, silver ear, (Lin *et al.*, 2008; Tseng *et al.*, 2010), mushroom mycelia (Ulziijargal *et al.*, 2013) and maitake mushroom (Seghchi *et al.*, 2001) on bread properties.

In this study, white button (*Agaricus bisporus*), shiitake (*Lentinula edodes*) and porcini mushroom (*Boletus edulis*) were incorporated into wheat bread at 5 %, 10 % and 15 % substitution levels, respectively. The influence of such inclusions on the nutritional characteristics and microstructure of breads were determined, and included total starch content, starch gelatinisation, *in vitro* starch digestibility, total phenolic contents and antioxidant capacities. The microstructure properties of both the bread crust and crumb were evaluated by scanning electron microscopy. These final novel breads may introduce more edible mushroom species to Western consumers' diets in both a simple to prepare and acceptable way.

7.2. Materials and methods

7.2.1. Materials

As described in 3.1.

7.2.2. Bread preparation

Bread samples were prepared as described in 3.2.2.

7.2.3. Fibre analysis

Fibre analysis was determined as described in 3.5.2.

7.2.4. Thermal properties

Thermal properties of bread samples were determined as described in 3.8.

7.2.5. Starch content and *in vitro* digestion analysis

The method used for starch content analysis was mentioned in 3.5.1. The method used for starch *in vitro* digestion was mentioned in 3.9.

7.2.6. Antioxidant analysis

Antioxidant analysis was determined as described in 3.10.

7.2.7. Microstructure

The microstructure analysis of bread crust and crumb was evaluated by scanning electron

microscope (SEM) on samples at 1000× magnification level. The analysis of microstructure was determined as described in 3.11.

7.2.8. Statistical analysis

Statistical analysis was carried out as described in 3.12.

7.3. Results and discussion

7.3.1. Effect of mushroom powder on the thermal properties of bread

Previous research has shown that variations in gelatinisation temperatures that within a dough and bread sample matrix may be important factors to predict bread quality (Collar *et al.*, 2015). The melting enthalpy (ΔH) was determined for all bread samples to represent the extent of the starch granule gelatinised, or dextrinised, during the bread making process (Parada *et al.*, 2011). Table 7.1 illustrates that the ΔH values of most of the mushroom bread samples were slightly higher than those of the control, and these values increased with the increasing mushroom powder contents. Table 7.2 illustrates that the inclusion of mushroom powder significantly increased the fibre content of the bread. It has been reported that the limited levels of fibre and sugars in the breads made for greater water availability than for starch alone and this contributed to its complete gelatinisation (Karkle *et al.*, 2012). This in turn may result in an increased ΔH (Aravind *et al.*, 2012 b). Table 7.3 shows that there were significant positive correlations between ΔH and all IDF, SDF and TDF ($r = 0.580, 0.620$ and 0.653 , respectively, at

$p \leq 0.001$). Such a phenomenon may be due to the limited water content during bread making and competitive action by all the hydrophilic components in the mushroom powder being able to retard the starch granules' swelling. A similar observation regards for the reduced degree of gelatinisation has been recorded (Aravind *et al.*, 2012 b). It is possible that the presence of hydrocolloids, for example, pectin and mushroom contain some specific lectins, such as *Agaricus bisporus* agglutinin from edible mushrooms (Wu *et al.*, 2003), could reduce the hydration of starch (Witczak *et al.*, 2012).

Table 7.1 The effect of different mushroom powder on the thermal properties of bread

Sample	T _{onset} °C	T _{gelatinisation} °C	T _{endset} °C	ΔH J/g	ΔTr °C
Control bread	58.06±0.84	64.41±1.73	69.85±1.17	0.42±0.08	11.80±0.33
5 % White button mushroom bread	55.96±1.07	60.14±0.85	67.19±2.51	0.38±0.07	11.23±3.59
10 % White button mushroom bread	55.64±2.17	59.62±0.09	65.92±2.19	0.40±0.14	10.29±0.02
15 % White button mushroom bread	52.71±1.70	59.84±0.36	70.59±0.68	0.86±0.10	17.89±2.38
5 % Shiitake mushroom bread	56.34±2.82	61.31±0.28	68.79±1.83	0.44±0.08	12.45±4.65
10 % Shiitake mushroom bread	53.79±0.81	61.16±0.49	73.14±0.60	0.73±0.10	19.35±0.21
15 % Shiitake mushroom bread	52.97±1.94	59.59±1.58	68.31±1.69	0.64±0.09	15.34±0.25
5 % Porcini mushroom bread	54.2±0.16	61.01±0.07	69.88±0.82	0.42±0.02	15.68±0.98
10 % Porcini mushroom bread	54.30±1.01	60.14±0.80	68.93±0.26	0.56±0.03	14.63±1.27
15 % Porcini mushroom bread	52.74±1.41	57.10±0.73	67.13±0.96	1.27±0.2	14.39±2.38
Mean ± standard deviation.					



Figure 7.1 The photo of bread samples

Slices of the control bread (CB) and all mushroom powder enriched bread samples: white button mushroom bread (WBB); shiitake mushroom bread (SB) and porcini mushroom bread (PB).

Table 7.2 Fibre content of bread products

Sample	IDF %	SDF %	TDF %
Control bread	2.24±0.17 ^d	2.67±0.085 ^b	4.91±0.087 ^d
5 % White button mushroom bread	2.55±0.33 ^d	2.81±0.23 ^{ab}	5.36±0.10 ^{cd}
10 % White button mushroom bread	3.50±0.28 ^{bcd}	3.12±0.20 ^{ab}	6.62±0.48 ^{bc}
15 % White button mushroom bread	4.02±0.82 ^{abc}	3.20±0.060 ^{ab}	7.23±0.76 ^{ab}
5 % Shiitake mushroom bread	3.29±0.024 ^{bcd}	2.88±0.54 ^{ab}	6.16±0.52 ^{bcd}
10 % Shiitake mushroom bread	4.44±0.18 ^{ab}	3.09±0.24 ^{ab}	7.53±0.062 ^{ab}
15 % Shiitake mushroom bread	5.19±0.30 ^a	3.28±0.020 ^{ab}	8.47±0.28 ^a
5 % Porcini mushroom bread	2.88±0.07 ^{cd}	3.36±0.19 ^{ab}	6.24±0.12 ^{bcd}
10 % Porcini mushroom bread	3.99±0.47 ^{abc}	3.57±0.14 ^a	7.56±0.34 ^{ab}
15 % Porcini mushroom bread	4.39±0.0071 ^{ab}	3.69±0.0064 ^a	8.07±0.014 ^a

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$).

Table 7.3 Pearson's correlation coefficient (r) of physicochemical and nutritional properties of bread samples

	AUC	TPC	DPPH	ORAC	ΔH	IDF	SDF	TDF
ST	0.699***	-0.409*	-0.380*	-0.490**	-0.773***	-0.618***	-0.365*	-0.608***
AUC		-0.355	-0.232	-0.412*	-0.586***	-0.858***	-0.368*	-0.802***
TPC			0.960***	0.979***	0.493**	0.432*	0.752***	0.573***
DPPH				0.966***	0.484**	0.342	0.764***	0.504**
ORAC					0.551**	0.509**	0.776***	0.642***
ΔH						0.580***	0.620***	0.653***
IDF							0.539**	0.968***
SDF								0.734***

ST, starch; AUC, in vitro area under the curve value; TPC, total phenolic content; DPPH, the DPPH. scavenging activities; ORAC, the ORAC assay results; ΔH, the enthalpy of the transition; IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre. *, significant at $p \leq 0.05$. **, significant at $p \leq 0.01$. ***, significant at $p \leq 0.001$.

7.3.2. Effect of mushroom powder on the starch content and digestibility of bread

Figure 7.2 illustrates the total starch content of bread samples. The substitution of wheat flour with mushroom powder caused a decrease in the total starch content (dry basis). Negative correlations were observed between starch content and IDF ($r = -0.618$; $p \leq 0.001$), SDF ($r = -0.365$; $p \leq 0.05$), and TDF ($r = -0.608$; $p \leq 0.001$) (Table 7.3). Fig. 7.3 illustrates the reducing

sugars released over a 120 min *in vitro* bread digestion. Fewer reducing sugars were released from all the mushroom enriched breads compared to the control. The levels of reducing sugars in the control bread were significantly higher, at 60 and 120 min, in the *in vitro* digestion. The strongest decrease of reducing sugar release was observed in the 15 % substitution level. The rapidly digested starch during the first 20 min in the mushroom enriched samples was reduced compared to the control. The amounts of the slowly digested starch fractions, over 20–120 min, were much lower in the mushroom bread samples when compared to the control. Fig. 7.3 (d) represents the influence of mushroom powder inclusions on the standardised AUC bread values compared to the control sample. Increasing mushroom powder substitution levels decreased the AUC values. The values of the 15 % mushroom bread samples and 10 % shiitake mushroom bread were significantly lower than the control. These phenomena might be partly attributed to the significantly lower total starch content (Figure 7.2), as well as the reduced degree of starch gelatinisation (Table 7.1) and more resistant starch levels in the mushroom breads than in the control. A significant positive correlation existed between the total starch content and AUC value ($r = 0.699$, at $p \leq 0.001$), whereas a negative correlation existed between ΔH and both the total starch content and AUC ($r = -0.773$ and -0.586 , respectively, at $p \leq 0.001$).

The nature of starch (granule size and amylose/amylopectin ratio) plays a principal role in the glycaemic response of breads (Wolter *et al.*, 2013), in that smaller starch granules with a high specific surface area are susceptible to enzymatic attack, thus increasing the rate of starch digestibility. Starch gelatinisation can lead to reduced crystallinity during bread baking, which

made the starch granules easier to digest by the endogenous enzymes (Fardet *et al.*, 2006). Table 7.1 shows that the addition of mushroom powder inhibited starch gelatinisation of the bread. The composition of starch is also an important factor in the starch hydrolysis rate. It has been reported that amylose molecules form a compact amylose matrix by recrystallisation, that then limits the mobility of the enzymes (Wolter *et al.*, 2013). Compared to amylose, every amylopectin molecule has a large surface area, which makes it more enzymatically susceptible (Singh *et al.*, 2010). Previous research has illustrated that fibre-enriched flour affects starch digestibility through fibre associating with amylose (Wolter *et al.*, 2013).

Dietary fibre is resistant to the digestion within the human small intestine and benefits the “good” microfauna growth through fermentation in the human colon (Ho *et al.*, 2013). Several researchers have investigated the effect of the inclusion of fibre on the glycaemic response of foods (Brennan *et al.*, 1996; Brennan and Tudorică, 2008; Foschia *et al.*, 2015 b; Ho *et al.*, 2015; Liu *et al.*, 2016). The three species of mushroom powder used in this study have much higher total dietary fibre contents than wheat flour. Table 7.3 illustrated significant negative correlations between AUC and all IDF, SDF and TDF ($p \leq 0.001$, 0.05 and 0.001, respectively). Interestingly, compared with SDF, both IDF and TDF played more important roles in modulating the starch digestibility of the final bread samples. This may be attributed to the fact that fibre has the capacity to protect the starch granules from human physical digestive actions inside the stomach by increasing the viscosity of the digesta and form a physical “barrier” to the digestion of starch granules (Brennan *et al.*, 1996; Ho *et al.*, 2015). In addition, dietary fibre could form a close and compact matrix with other ingredients in bread, such as protein and

starch, and such complex formations played a pivotal role in inhibiting enzyme accessibility to starch granules (Cleary and Brennan, 2006).

As mentioned above, the three mushroom powders used in this experiment contained more protein than wheat flour, which may be another reason for the reduced glycaemic response of the mushroom enriched breads. It has been observed that even small amounts of protein in food products were enough to alter the starch digestibility and other functional properties (Singh *et al.*, 2010) in that the protein matrix is able to entrap the starch granules and so reduce accessibility to enzyme attack (Srichamroen, 2014). Continuous protein strands have an encapsulation effect on large starch granules, resulting in well-formed protein-starch complexes; thus, limiting starch degradation and sugar liberation (Cleary and Brennan, 2006).

The results of our previous work have shown that the fat contents of all three mushroom species powders were higher than those of wheat flour. It has also been shown that the proportion of polyunsaturated fatty acids in the mushroom fat can be as high as around 75 %; including 68.8–84 % linoleic acid; 19.2 % palmitic acid and 8.3 % oleic acid (Cheung, 2008). Singh *et al.* (2010) illustrated that the inclusion of palmitic and oleic acids was a way to limit amylose accessibility to hydrolysis enzymes by 35 % inhibiting gelatinisation, delaying retrogradation and reducing susceptibility to digestive enzymes.

Oleic and linoleic acids in the mushroom extracts have been shown to have a strong anti- α -glucosidase capability (Su *et al.*, 2013). Furthermore, edible mushrooms are a good sources of

organic acids and contain at least five organic acids (Valentão *et al.*, 2005), it in turn organic acids limit starch digestibility and, subsequently, decrease glycaemic response of foods (Shumoy and Raes, 2017). This effect may be attributed to the ability of organic acids to slow the gastric emptying rate and strengthen the protein-starch formation (Fardet *et al.*, 2006). In addition, polyphenolic compounds or phenolic acids in the added mushroom powder (Fig. 7.4a) could act as non-proteinaceous amylase inhibitors (Singh *et al.*, 2010). Choo and Aziz (2010) reported that polyphenols reduced starch hydrolysis by binding with amylases.

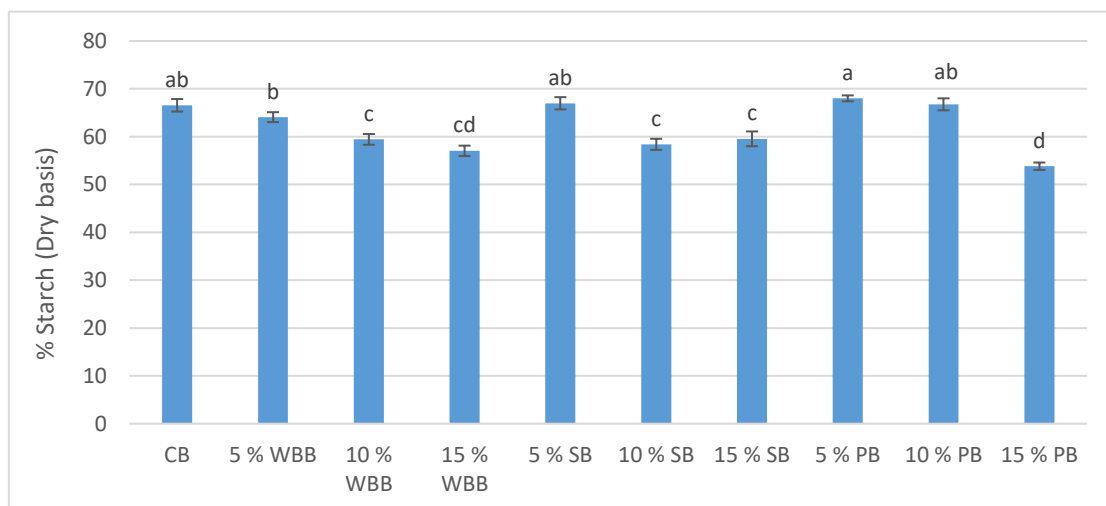


Figure 7.2 The total starch content

Comparing the control to 5 %, 10 %, 15 % white button mushroom bread (a); shiitake mushroom bread (b); and porcini mushroom bread (c): white button mushroom bread (WBB); shiitake mushroom bread (SB) and porcini mushroom bread (PB). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$).

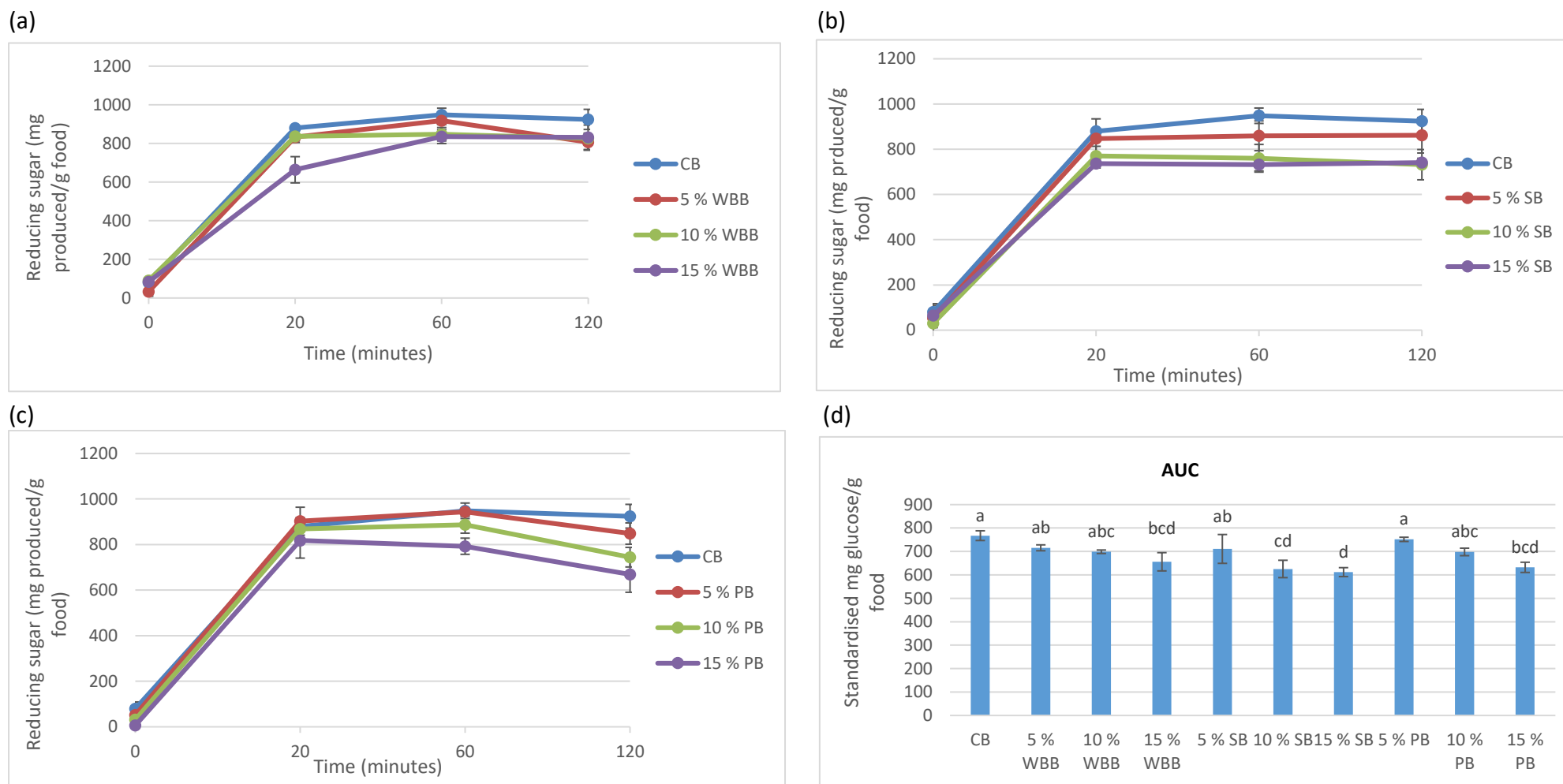


Figure 7.3 Levels of reducing sugars released during *in vitro* digestion

Comparing the control to 5 %, 10 %, 15 % white button mushroom bread (a); shiitake mushroom bread (b); and porcini mushroom bread (c). Values for area under the curve (AUC) (d): white button mushroom bread (WBB); shiitake mushroom bread (SB) and porcini mushroom bread (PB). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$).

7.3.3. Effect of mushroom powder on the total phenolic contents and antioxidant properties of bread

Fig. 7.4 shows the total phenolic content, DPPH radical-scavenging and the oxygen radical absorbance capacity (ORAC) of the fortified breads. Bread made with 100 % high grade wheat flour had the lowest total phenolic, DPPH radical-scavenging and ORAC levels of all samples. Mushroom powder substitute for wheat flour enhanced the total phenolic content significantly and the antioxidant capacities of the fortified bread samples. Table 7.3 shows significant positive correlations between TPC and both DPPH and ORAC ($r = 0.960$ and 0.979 , respectively; at $p \leq 0.001$), and between DPPH and ORAC ($r = 0.966$, $p \leq 0.001$).

Bread production processing, including mixing, fermentation and baking has been shown to influence the final phenolic contents and activity with research indicating that the antioxidant capacity of bread decreased after baking (Gawlik-Dziki *et al.*, 2013). This observation was mainly due to the high temperature that led to a decrease in, or oxidation of, some of the antioxidant compounds that were not thermally stable. Thermal treatment may also liberate part of the insoluble bound phenolics and enhance the antioxidant properties (Swieca *et al.*, 2014). In addition, the products of the Maillard reaction are able to affect the antioxidant capacity of bread (Gawlik-Dziki *et al.*, 2013). The total phenolic contents of bread were about twice as high as that of flour, possibly due to the positive ability of dough fermentation on the release of phenolics from flour (Swieca *et al.*, 2017). Mushrooms have always been regarded as a good source of phenolic compounds, which has been associated with their antioxidant,

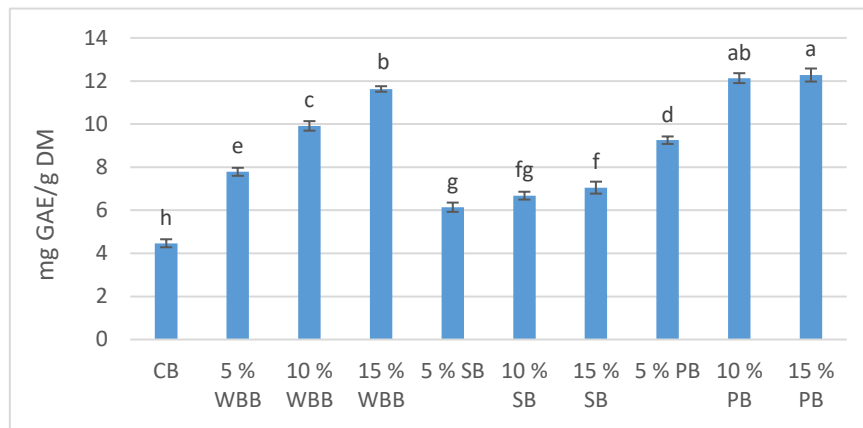
anti-inflammatory or anti-tumour abilities (Lo and Cheung, 2005; Mattila *et al.*, 2001; Palacios *et al.*, 2011). Mushrooms contain antioxidant compounds, including organic acids, alkaloids and ergothioneine (Ribeiro *et al.*, 2008; Sanchez *et al.*, 2015). In this study, the mushroom powders increased the level of bioactive components in the final breads, even after fermentation and baking, increased the antioxidant capacities of the final products.

Except for the white button mushroom breads, there were no significant differences between the 10 and 15 % substitution levels for the DPPH and ORAC assay results in porcini mushroom breads and in the DPPH of shiitake breads (Fig. 7.4), which may be due to the same trend as their total phenolic contents results. Researchers have indicated that the occurrence of phenolic-bread formation during the bread making process may lower the phenolic bioaccessibility index of the phenolic fortified bread (Swieca and Baraniak, 2014). Therefore, the interaction of mushroom phenolics with wheat components (such as starch and protein) acted as one of the most important factors in the antioxidant activity of the final breads, and can include the phenolic-phenolic, phenolic-starch and phenolic-protein matrices. Ozdal *et al.* (2013) indicated that the formation between phenolics (extracted from tea, coffee and cocoa) and milk proteins favoured the reduction of the free phenolic contents and negatively correlated with the antioxidant activity of dairy products. Table 7.3 demonstrates the strong positive correlations between TPC and all IDF, SDF and TDF (at $p \leq 0.05$, 0.001 and 0.001, respectively), between DPPH and both SDF and TDF ($p \leq 0.001$ and 0.01 respectively), and between ORAC and all IDF, SDF and TDF (at $p \leq 0.01$, 0.001 and 0.001, respectively). This indicates a synergistic effect of dietary fibre and antioxidant activity possibly through carrier bioactive compounds. Similar

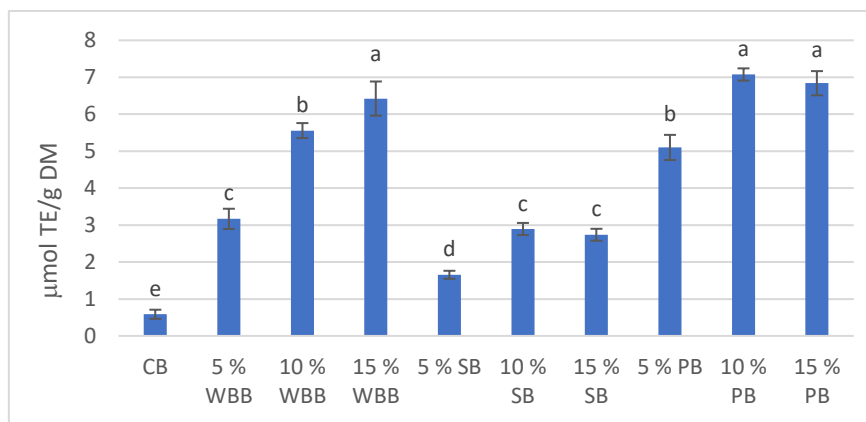
results were found, showing that the addition of carob fibre into wheat flour could increase the TPC and antioxidant capability of the pasta made with the flour (Biernacka *et al.*, 2017).

What is of added interest is that there were significant and positive correlations between ΔH and all TPC, DPPH and ORAC ($r = 0.493, 0.484$ and 0.551 , respectively, at $p \leq 0.01$), whereas negative correlations existed between AUC and ORAC ($r = -0.412$; $p \leq 0.05$), the starch content and all TPC, DPPH and ORAC ($r = -0.409, -0.380$ and -0.490 , respectively; at $p \leq 0.05, 0.05$ and 0.01 , respectively). The results of the *in vitro* starch digestibility and the antioxidant properties of mushroom enriched breads were in agreement with the work of Swieca *et al.* (2014), who added quinoa leaves (a bioavailable phenolic-rich material) to bread and found that the phenolic-protein matrix might lead to blocking of the substrate and/or limitation enzymes. Similarly, green leafy vegetables are good sources of antioxidants and can lower starch digestion by phenolic-starch interaction (Tiwari *et al.*, 2013).

(a)



(b)



(c)

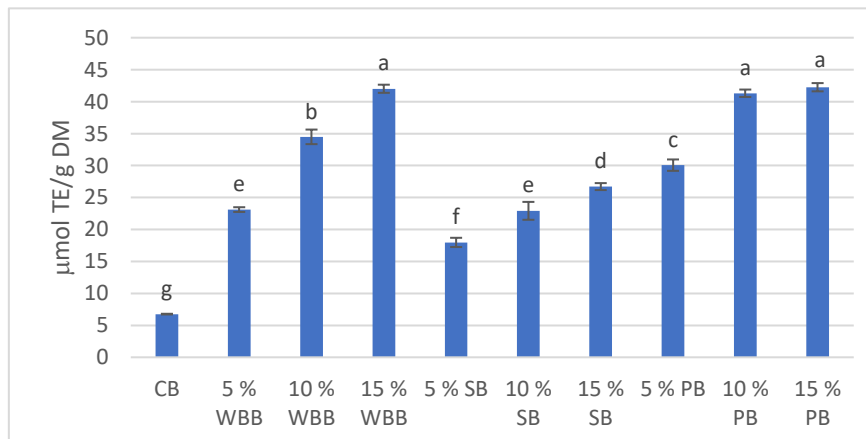


Figure 7.4 Values for total phenolic component and antioxidant capacities of bread samples. Values for total phenolic component (TPC) (a); antioxidant capacities: the DPPH. scavenging activities (b); and the ORAC assay results (c). Comparing the control bread (CB) to all mushroom powder enriched bread samples: white button mushroom bread (WBB); shiitake mushroom bread (SB) and porcini mushroom bread (PB). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$).

7.3.4. The effect of mushroom powder on the microstructure of the bread crust and crumb

Scanning electron microscopy of the control and all mushroom enriched bread crumbs and crusts are shown in Fig. 7.5. The pictures illustrate the effect of the inclusion of mushroom powder on the morphological changes in bread structure during the whole bread making process. The small starch granules were observed as spherical or oval shapes that protruded from the gluten matrix (Srichamroen, 2014) and the large granules, lenticular in shape, were dispersed in the gluten-protein matrix (Ammar *et al.*, 2016). Fig. 7.5a shows that the starch-protein matrix of the control bread crumb was well formed and there was a strong and complex connection in all the components, with some small cavities. The mushroom powder incorporated samples had different protein-starch binding patterns. With the increase in mushroom supplementation, some large starch granules were seen to move to the exterior surface, which might reflect their lower degree of gelatinisation, and this observation was in agreement with the results of DSC (Table 7.1) and is similar to the observations regarding the surface starchy granules observed in protein matrix recorded by Bahal *et al.* (2013). These factors may be related to the limited starch digestibility of the mushroom fortified breads. Increasing amounts of both white button and porcini mushroom made the crumb structures more porous, rough and less dense. While, for the shiitake mushroom additions, the crumb structure started to be less compact from the 5 % addition level upwards. All three mushroom species had higher insoluble dietary fibre, protein and fat contents than wheat flour alone. It has been shown that insoluble dietary fibre could disrupt the gluten network and result in a discontinuous structure in the bread crumb (Ronda *et al.*, 2012). Besides, the different levels

and sizes of insoluble fibre from the different mushroom species could be considered as a reason for their different structures at the same substitution levels (Ronda *et al.*, 2012). These observations about shiitake mushroom enriched breads may have contributed to their slightly higher insoluble fibre and lower protein amounts than the other two species. The 5 %, 10 % white button mushroom breads and the 5 % porcini mushroom bread had a smooth surface and more compact appearance not dissimilar to the control (5 % porcini mushroom bread looked even more compact than the control). This illustrated the continuous structure of the starch granules enveloped into the protein matrix. This might be due to the abundance of proteins the white button and porcini mushrooms provided to breads, which was supported by the findings of Cleary and Brennan (2006) who showed that protein could help develop a diffused and coagulated protein network to entrap starch granules. Compared with the control, the three mushroom supplemented samples showed a continuous sheet formation instead of wrapping, which may be attributed to the presence of large areas of sheet-like protein structures covering the starch granules (Ammar *et al.*, 2016). In the case of breads with additional lipids, the structures tended to be smoother and nonporous without any phase separation (Altamirano-Fortoul *et al.*, 2015). So, the antagonistic effects of additional insoluble fibre, protein and fat, and their interactions with starch granules could be responsible for the final different structures of the bread crumb.

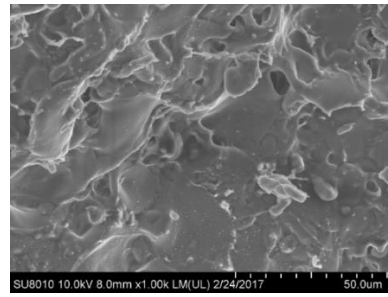
Fig. 7.5b shows the scanning electron micrographs of bread crust sections. The control crust layer represented a continuous veil-like film, with large starch granules and some small ones, which were slightly deformed, encapsulated completely and uniformly distributed in the

lipoprotein network. In terms of the mushroom enriched samples, loose starch granules were visible on the surface of the network with large areas of heterogeneous starch structures in a discontinuous phase. The starch granules possibly could, therefore, have altered the bread architecture in different ways (Altamirano-Fortoul *et al.*, 2015). The inclusion of mushroom powder made some starch granules lose their identity and aggregate in polymeric areas of protein.

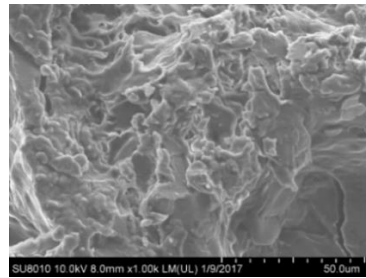
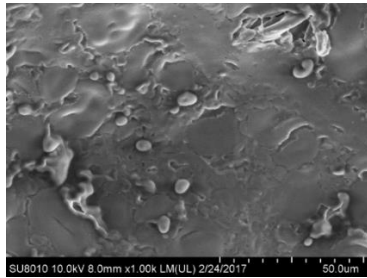
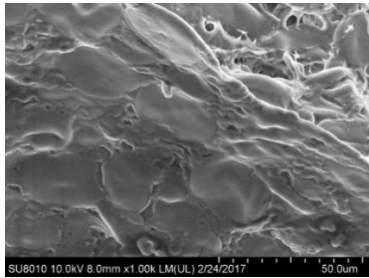
7.4. Conclusion

The 15 % white button, 10 % and 15 % shiitake and 15 % porcini mushroom enriched breads resulted in lower glycaemic indexes than the control. Increased mushroom powder inclusion levels led to progressive increase in the total phenolic content and antioxidant capacities for most samples (the 10 % and 15 % levels did not show any obvious difference). Furthermore, the mushroom powder disrupted the initial intact starch-protein matrix and reconstructed the bread crust and crumb microstructures. The SEM images represented the results of multiple interactions (synergistic or antagonistic) between the additional nutrients mushroom powder provided and the inherent constituents.

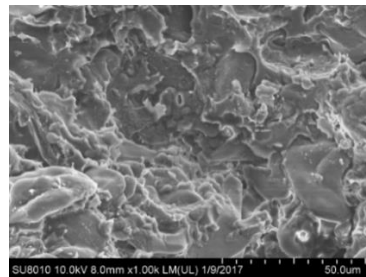
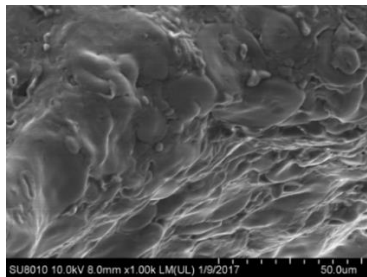
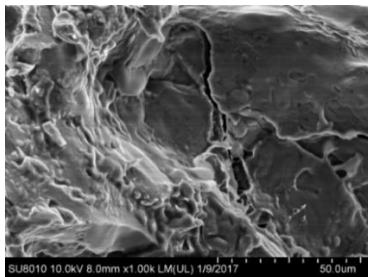
(a)



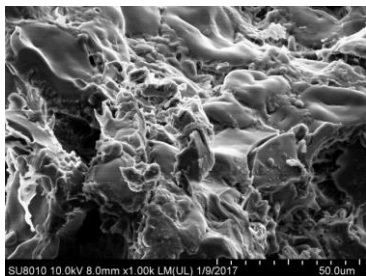
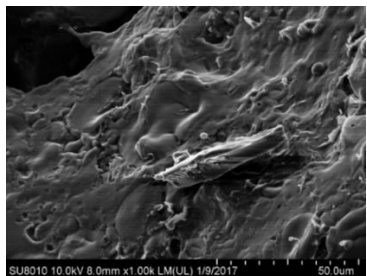
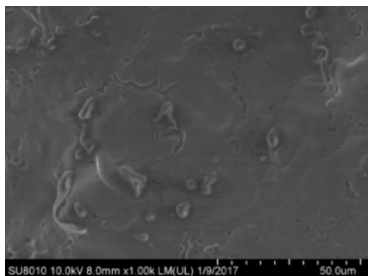
CB



WBB



SB



PB

(b)

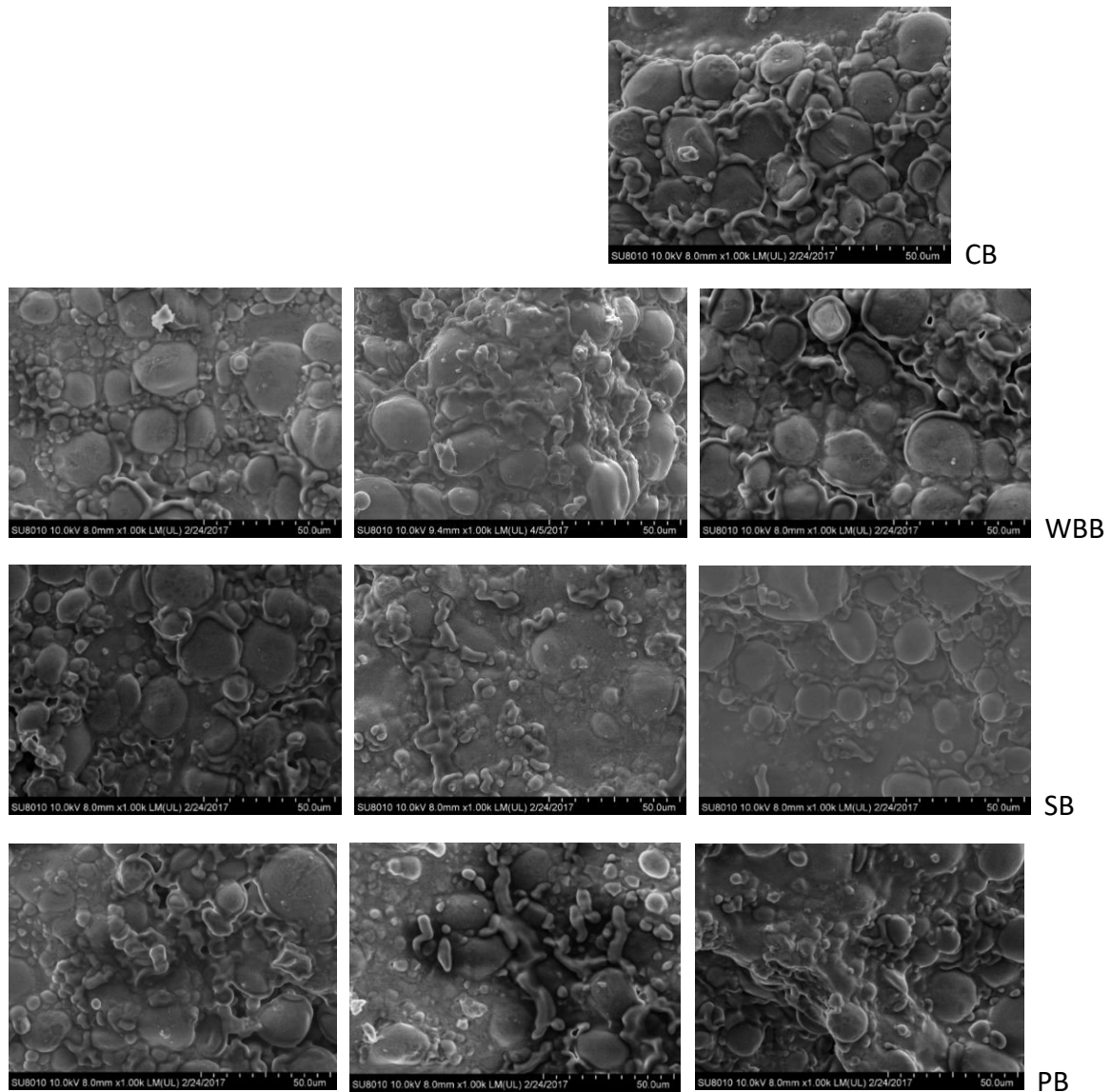


Figure 7.5 Scanning electron micrographs of bread samples

Scanning electron micrographs of bread crumb (a) and bread crust (b) at 1000× magnification: the control bread (CB); white button mushroom bread (WBB); shiitake mushroom bread (SB) and porcini mushroom bread (PB). From left to right: 5 %; 10 % and 15 % substituent level.

Chapter 8

Matrix transformation of fortified extruded snacks: effect of different mushroom powders on texture, macrostructure, nutrition, microstructure and digestibility

(Submitted to the Journal of Cereal Science)

Abstract

In this study, white button mushroom (*Agaricus bisporus*), shiitake (*Lentinula edodes*) and porcini mushrooms (*Boletus edulis*) were incorporated into semolina to produce hot extruded snacks. These final products may introduce more edible mushroom species to western consumers' diet using a relatively both simple prepare and accepting approach. A series of analysis were conducted in order to evaluate these functional products comprehensively, including the nutritional profile, macrostructure, colour, textural properties, microstructure, starch characteristics (content, gelatinisation and digestibility) and antioxidant activities (total phenolic content, DPPH and ORAC). The results indicated that the three different mushroom powders made different effects on the physical properties. While all the mushroom addition tended to reduce the expansion, decrease water absorption index, increase water solubility index and altered microstructure characteristics. The mushroom enriched samples represented higher fibre content and delayed starch gelatinisation, which may be responsible for the decreased glycaemic response. What's more, such inclusion resulted in delivering more phenolic compounds and antioxidant benefits to consumers. Overall, even though mushroom powder could bring about changes in the appearance, macrostructure and textural properties

of the final snacks, mushroom powders could be incorporated into hot extruded products to provide their functional health effects.

8.1. Introduction

Hot extrusion is a multistep, thermal food process that includes mixing, forming, texturising and cooking operations (Brennan *et al.*, 2013 b). The popularity of hot extruded snack products is increasing due to their puffy, crisp and crunchy textures (Brennan *et al.*, 2008 a). Extrusion processing has recently changed to being an extremely versatile and well-reputed industrial food technology, especially when used in the preparation of ready-to-eat snack products (Wani and Kumar, 2016). There is a large variety of snack products available in a wide range of sizes, shapes, flavours and colours. While most of these hot extruded snacks are derived from cereals, such as wheat, rice, and potato, they are often nutritionally unbalanced; for example, they lack of vitamins, minor minerals, dietary fibre, essential amino acids and other bioactive compounds (Brennan *et al.*, 2013 a; Parada *et al.*, 2011). Another disadvantage of hot extrudates in terms of nutrition may be the decreased starch digestibility, which could be attributed to starch gelatinisation and fragmentation by the shearing process. Thus, in general, hot extruded snacks are treated as high glycaemic index foods (Brennan *et al.*, 2012 a; Brennan *et al.*, 2013 b; Onwulata *et al.*, 2010). As healthier snack products appeal to consumers, the addition of bioactive phytochemicals provides the opportunity to modify the nutritional properties by decreasing, or preventing, the destruction of functional nutrients and, even, increasing some nutritional components (Wani and Kumar, 2016).

In this study, three different edible mushroom powders were incorporated into hot extruded products. The white button mushroom (*Agaricus bisporus*) is the most commonly consumed edible mushroom in the US and European countries (Calvo *et al.*, 2016) and occupies slightly more than half of the total world mushroom production (Breene, 1990). The other two species, shiitake (*Lentinula edodes*) and porcini mushrooms (*Boletus edulis*), are specialty mushrooms that are grown and consumed in Asian countries, such as China, Korea and Japan (Chang and Miles, 1984; Farr, 1983). They are relatively unfamiliar to western consumers. Edible mushrooms are generally good sources of high-quality protein, bioactive polysaccharides, vitamins, minor minerals, dietary fibre, unsaturated fatty acids and other bioactive components (Breene, 1990; Calvo *et al.*, 2016; Lu *et al.*, 2016). In particular, they contain ergothioneine, which humans cannot synthesise themselves (Weigand-Heller *et al.*, 2012). All these compounds may play important roles in the medicinal and therapeutic values of edible mushrooms. Based on clinical studies, roles include the prevention of cancer, anti-influenza, anti-hypercholesterolemia and anti-polio effects, the treatment of blood platelet aggregation, the relief of muscle and joint tension, and antioxidant and anti-hypertensive effects (Breene, 1990; Calvo *et al.*, 2016; Lu *et al.*, 2016). In order to introduce more edible mushroom choices to western consumers that are relatively simple to prepare in an easily accepted way, the incorporation of mushroom powder into hot extruded products provides an opportunity. In addition, for some mushroom species, like shiitake mushroom, their umami flavours could be improved during drying by the breakdown of proteins into amino acids (Lu *et al.*, 2016). Such additions may not only enhance the nutritional benefits of the extrudates, but also result in better flavours and taste.

The objectives of the current study were to incorporate novel food ingredients from three different mushroom species into hot extruded snacks and to confirm the health benefits of such additions after undergoing hot extrusion processing. In this study, the nutritional components, calorie contents, starch gelatinisation and digestibility, and antioxidant properties of the final extrudates were compared with the control (no mushroom powder). At the same time, we wanted to know whether such inclusion compromises the inherently attractive physical and textural properties of hot extruded products, such as colour, physical properties, hardness, crunchiness and microstructure characteristics. The results would be valuable to evaluate how different mushroom species affect, and modify, the quality and functional properties of hot extruded snacks for value-added food applications.

8.2. Materials and methods

8.2.1. Materials

As described in 3.1.1.

8.2.2. Extrusion processing

The extrusion processing was carried out as described in 3.2.3. The operating details are summarised in Table 8.1.

Table 8.1 The processing parameters of hot extruded cooking

Sample	Shaft speed (rpm)	Feed rate	Current Amps	Torque
CHE	200	20	5.53	56.5
5 % WBHE	200	25	5.77	60
10 % WBHE	200	25	5.98	64.5
15 % WBHE	200	25	5.95	61.7
5 % SHE	200	15	5.44	46
10 % SHE	200	15	4.92	42
15 % SHE	200	15	3.65	48.4
5 % PHE	200	25	6.04	70
10 % PHE	200	30	6.17	66.8
15 % PHE	200	30	6.22	62.8

Abbreviations: CHE=control hot extruded; WBHE=white button mushroom hot extruded; SHE=shiitake mushroom hot extruded; PHE=porcini mushroom hot extruded

8.2.3. Near infrared spectroscopy

The near infrared spectroscopy analysis was determined as described in 3.7.

8.2.4. Physicochemical characteristics

The physical properties of hot extruded samples were determined as described in 3.3.3.

The textural properties of hot extruded samples were determined as described in 3.4.4.

The method used for fibre analysis was mentioned in 3.5.2 and the method used for protein analysis was mentioned in 3.5.3.

8.2.5. Colour

The colour of samples was determined as described in 3.3.5.

8.2.6. Differential scanning calorimetry (DSC)

The DSC analysis were determined as described in 3.8.

8.2.7. Total phenolic content and antioxidant capabilities

The total phenolic content and antioxidant analysis were determined as described in 3.10.

8.2.8. The starch content and *in vitro* starch digestibility analysis

The method used for starch content analysis was mentioned in 3.5.1. and the method used for starch digestion was mentioned in 3.9.

8.2.9. Microscopy

The microscopy analysis was determined as described in 3.11.

8.2.10. Statistical analysis

Statistical analysis was carried out as described in 3.12.

8.3. Results and discussion

8.3.1. Near infrared spectroscopy

The fat, carbohydrate, water and calorie contents of the control and mushroom supplemented hot extruded samples were measured using a Calorie Answer™ machine (Table 8.2); a rapid, reproducible, cost-effective and economical method of analysis with an acceptable accuracy range. Other advantages of such a method, as an alternative to conventional bomb calorimetry, were minimal sample preparation, the capability to analyse a wide range of foods, as well saving time and effort (Lau *et al.*, 2016).

The carbohydrate content of most of the mushroom enriched samples did not show any significant differences from the control. The fat content of all the hot mushroom extruded products was significantly higher than that the control, and increased gradually with increasing mushroom powder content. According to our previous study, all three mushroom species contained more fat than semolina on a dry weight basis: (white button [2.42 ± 0.04 %], shiitake mushroom [1.48 ± 0.02 %], porcini mushroom [3.03 ± 0.03 %] and semolina [1.17 ± 0.09 %]). This finding for hot extruded samples confirmed our expectations. Fortunately, 75 % of mushroom fat is in the form of polyunsaturated fatty acids (Cheung, 2008). However, during the hot extrusion process the samples suffered from high temperatures (usually above 70°C) (Brennan *et al.*, 2013 b), and both the degree of unsaturated fatty acids and the presence of a high temperature were factors that promoted lipid oxidation (Achir *et al.*, 2006). Mushrooms are good sources of various antioxidants, including phenolic compounds and polysaccharides

(Alam *et al.*, 2011). The antioxidants in mushroom powder could inhibit oxidation activity by reacting with lipid radicals to prevent them from damaging other fatty acids, deactivate reactive oxygen species, or chelate transition metals (Roman *et al.*, 2013). As a result, it was difficult to differentiate the individual influences of these factors on the nutritional benefits of the final samples in this study. Further study may be needed in the future.

According to Table 8.2, in terms of the calorie content, the addition of mushroom powder did significantly and gradually increase the samples' energy compared to the control. Such an increase may be attributed to similar trends in the fat content. Significant positive correlation (Table 8.3) exists between sample energy and fat content ($r = 0.857$, at $p \leq 0.001$). This finding may illustrate that the inclusion of mushroom powder has a negative effect on the health properties of hot extruded products, especially for obesity control. A high unsaturated fat diet was recommended to reduce total cholesterol, low density lipoproteins and cholesterol, and to control the glycaemic response, improve insulin sensitivity and fasting plasma triglycerides contents (Dessein *et al.*, 2000; Gardner and Kraemer, 1995; Parillo *et al.*, 1992). The point about the glycaemic index was in agreement with our results from an *in vitro* starch digestibility study below. There were significant negative correlations between energy content and both starch content and AUC values ($r = -0.906$ and -0.768 , respectively, at $p \leq 0.001$). What is of added interest is that there were positive correlations between energy content and TPC, DPPH and ORAC values. One can speculate that the higher calorie content may play an active role in the antioxidant benefits of final products.

Table 8.2 The nutrient components of hot extruded samples

Sample	Energy kcal	Fat %	Carbohydrate %	Water %	Protein %	IDF %	SDF %	TDF %
CHE	355.33±1.53 ^e	0.37±0.058 ^e	78.73±0.50 ^a	7.13±0.25 ^{bc}	11.46±0.13 ⁱ	2.44±0.19 ^d	2.65±0.045 ^{ab}	5.09±0.15 ^e
5 % WBHE	357±0 ^e	1.1±0.1 ^d	78.2±0.36 ^{ab}	7.13±0.15 ^{bc}	12.61±0.020 ^{ef}	3.00±0.097 ^{cd}	2.27±0.30 ^b	5.27±0.21 ^e
10 % WBHE	360.67±0.58 ^{cd}	1.53±0.12 ^{bc}	77.67±0.51 ^{abc}	6.6±0.1 ^{cd}	14.01±0.12 ^c	4.45±0.17 ^{bc}	2.49±0.18 ^b	6.94±0.34 ^{cd}
15 % WBHE	364.67±0.58 ^a	1.83±0.058 ^{ab}	76.8±0.17 ^c	5.83±0.058 ^e	15.80±0.028 ^a	5.23±0.25 ^b	2.24±0.10 ^b	7.47±0.15 ^{bc}
5 % SHE	359.67±0.58 ^d	1.27±0.058 ^{cd}	76.87±0.29 ^c	6.53±0.15 ^d	12.35±0.074 ^{efg}	3.53±0.16 ^{cd}	2.42±0.44 ^b	5.96±0.60 ^{de}
10 % SHE	362±0 ^{bc}	1.4±0.1 ^{cd}	78.03±0.25 ^{ab}	6.43±0.29 ^d	12.76±0.088 ^e	5.46±0.85 ^b	2.64±0.19 ^{ab}	8.10±0.66 ^{bc}
15 % SHE	363.33±0.58 ^{ab}	2±0.1 ^a	78.13±0.47 ^{ab}	6.6±0.1 ^{cd}	12.52±0.11 ^{ef}	8.00±0.21 ^a	2.94±0.25 ^{ab}	10.93±0.46 ^a
5 % PHE	355.67±0.58 ^e	1.1±0.17 ^d	78.33±0.50 ^{ab}	7.73±0.25 ^a	12.22±0.18 ^{fg}	3.15±0.19 ^{cd}	2.75±0.0032 ^{ab}	5.90±0.20 ^{de}
10 % PHE	359.33±0.58 ^d	1.4±0.2 ^{cd}	78.07±0.23 ^{ab}	7.4±0.36 ^{ab}	13.43±0.077 ^d	4.34±0.63 ^{bc}	3.13±0.25 ^{ab}	7.47±0.39 ^{bc}
15 % PHE	361±0 ^{cd}	1.6±0.1 ^{bc}	77.6±0.26 ^{bc}	7.17±0.12 ^{abc}	14.58±0.22 ^b	5.23±0.49 ^b	3.62±0.44 ^a	8.85±0.057 ^b

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CHE=control hot extruded; WBHE=white button mushroom hot extruded; SHE=shiitake mushroom hot extruded; PHE=porcini mushroom hot extruded; IDF=insoluble dietary fibre; SDF=soluble dietary fibre; TDF=total dietary fibre.

Table 8.3 Pearson's correlation coefficient (r) of physicochemical and nutritional properties of hot extruded products

	DPPH	ORAC	AUC	ST	EN	FT	PT	MS	DS	CRU	HAR	H	EXP	ER	Lsp	IDF	SDF	TDF	WAI	WSI
TPC	0.973***	0.822***	-0.671***	-0.459*	0.417*	0.520**	0.763***	-0.28	0.836***	-0.54**	0.548**	0.390*	-0.729***	-0.712***	0.139	0.344	0.626***	0.466**	-0.753***	0.069
DPPH		0.882***	-0.686***	-0.459*	0.432*	0.581***	0.781***	-0.305	0.860***	-0.506**	0.533**	0.313	-0.724***	-0.708***	0.158	0.369*	0.557***	0.472**	-0.762***	0.056
ORAC			-0.776***	-0.655***	0.608***	0.784***	0.828***	-0.562***	0.635***	-0.13	0.156	0.301	-0.504**	-0.488**	0.272	0.531**	0.327	0.561***	-0.727***	0.137
AUC				0.826***	-0.768***	-0.855***	-0.600***	0.718***	-0.336	-0.07	0.102	-0.557***	0.593***	0.589***	-0.694***	-0.799***	-0.452*	-0.833***	0.800***	-0.575***
ST					-0.906***	-0.862***	-0.572***	0.910***	-0.031	-0.361*	0.372*	-0.597***	0.338	0.33	-0.721***	-0.9***	-0.223	-0.867***	0.634***	-0.605***
EN						0.857***	0.669***	-0.891***	0.032	0.262	-0.254	0.437*	-0.411*	-0.401*	0.724***	0.816***	0.025	0.742***	-0.539**	0.538**
FT							0.633***	-0.810***	0.183	0.278	-0.248	0.469**	-0.420*	-0.410*	0.660***	0.823***	0.152	0.78***	-0.615***	0.503**
PT								-0.513**	0.577***	-0.308	0.341	0.11	-0.556***	-0.534**	0.111	0.35	0.066	0.332	-0.469**	-0.093
MS									0.136	-0.478**	0.466**	-0.524**	0.195	0.185	-0.754***	-0.871***	-0.042	-0.796***	0.523**	-0.600***
DS										-0.752***	0.787***	0.035	-0.669***	0.658***	-0.197	-0.049	0.484**	0.077	-0.521**	-0.274
CRU											-0.973***	0.249	0.620***	0.613***	0.399*	0.414*	-0.318	0.294	0.095	0.474**
HAR												-0.23	-0.594***	-0.583***	-0.428*	-0.418*	0.271	-0.31	-0.104	-0.507**
H													-0.287	-0.285	0.649***	0.739***	0.550**	0.803***	-0.700***	0.648***
EXP														0.999***	-0.337	-0.378*	-0.468**	-0.458*	0.559***	-0.116
ER															-0.342	-0.376*	-0.472**	-0.457*	0.553**	-0.123
Lsp																0.888***	0.229	0.858***	-0.586***	0.882***
IDF																	0.278	0.971***	-0.694***	0.747***
SDF																		0.5**	-0.654***	0.345
TDF																			-0.788***	0.759***
WAI																				-0.513**

TPC, mg GAE/g DM; DPPH, $\mu\text{M TE/g DM}$; ORAC, $\mu\text{M TE/g DM}$; AUC, In vitro area under the curve value; ST, Starch %; EN, Energy (kcal); FT, Fat %; PT, Protein %; MS, Moisture %; DS, Density g/L; CRU, Cruchiness/Number of peaks; HAR, Peak force/Hardness of product; H, $\Delta\text{H J/g}$; EXP, Expansion; ER, Radial expansion ratio; Lsp, Specific length; IDF, Insoluble dietary fibre %; SDF, Soluble dietary fibre %; TDF, Total dietary fibre %; WAI, Water absorption index; WSI, Water solubility index. *, significant at $p \leq 0.05$. **, significant at $p \leq 0.01$. ***, significant at $p \leq 0.001$.

Table 8.4 Physical and textural properties of extruded snack products

Sample	% Expansion	ER	Lsp mm/g	Product Density g/l	WAI	WSI	Moisture content %
CHE	394.9 ± 31.02 ^{bcd}	15.69±2.51 ^{bcd}	45.36±2.50 ^e	138.83±5.13 ^{cd}	6.55 ± 0.41 ^a	11.063 ± 1.029 ^e	8.24±0.05 ^a
5 % WBHE	446.68±32.46 ^a	20.05±2.98 ^a	44.01±3.85 ^e	139.37±3.51 ^{cd}	6.31 ± 0.098 ^{ab}	11.21 ± 1.35 ^e	7.80±0.20 ^{cd}
10 % WBHE	406.52±23.33 ^{bc}	16.58±1.91 ^{bc}	45.04±2.94 ^e	148.31±4.28 ^c	6.027 ± 0.22 ^{abc}	11.32 ± 0.37 ^e	7.46±0.08 ^{def}
15 % WBHE	346.18±16.23 ^g	12.01±1.12 ^g	52.43±3.01 ^{bc}	162.95±2.26 ^b	5.71 ± 0.092 ^{bcd}	12.018 ± 0.018 ^{cde}	7.18±0.22 ^{fg}
5 % SHE	400.45±26.46 ^{bcd}	16.1±2.13 ^{bcd}	51±4.60 ^{bcd}	138.68±1.85 ^{cd}	6.27 ± 0.18 ^{ab}	14.72 ± 1.16 ^{bc}	7.86±0.12 ^{bc}
10 % SHE	420.07±25.14 ^b	17.71±2.14 ^b	54.18±7.30 ^b	131.28±4.68 ^d	5.61 ± 0.18 ^{cd}	17.49 ± 0.79 ^{ab}	7.20±0.03 ^{efg}
15 % SHE	375.45±19.62 ^{def}	14.13±1.48 ^{def}	60.97±4.85 ^a	135.01±2.45 ^d	5.31 ± 0.096 ^{de}	18.89 ± 0.59 ^a	6.96±0.18 ^g
5 % PHE	388.73±22.87 ^{cde}	15.16±1.82 ^{cde}	47.55±2.66 ^{de}	165.17±7.04 ^b	5.80 ± 0.051 ^{bcd}	11.48 ± 1.37 ^{de}	8.18±0.10 ^{ab}
10 % PHE	366.3±25.28 ^{efg}	13.48±1.81 ^{efg}	50.27±3.08 ^{bcd}	167.41±1.61 ^{ab}	5.42 ± 0.22 ^{cde}	14.28 ± 1.08 ^{cd}	7.74±0.03 ^{cd}
15 % PHE	359.18±22.08 ^{fg}	12.95±1.65 ^{fg}	49.69±4.44 ^{cd}	177.66±6.36 ^a	4.97 ± 0.28 ^e	14.40 ± 0.34 ^c	7.55±0.03 ^{cde}

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CHE=control hot extruded; WBHE=white button mushroom hot extruded; SHE=shiitake mushroom hot extruded; PHE=porcini mushroom hot extruded.

8.3.2. Physicochemical properties

In this study, the expansion ratio of the three different mushroom species of enriched samples showed an inverse relationship with the amount of enriched mushroom powder incorporated. These results were all significantly lower, or similar, to the control except for the 5 % white button mushroom substitution (Table 8.4). The amount of water available at the die face of the extruder to be flashed off was an important factor in the expansion ratio of the final products (Brennan *et al.*, 2008 a). In addition, according to our previous study, all three mushroom species contained significant higher total dietary fibre than semolina (semolina [3.95 ± 0.44 %], white button [24.61 ± 2.34 %], shiitake mushroom [41.97 ± 1.08 %] and porcini mushroom [26.26 ± 1.13 %] on a dry weight basis). The IDF and TDF contents of mushroom enriched hot extruded products increased significantly and gradually compared to control with the increase of substituent level (Table 8.2). Such higher dietary fibre contents and the relative absence of starch could decrease the free water of the raw materials in the extruder barrel. This, in turn, limited the expansion of the products (Brennan *et al.*, 2013 a). This hypothesis is confirmed there are negative correlations between the expansion of sample and all IDF, SDF and TDF (Table 8.3). Furthermore, the reduced expansion ratio of the mushroom enriched samples may be due to the higher fat contents in the mushroom powder than in the semolina; thus, reducing the extrudate viscosity and, consequently, reducing the pressure difference the pre- and post- the die face (Robin *et al.*, 2011). Table 8.3 demonstrates a negative correlation between sample expansion and fat content ($r = -0.420$, $p \leq 0.05$). Generally, the sample density showed an inverse trend of expansion ratio in this study ($r = -0.669$, $p \leq 0.001$); this was

supported by the work of Karkle *et al.* (2012). However, the specific length of the extruded samples increased significantly compared to the control. Some researchers have reported similar results, showing that the inclusion of fibre rich materials resulted in increased longitudinal expansion (Jin *et al.*, 1994; Karkle *et al.*, 2010; Lue *et al.*, 1991). This observation is consistent with that of Table 8.3 illustrating strong positive correlations between the specific length and both IDF and TDF ($r = 0.888, 0.858$, respectively, at $p \leq 0.001$). Furthermore, a significant positive correlation is observed between specific length and fat content (Table 8.3). This phenomenon may be explained by the reduced viscosity caused by enriched lipids or other ingredients changing the expansion direction.

In this study, water absorption index (WAI) values decreased significantly compared to the control. In contrast, the water solubility index (WSI) values of the white button enriched samples increased slightly and the other two mushroom samples increased significantly and gradually when compared to the control (Table 8.4). The WAI results were supported by the work of Brennan *et al.* (2012 b), who investigated the influence of the inclusion of chestnut mushroom co-products on extruded snack products, where the WAI values decreased significantly and gradually with increasing levels of substitution. There are strong negative correlations between WAI and the proportion of IDF, SDF and TDF (Table 8.3), by increasing water holding capacity of enriched fibre to the final products. In fact, the WAI is a parameter that measures the proportion of intact starch granules that were fully gelatinised; in particular, there is a positive relationship between the degree of starch fragmentation and gelatinisation, and the WAI (Brahma *et al.*, 2016; Sompong *et al.*, 2011). This fact is further supported by the

significant negative correlation between ΔH and WAI ($r = -0.7, p \leq 0.001$) found in this study. A higher WAI means there were more shorter starch chains from the fragmentation of the long chains during the extrusion screwing, with the shorter chains having stronger solubility capacity (Hagenimana *et al.*, 2006). This could be explained as the enriched fibre materials preventing the starch from fragmenting and gelatinising (Brennan *et al.*, 2012 a). Furthermore, it has been reported that a lower starch content could be a reason for the decreased WAI (Brennan *et al.*, 2012 b). Table 8.3 illustrates a positive correlation between starch content and WAI ($r = 0.634, p \leq 0.001$). Indeed, the starch contents of the three mushroom powders were much lower than those of semolina (semolina [$73.11 \pm 1.17\%$]; white button [$1.90 \pm 0.02\%$]; shiitake mushroom [$2.45 \pm 0.14\%$] and porcine mushroom [$2.62 \pm 0.08\%$] on a dry weight basis). The WSI indicates the amount of soluble starch present after extrusion (Brahma *et al.*, 2016). It is also an indicator of the amount of protein in the proteinaceous blends, and the present state of denatured proteins within the food matrix (Ghumman *et al.*, 2016). In addition, in the control sample, the proteins can prevent amylose solubilisation by crossing linking with starch granules to some extent, thereby obtaining a relatively lower WSI (Singh and Muthukumarappan, 2016). According to Table 8.2, the protein content of final extruded products increased significantly compared to control. While no significant correlation was observed between the protein content and WSI in this study. Furthermore, there were strong positive correlations between the WSI and both IDF and TDF ($r = 0.747$ and 0.759 , respectively, at $p \leq 0.001$). One can speculate that the additional mushroom fibre may interrupt the protein and starch networks, thus increasing the WSI. The WAI was inversely related to the WSI values in this study ($r = -0.513, p \leq 0.01$) and similar findings have been reported by Peęksa *et al.* (2016)

and Singh and Muthukumarappan (2016). Interestingly, Peęksa *et al.* (2016) reported that there was no obvious relationship between WAI and the textural attributes of the hot extruded samples. While this study found different results that significant positive correlations exist between WAI and both sample expansion and radical expansion ratio, meanwhile negative correlations exist between WAI and both sample density and specific length (Table 8.3). Additionally, an increased WSI has been reported previously along with structural improvements, such as uniform and adequate porosity (Peęksa *et al.*, 2016). Certainly, strong positive correlations were illustrated between WSI and both the crunchiness and specific length of hot extruded samples, whereas negative correlation was found between WSI and the hardness of products (Table 8.3).

8.3.3. Textural properties

Both the ingredients and the formulations were important factors in the final textural properties of hot extruded products, as they play an major role in their acceptability by consumers (Brennan *et al.*, 2013 b). Generally, one extruded sample can be divided into two in terms of structure and configuration. The outer coat, formed by a layer of collapsed gas cells, was relatively hard. In contrast, the inner section was formed by gas cells, and so was relatively larger. In this way, the integrity of the outer coat formation and the expansion extent of the extrudate determined the textural characteristics (Brennan *et al.*, 2013 b; Pai *et al.*, 2009). The hardness of the hot white button mushroom extrudate decreased initially then increased dramatically compared to the control. The shiitake mushroom samples decreased significantly

and progressively while the porcini mushroom substitution showed no obvious difference from the control (Fig. 8.2). These differences could be attributed to the differences in concentrations of the ingredients and the interactions between these components within the final products. As all these factors could affect the moisture, molecular gelatinisation, degradation and reassociation under the hot extrusion process this, then, resulted in changes in the textural quality (Korkerd *et al.*, 2015). It has been shown that the inclusion of high amounts of protein and dietary fibre into extruded products led to increased hardness (Korkerd *et al.*, 2015), which would support the results of the white button mushroom samples. The decreased hardness of shiitake mushroom products may be associated with their lower amylose contents, increased pores' size and/or number, and weaker interactions between amylose-amylose and amylose-amylopectin molecules (Vanier *et al.*, 2016). The crunchiness results represented an inverse trend to hardness (Table 8.3, $r = -0.973$, at $p \leq 0.001$). The crisp texture indicated a connected internal structure with fine gas cells (Brennan *et al.*, 2013 b). Table 8.3 further demonstrates the strong positive correlations between crunchiness and expansion ($r = 0.620$, $p \leq 0.001$), radical expansion ($r = 0.613$, $p \leq 0.001$) and specific length ($r = 0.399$, $p \leq 0.05$).

Decreased product hardness and increased crunchiness always has a positive effect on the consumer acceptance of samples (Brennan *et al.*, 2013 b; Hsieh *et al.*, 1989; Korkerd *et al.*, 2015), so most of these mushroom enriched products had satisfactory textural quality. It has been found that the increased WSI was an indicator to the improved structure of the extruded products (Peęksa *et al.*, 2016). The results for the WSI of the shiitake and porcini mushroom

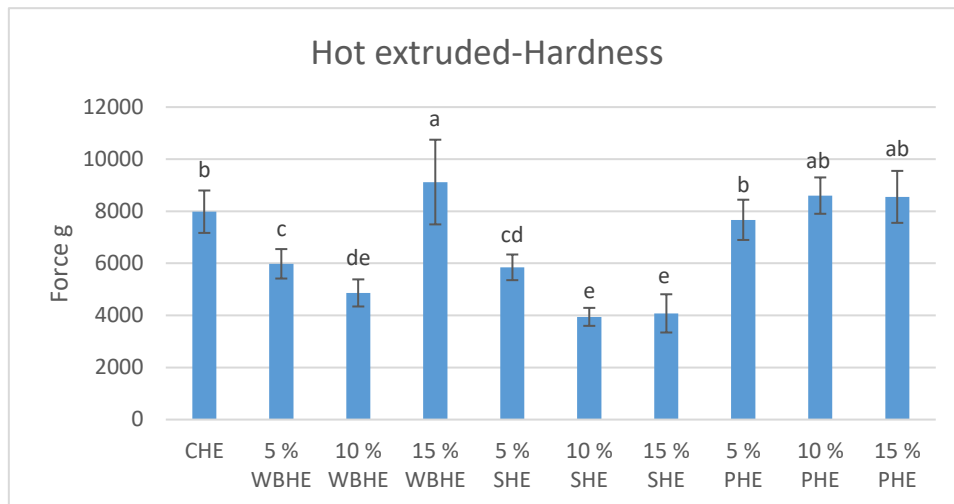
samples were significantly larger than in the control, which was consistent with the results for hardness and crunchiness.



Figure 8.1 The photos of hot extruded samples

From top to bottom (hot extruded products): control hot extruded products, white button mushroom hot extruded products, shiitake mushroom hot extruded products and porcini mushroom hot extruded products. From left to right (except first row): 5 %; 10 % and 15 % mushroom powder substitution levels.

(a)



(b)

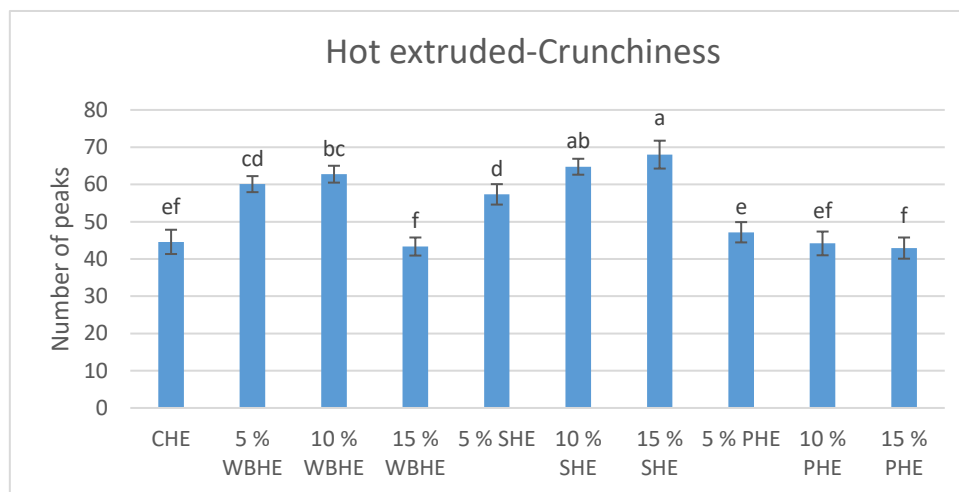


Figure 8.2 Textural properties of hot extruded samples

Values for the hardness (a) and crunchiness (b). Comparing the control (CHE) to all mushroom powder enriched hot extruded samples: white button mushroom hot extruded products (WBHE); shiitake mushroom hot extruded products (SHE) and porcini mushroom hot extruded products (PHE). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$).

8.3.4. Colour

According to Table 8.5, the lightness/darkness (L^*) of some of the mushroom enriched samples decreased significantly compared to the control, which was darker in colour (Ghumman *et al.*, 2016), except for the colour differences between semolina and mushroom powder. In this

study, the screw speed and temperature conditions remained constant, so the moisture was an important factor in the lightness of the samples. According to our previous research, the moisture content of the three mushroom powders showed no significant differences from each other, but they were slightly lower than semolina. In addition, the lower L^* values may be due to the reduced expansion and porosity of the extrudates instead of chemical reactions (Yu *et al.*, 2013). Both the redness (a^*) and yellowness (b^*) values increased dramatically compared to the control. All the a^* values in this study were negative, representing a green colour, and no red hues were found in these hot extruded snacks (Ho *et al.*, 2013). The increased yellowness could be attributed to the higher protein levels in the formulations (Brnčić *et al.*, 2009). All three mushroom samples provided more protein to the final products than semolina (semolina [11.32 ± 0.28 %]; white button [30.52 ± 0.13 %]; shiitake mushroom [15.04 ± 0.04 %] and porcini mushroom [23.83 ± 0.19 %] on a dry weight basis). Furthermore, the other factors involved in these colour differences may be the Maillard reactions during the hot extrusion process or the oxidation of mushroom fat under thermal treatment (Xu *et al.*, 2016). The ΔE^* values of the mushroom enriched samples were all above three units and increased progressively, which indicated that these colour differences could be viewed with the naked eye (Martínez-Cervera *et al.*, 2012). Fortunately, from the perspective of the naked eye, there were no strange colours that appeared in these food products.

Table 8.5 Colour measurements of hot extruded products

Sample	L*	a*	b*	ΔE^*
CHE	96.7±0.04 ^a	-10.7±0.15 ^d	26.77±0.28 ^h	0
5 % WBHE	91.43±0.48 ^{bc}	-8.06±0.03 ^{bc}	36.72±0.55 ^{bcd}	11.56
10 % WBHE	83.52±1.23 ^d	-6.25±0.85 ^a	31.5±0.68 ^f	14.69
15 % WBHE	82.7±0.22 ^d	-6.91±0.24 ^{ab}	29.34±0.27 ^g	14.73
5 % SHE	95±0.24 ^a	-12.8±0.12 ^e	34.98±0.39 ^e	8.65
10 % SHE	90.41±0.73 ^c	-8.88±0.26 ^c	35.09±1.08 ^{de}	10.59
15 % SHE	90.43±0.36 ^c	-7.91±0.18 ^{bc}	35.91±0.24 ^{cde}	11.43
5 % PHE	92.82±0.62 ^b	-8.56±0.43 ^c	36.97±0.20 ^{bc}	11.11
10 % PHE	92.6±0.65 ^b	-8.43±0.98 ^c	38.82±0.38 ^a	12.93
15 % PHE	89.81±0.62 ^c	-5.99±0.18 ^a	38.24±0.95 ^{ab}	14.18

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CHE=control hot extruded; WBHE=white button mushroom hot extruded; SHE=shiitake mushroom hot extruded; PHE=porcini mushroom hot extruded

8.3.5. Thermal properties

The ΔH values of most of the products were zero, indicating that the hot extrusion treatment resulted in complete starch gelatinisation or dextrinisation, and protein denaturation (no peaks) (Ai *et al.*, 2016; Parada *et al.*, 2011) (Table 8.6). During the extrusion process, the food materials suffered from high temperatures and shearing; thus, the starch was disrupted totally and its macromolecules would be thoroughly fractionated (Bryant *et al.*, 2001; Parada *et al.*, 2011). A strong negative correlation exists between ΔH and starch content ($r = -0.597$, $p \leq 0.001$). In addition, the 10 %, 15 % shiitake mushroom and 15 % porcini mushroom enriched samples had low values. These results showed a similar trend to that of the total dietary fibre contents in semolina and the other mushroom powders. Positive correlations were also observed between ΔH and IDF ($r = 0.739$; $p \leq 0.001$), SDF ($r = 0.550$; $p \leq 0.01$) and TDF ($r = 0.803$; $p \leq 0.001$). This phenomenon was in agreement with the work of (Aravind *et al.*, 2012 b), who showed that the inclusion of insoluble dietary fibre led to an increase in all DSC

parameters. This trend was also consistent with the WAI values, as mentioned above. Overall, the variations in these data were not significant.

Table 8.6 Thermal properties (DSC measurements) for hot extruded products

Sample	T onset °C	T gelatinisation °C	T endset °C	ΔH J/g	ΔT r °C
CHE	None	None	None	None	None
5 % WBHE	None	None	None	None	None
10 % WBHE	None	None	None	None	None
15 % WBHE	None	None	None	None	None
5 % SHE	None	None	None	None	None
10 % SHE	59.61±0.25	64.32±0.57	69.25±1.29	0.019±0.002	9.65±1.04
15 % SHE	57.57±1.16	62.23±2.61	71.57±1.82	0.047±0.003	14±2.98
5 % PHE	None	None	None	None	None
10 % PHE	None	None	None	None	None
15 % PHE	57.73±0.47	61.96±1.79	71.50±2.48	0.056±0.019	13.77±2.02

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CHE=control hot extruded; WBHE=white button mushroom hot extruded; SHE=shiitake mushroom hot extruded; PHE=porcini mushroom hot extruded

8.3.6. Total phenolic contents and antioxidant capabilities

The total phenolics content and antioxidant activities of the control and all mushroom enriched hot extruded samples were studied (Fig. 8.3). As expected, the inclusion of mushroom powder into the hot extruded snacks had a significant effect on the total phenolic contents and antioxidant activities of the samples. According to Fig. 8.3, the results of the total phenolic contents and the DPPH and ORAC assays showed similar trends. Table 8.3 illustrates significant positive correlations between TPC and both DPPH and ORAC values ($r = 0.973$ and 0.822 , respectively; $p \leq 0.001$). The relationship between the total phenolic contents of the samples and their antioxidant activities agreed with several earlier reports (Duan *et al.*, 2007; Jaworska *et al.*, 2015; Pan *et al.*, 2010). All the mushroom enriched samples showed

significantly higher total phenolic contents and stronger antioxidant capacities than the control. These values also increased gradually with increasing mushroom powder substitution levels. The high phenolic contents in edible mushrooms have been reported by several researchers (Alam *et al.*, 2009; Mattila *et al.*, 2001; Palacios *et al.*, 2011), resulting in high interest in the antioxidant ability of mushroom enriched snacks. These observations of the antioxidant abilities were attributable to the bioactive components (that frequently acted as antioxidants) in the added mushroom powders. Edible mushrooms have been proven to play an important role in protection against oxidant stress (Sanchez *et al.*, 2015), the inhibition of cancerous cells (like HT-29 cells) (Kim *et al.*, 2009 a), and in extending longevity (Sanchez *et al.*, 2015). Except for the phenolic contents, the antioxidant properties of edible mushrooms were due to other phytochemicals they contained (Alam *et al.*, 2011), such as gallic acid, ascorbic acid, tocopherols, flavonoids, carotenoids and selenium (Jaworska *et al.*, 2015; Lo and Cheung, 2005; Ribeiro *et al.*, 2008). Furthermore, the primary source of ergothioneine was in mushrooms. This sulphur amino acid could act as an antioxidant, and is present in levels of up to 0.4–2.0 mg/g in dry mushrooms (Weigand-Heller *et al.*, 2012). The antioxidant activity of ergothioneine was based on multiple mechanisms among which the capability to scavenge free radicals was quite powerful (Colognato *et al.*, 2006). This may be a reason to explain the increased DPPH scavenging activity and oxygen radical absorbance capacity of the mushroom enriched samples in this study. In addition, other changes that the inclusion of mushrooms brought about were factors that influenced the anti-radical properties of the final products, such as the amount of fat, sugar and dietary fibre. The fat content of mushroom enriched hot extruded samples increased significantly and gradually with the increase of mushroom powder

content compared to control (Table 8.2), while the material with enhanced fat content and low sugar content probably created a lower mechanical energy during the hot extrusion (Silva *et al.*, 2014); thus, inhibiting the effect of mushroom inclusion on the antioxidant capacities of snack products (Peřsa *et al.*, 2016). However, this study found strong positive correlations between fat content and TPC ($r = 0.520$, $p \leq 0.01$), DPPH ($r = 0.581$, $p \leq 0.001$) and ORAC ($r = 0.784$, $p \leq 0.001$). It has been shown that the fibre in some mushroom fruiting bodies could promote the antioxidant ability, to some extent (Sanchez *et al.*, 2015). Similar results were demonstrated in Table 8.3 that there were strong positive correlations between antioxidant capabilities (DPPH and ORAC) and all IDF, SDF and TDF fibre contents, even though no correlation was observed between ORAC and SDF. According to Fig. 8.3, it was obvious that, under the same substitution levels, there were still significant differences between different species of mushroom enriched samples. The different antioxidant components and other bioactive compounds of the three mushroom species played important roles in this observation. In this study, significant positive correlations exist between protein content and antioxidant activities, whereas negative correlations exist between starch content and both DPPH and ORAC values (Table 8.3).

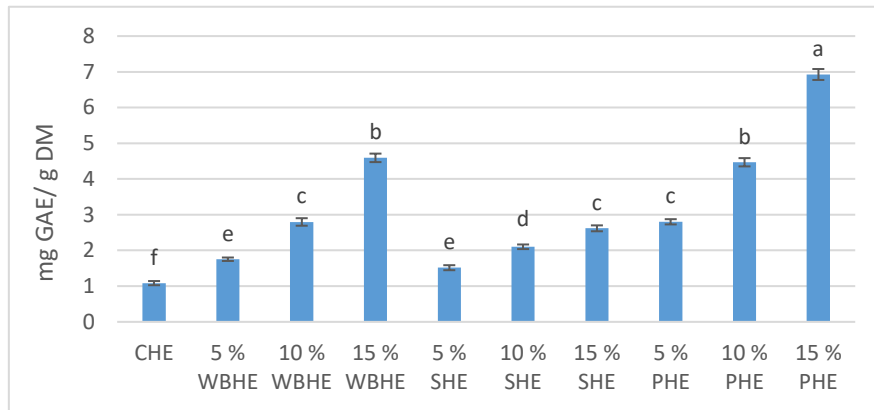
During hot extrusion processing the raw materials suffered from high temperatures and this tended to decrease the total phenolics compounds and antioxidant capacities of the samples because of the increased oxidation of the total phenolics (Korkerd *et al.*, 2015). However, it has also been shown that the extrusion conditions (the screw speed and extrusion temperature) may have stimulated the polymerisation of the phenolic compounds and this led

to its improved extractability from the raw materials, thus improving their antioxidant activities (Brennan *et al.*, 2011; Peęsa *et al.*, 2016). Because the increased speed of screw rotation and processing temperature could enhance the amount of mechanically damaged plant material cells, this then promoted the release of polyphenols from the food matrix (Peęsa *et al.*, 2016). While hot extrusion may create melanoidin, which is a kind of polymeric compound that possibly has antioxidant properties (such as hydroxymethyl furfural) (Brennan *et al.*, 2011), little evidence has been found to show the addition of mushroom powder would favour the former or the latter. On the other hand, compared to fresh mushrooms, culinary treatments, especially long exposure to high temperatures, brought about a 77 % reduction in total polyphenols, flavonoids, vitamins and carotenoids and a 21–69 % reduction in the DPPH assay results (Jaworska *et al.*, 2015). Little work has been undertaken to study the effect of hot extrusion on mushroom bioactivity.

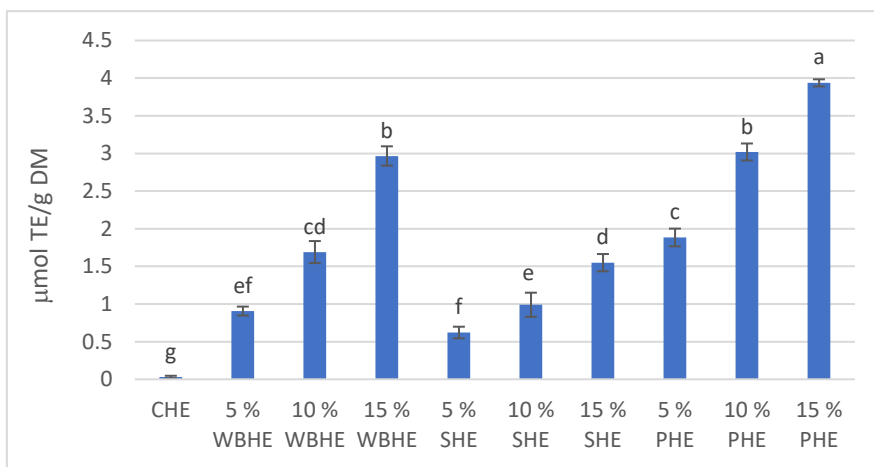
8.3.7. The starch content and *in vitro* digestibility

According to Fig. 8.4, the addition of mushroom powder into hot extruded products lowered the starch contents compared to the control. With the increasing amounts of mushroom powder, the starch content decreased gradually, as shown by the results of the semolina and mushroom starch contents. Strong negative correlations were observed between starch content and fat, protein, IDF and TDF contents ($p \leq 0.001$), we can suggest that the dramatically higher protein, fat and dietary fibre contents diluted the starch within the mushroom enriched samples.

(a)



(b)



(c)

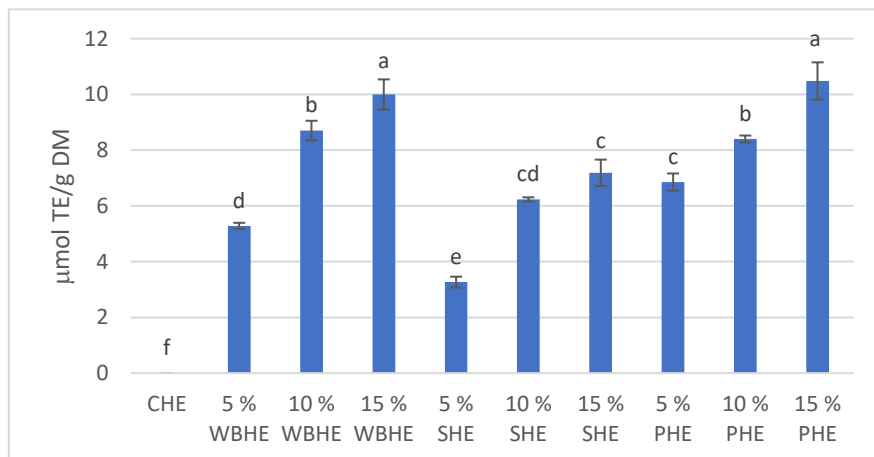


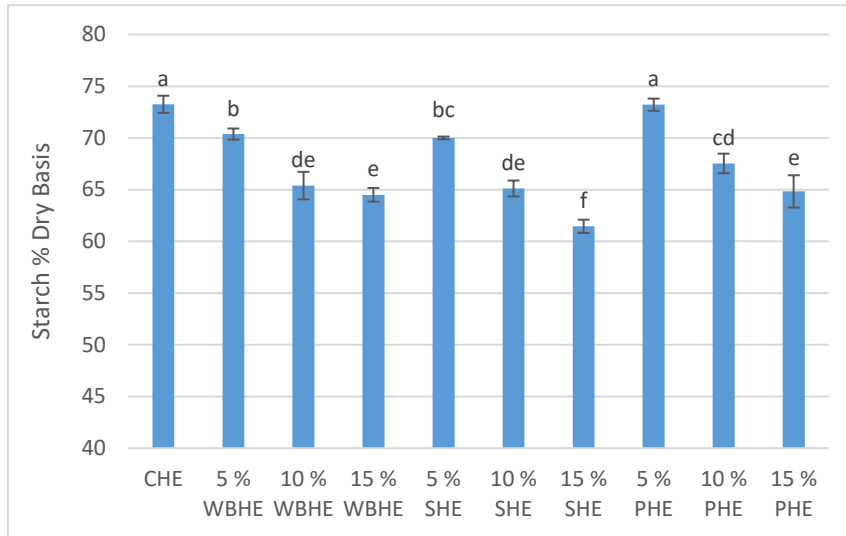
Figure 8.3 Values for total phenolic component and antioxidant capacities of hot extruded samples

Total phenolic component (TPC) (a); antioxidant capacities: the DPPH. scavenging activities (b); and the ORAC assay results (c). Comparing the control (CHE) to all mushroom powder enriched hot extruded samples: white button mushroom hot extruded products (WBHE); shiitake mushroom hot extruded products (SHE) and porcini mushroom hot extruded products (PHE). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$).

The reducing sugars released from mushroom enriched products over 120 min of *in vitro* digestion were much lower than that of the control and the plasma concentration-time curve (AUC) bar chart represented this trend more clearly (Fig. 8.4 and 8.5). With the increased mushroom powder contents, the AUC values decreased significantly and gradually. This observation is consistent with the lower starch content of mushroom enriched samples, which could be confirmed by the strong positive correlation between AUC and starch content ($r = 0.826$; $p \leq 0.001$) found in this study. Rapidly digested starch (RDS) is the starch digested in the first 20 min, which rapidly increases the blood glucose levels (Englyst *et al.*, 1992). According to Fig. 8.5, all the mushroom enriched snack samples (except at the 5 % substitution level) had lower RDS levels compared to the control. Slowly digested starch (SDS) is the amount of starch completely digested in 20 to 120 min in the human small intestine (Englyst *et al.*, 1992). Inclusion of mushroom powder did reduce the SDS fractions of the hot extruded products. Thus, the decrease in the predicted glycaemic response may be attributed to the higher amounts of resistant starch (RS) in the mushroom enriched samples. When chestnut mushroom co-products were added to the hot extruded snack products and exerted significantly reduced glucose which was released throughout the digestion (Brennan *et al.*, 2012 b). This result proved that inclusion of mushroom powder into hot extruded products could reduce the rate and extent of the predicted glycaemic response and provide a kind of nutritionally sound product that could bring health benefits to consumers. According to Table 8.2, mushroom enriched samples contain significantly higher contents of IDF and TDF than control, and many authors have reported that the fibre fractions were able to encapsulate starch granules and prevent the penetration of enzymes; thus, inhibiting the release of glucose

(Brahma *et al.*, 2016; Brennan *et al.*, 1996; Brennan *et al.*, 2012 a; Brennan *et al.*, 2013 a). In addition, the viscous nature of the fibres possibly inhibited the activity and efficiency of the digestive enzymes (Aravind *et al.*, 2012 c; Brennan *et al.*, 2013 a; Kendall *et al.*, 2008). Certainly the negative correlations observed between AUC and IDF ($r = -0.799$; $p \leq 0.001$), SDF ($r = -0.452$; $p \leq 0,05$) and TDF ($r = -0.833$; $p \leq 0.001$). Furthermore, the higher protein contents of the added mushroom powders may be another reason for the decreased glycaemic index. Table 8.3 further demonstrates the strong negative correlation between AUC value and protein content ($r = -0.6$; $p \leq 0.001$). One can speculate that hot extrusion produced a very close protein network and enhanced the entrapment of starch granules; thus, damaging the activity of enzymes (Brennan and Tudorică, 2008). At the microscopic level, the tortuosity protein matrix could restrict the movability of α -amylase through extrudate formation (Fardet *et al.*, 1998). In addition, according to the results of Calorie Answer, all the mushroom enriched samples contained more fat than the control (Table 8.2). It has been shown that the interactions of lipids with other components resulted in decreasing susceptibility to digestive enzymes (Holm *et al.*, 1983). What is in agreement that there is significant negative correlation between AUC and fat content of samples ($r = -0.855$; $p \leq 0.001$). Besides the factors mentioned above, there are other factors that may play important roles in starch digestibility, including: polymorphism, structure of amylopectin/amylose and granular morphology (Lehmann and Robin, 2007; Robin *et al.*, 2016).

(a)



(b)

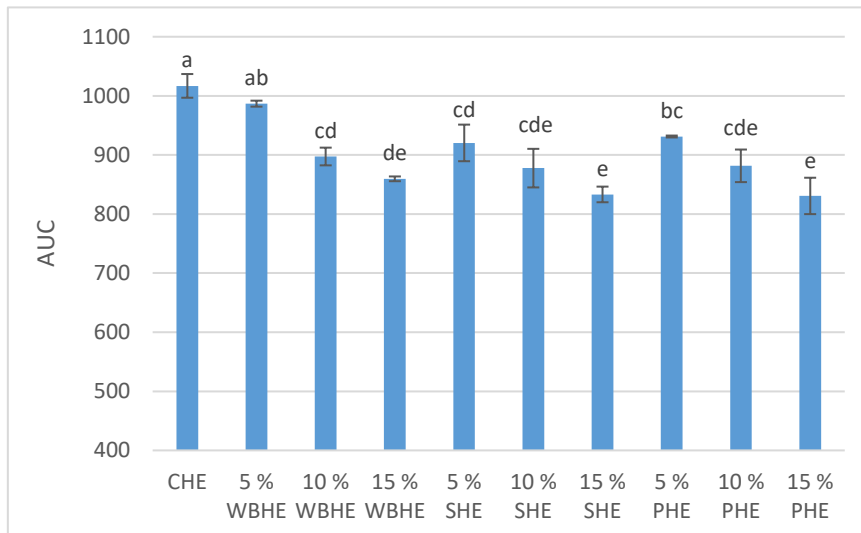
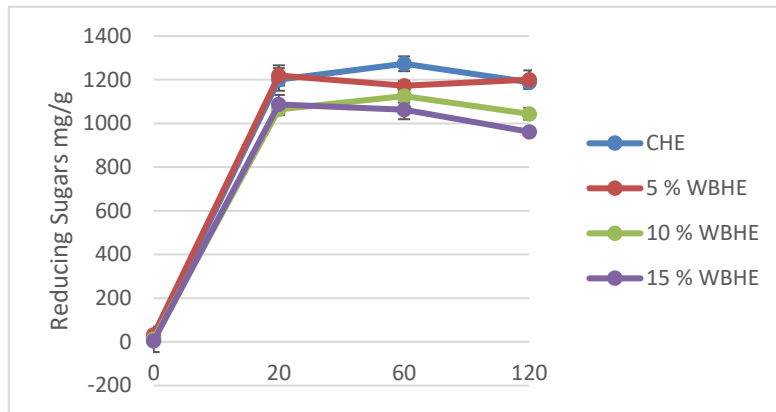


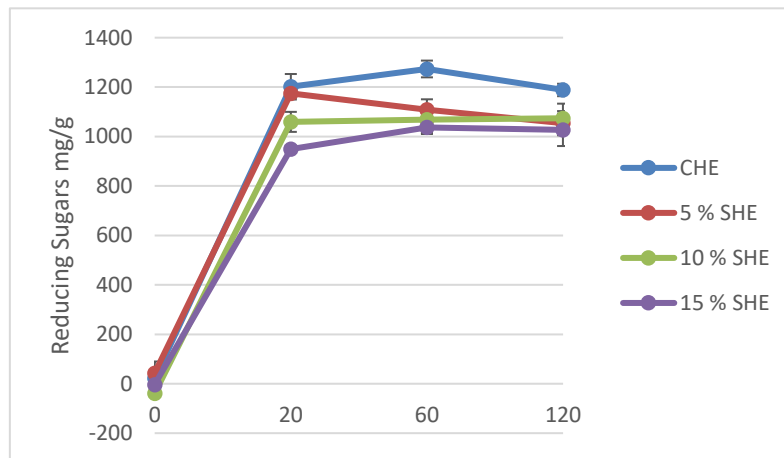
Figure 8.4 The total starch content and values for area under the curve (AUC) of hot extruded samples

Comparing the control (CHE) to all mushroom powder enriched hot extruded samples: white button mushroom hot extruded products (WBHE); shiitake mushroom hot extruded products (SHE) and porcini mushroom hot extruded products (PHE). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$).

(a)



(b)



(c)

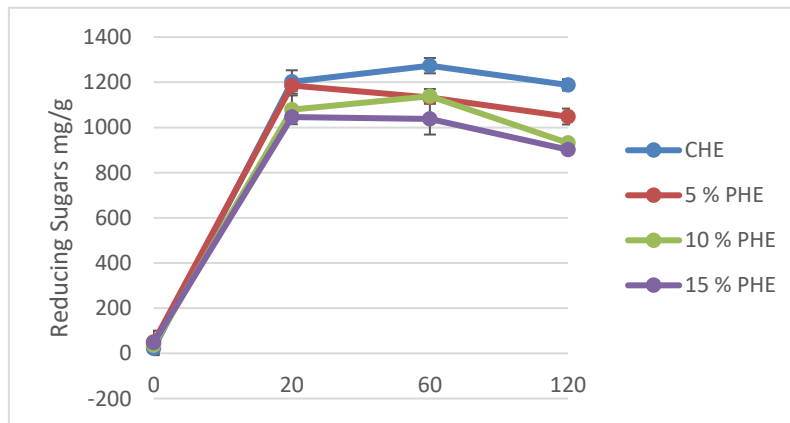


Figure 8.5 Levels of reducing sugars released during *in vitro* digestion

Comparing the control to 5 %, 10 %, 15 % white button mushroom hot extruded products (a); shiitake mushroom hot extruded products (b); and porcini mushroom hot extruded products (c): white button mushroom hot extruded products (WBHE); shiitake mushroom hot extruded products (SHE) and porcini mushroom hot extruded products (PHE).

8.3.8. The relationship between antioxidant activity and the glycaemic response

The results of the glycaemic response of all snack products in this study showed an inverse relationship with their antioxidant capabilities (Figs. 8.3, 8.4b and 8.5), Table 8.3 further illustrates strong negative correlations between AUC and DPPH ($r = -0.686$; $p \leq 0.001$) and ORAC ($r = -0.776$; $p \leq 0.001$). To be specific, the addition of mushroom powder increased the antioxidant properties of the final products and limited the release of reducing sugars. This observation may be ascribed to the ability of the mushroom enriched samples to decrease oxidative stress (Zemestani *et al.*, 2016). This relationship was supported by the work of Moraes *et al.* (2015), who showed that the glycaemic response was negatively correlated to the phenolic compounds and antioxidant capacities. This phenomenon may be due to the effect of the phenolic components on enzyme inhibition and starch molecule interactions, that result in impaired starch digestibility and increased levels of resistant starch (Mkandawire *et al.*, 2013). It has been shown that foods that are good sources of biological antioxidants could have inhibitory activity on pancreatic α -amylase, intestinal α -glucosidase and also in the production of haemoglobin glycation caused by glucose *in vitro* (Tiwari *et al.*, 2013).

Furthermore, for Type 2 diabetes patients, long exposure to hyperglycaemia resulted in oxidative stress with a reduction in endogenous antioxidant ability, which induced the release of reducing sugars. Thus, oxidative stress plays an important role in the pathogenesis of diabetes complications (Zemestani *et al.*, 2016). The mushroom enriched snack products will be a clever choice for diabetes patients because of their benefits for both glycaemic control and the promotion of antioxidant activities.

8.3.9. Scanning electron microscope (SEM)

Hot extrusion processing is a cooking method under severe conditions to destroy the granular structure of starch and even break the α -(1-4) and α -(1-6) bonds within starch granules (Bhatnagar *et al.*, 1997). The microstructure properties of control and mushroom enriched extrudates were described by SEM images given in Fig. 8.6. Relatively there was a more continuous matrix of amorphous starch formation within the control sample. The micrograph of mushroom enriched samples depicted that the cells were likely to get accumulated with huge mass-like formation and fissures between the huge mass of cellular structures. Generally, Fig. 8.6 showed that individual intact starch granules were almost disappeared completely after suffering the hot extrusion. However, based on the foregoing DSC results, although the amount was quite little, still some starch molecules were not gelatinised in the 10 % and 15 % shiitake mushroom extrudates. Fig. 8.6 illustrated more heterogeneous distributions of nuclei within these two samples, indicating a weaker structure than control. Both of them were more likely to appear fracture due to the discontinuous native granules. These were in agreement with the crunchiness of these two shiitake mushroom extrudates, with the increase of mushroom powder amount, the products were more crispy than control (Fig. 8.2). Furthermore, the image of 5 % white button sample represented fibrous-lamellar structure clearly, and 10 % white button and 5 % shiitake mushroom samples obtained a rippled structure and the structural orientation was random distributed with big fractures. This might play a role in their higher crunchiness values than control. An ordered arrangement of cells and fibres was existed in 15 % white button mushroom extrudate matrix, resulting in compacted and well-organised formation, and might lead to its highest hardness value compared to other

samples (Dar *et al.*, 2014). While even though different microstructures were observed, unique changes or interactions between different components within the extrudate matrix could be formed, thus still resulted in similar texture properties (Karkle *et al.*, 2012). For example, 15 % white button and all porcini mushroom extrudates showed no significant differences of textural properties with control, even though the structural characteristics of control were not preserved or retained in these mushroom enriched samples. In addition, the characterisation of microstructure, such as the average cell wall thickness and average cell size, has been proved to influence the digestibility of extruded products (Chanvrier *et al.*, 2007).

8.4. Conclusion

Substituting semolina in hot extruded snack formula with white button mushroom powder resulted in products with increased product density and significantly darker colour. For shiitake mushroom powder enriched samples, the colour and macrostructure changes were relatively not obvious, but did increase the crunchiness. In terms of porcini mushroom, such inclusion decreased the expansion ratio, increased specific length and product density. Furthermore, all the mushroom powders resulted in increased specific length, water solubility index, energy value, fat and total phenolic content, while decreased water absorption index, moisture and carbohydrate and total starch content. Inclusion of mushroom powder did adversely affect the starch gelatinisation and digestibility, while improved the antioxidant activities of snack samples. All these changes were correlated with the different inner structure alteration after

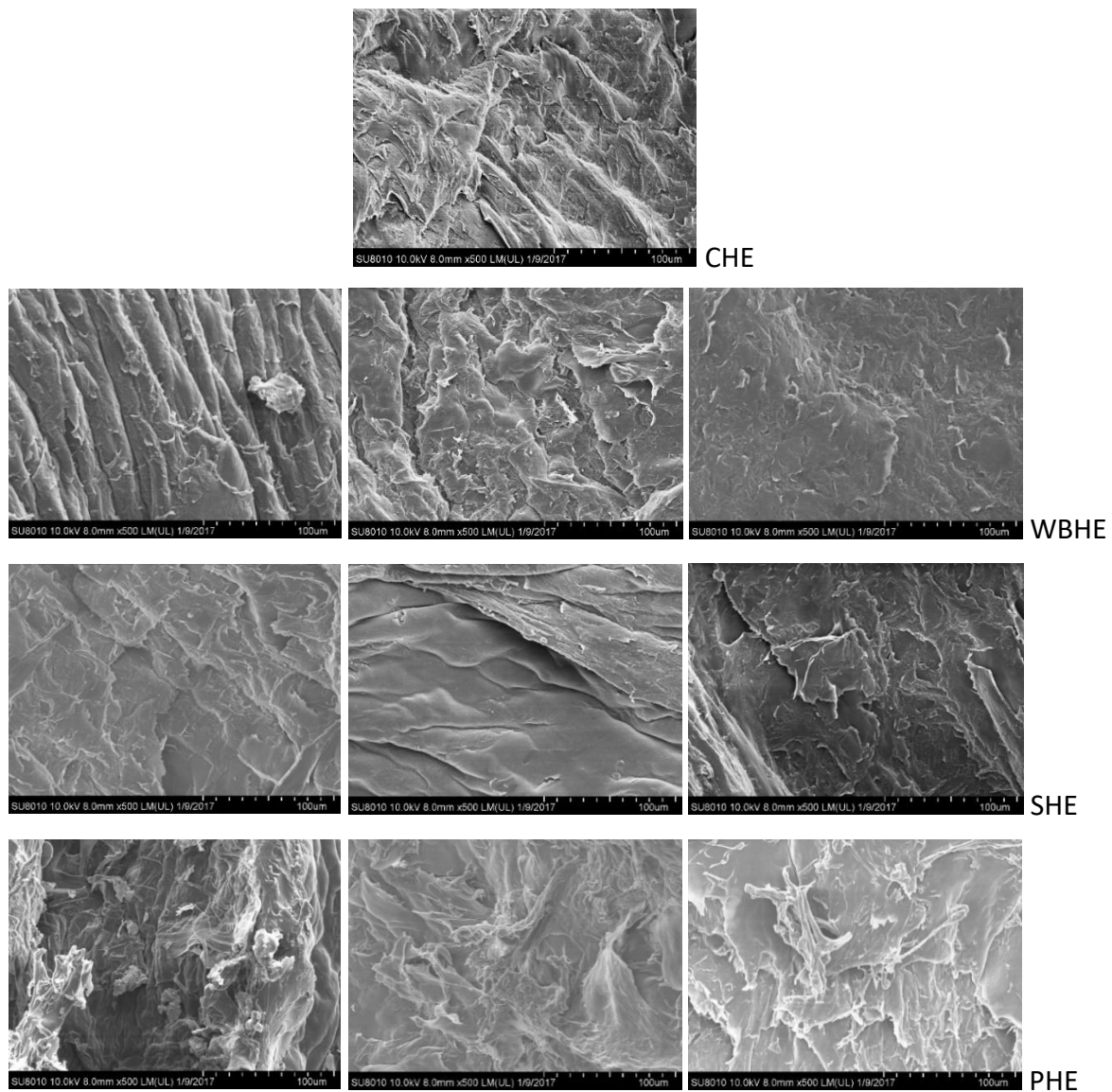


Figure 8.6 Scanning electron micrographs of hot extruded products at 500× magnification. The control (CHE); white button mushroom hot extruded products (WBHE); shiitake mushroom hot extruded products (SHE) and porcini mushroom hot extruded products (PHE). From left to right: 5 %; 10 % and 15 % substituent level.

the addition of mushroom powder. In conclusion, considering the different appearance and physical properties of mushroom substituent samples compared with control, it might be necessary to modify the formulation and process conditions to improve the preference of consumers to these novel snacks. Additionally, hot extruded products incorporated with mushroom powder could drastically provide more beneficial health influences.

Chapter 9

Effect of different thermal processes (bread pasta and extrusion) on the benefits of mushroom (*Auricularia auricula*) supplementation in cereal food products

(Submitted to Nutrients)

Abstract

This study investigated the characteristics of different cereal products (extruded snack, bread, pasta) incorporating varying amounts black ear mushroom powder (*Auricularia auricula*). After the addition of black ear mushrooms, the cooking losses, swelling index, water absorption and firmness of the pasta increased, while the bread dough stickiness, strength and extensibility decreased significantly compared with the control. The black ear mushroom enriched bread samples at the higher substitution levels had increased hardness, gumminess and chewiness, which reduced the springiness, cohesiveness, loaf height and volume of the samples. In terms of the hot extruded products, the addition of black ear mushroom powder decreased the expansion, crunchiness, L* and a* values and enhanced the density, hardness, b*, ΔE , WAI and WSI values. With increasing mushroom content, the insoluble and total dietary fibre contents of all cereal samples increased significantly, whereas the starch content decreased in all products. Addition of the black ear mushroom powder altered the properties inherent in the starch through the different cooking processes, thereby limiting starch gelatinisation. Such incorporation of these mushrooms could deliver more health benefits to consumers, such as enhanced antioxidant activities and a lower glycaemic index.

9.1. Introduction

Snack foods have become an indistinguishable part of modern, fast lifestyles as a platform to deliver energy and nutrients effectively to consumers. However, because of gradual increases in consumers' nutritional knowledge and the simultaneous rise of malnutrition, there is a considerable challenge in developing functional snack products as a popular platform to provide balanced nutrients rather than just deliver empty calories (Sharma *et al.*, 2017). Besides, the interest of consumers is not only focused on receiving an appropriate nutrient supply in order to achieve a healthy lifestyle, but also to prevent some diet-related diseases (Bustos *et al.*, 2013). *Auricularia auricula*, known as black ear, Jew's ear, or a number of other common names, grows on both dead and living wood. It has been used as a traditional Chinese medicine for many years and is widely used in Chinese cuisines (Luo *et al.*, 2009; Sun *et al.*, 2016). This fungus has been reported to have various pharmaceutical effects; such as, anti-tumour (Reza *et al.*, 2011); hypocholesterolaemia (Cheung, 1996); anti-virus (Nguyen *et al.*, 2012 a); anti-coagulant (Yoon *et al.*, 2003); anti-inflammatory (Damte *et al.*, 2011); hypoglycaemic (Takeuchi *et al.*, 2004); antioxidant activity (Acharya *et al.*, 2004); and it has immune-enhancing (Nguyen *et al.*, 2012 b) properties. These medicinal functions have been mostly attributed to the non-starch polysaccharides of black ear mushrooms, which consisted of three D-glucans and two acidic heteropolysaccharides (Luo *et al.*, 2009). It is, therefore, valuable to pay considerable attention to the exploitation of black ear mushrooms. These medical applications would result in an expansion of commercial demands in the food industry. *A. auricula* polysaccharides have been added to bread to increase the antioxidant activity and quality of the bread (Fan *et al.*, 2007). In addition, a novel functional formulation diet (*A.*

auricula and hawthorn) has been developed as an adjuvant dietetic food with increased *in vitro* and *in vivo* antioxidant and hypolipidaemic properties (Luo *et al.*, 2009).

The effect of cooking processing plays an important role in health benefits, in which additional ingredients are provided to the final products. Different treatments bring about differences in the gelatinisation of starch, denaturation of proteins, inactivation of enzymes, microbes and other anti-nutritional effects. All these are the significant factors used to determine the physicochemical and nutritional characteristics of snack products. Recently, extrusion cooking has been shown to play a central role in the modern cereal-based industry for the production of snacks, where they are mainly classified as being produced by cold extrusion or hot extrusion. Pasta is a representative food product produced through mixing, kneading and cold extrusion (Colonna *et al.*, 1990). Pasta is widely consumed throughout the world because of its low cost, attractive texture and long shelf life, which also makes pasta an optimal vehicle for health promotion (Ciccoritti *et al.*, 2017). Hot extruded products, which undergo hot temperatures and short processing times, are continuously combined, cooked and texturized quickly and efficiently; thus, they are commonly consumed not only as breakfast cereals, but also as instant mixes and novel centre-filled snacks (Singha and Muthukumarappan, 2017). During hot extrusion, food is subjected to a series of processing techniques, including mixing, shearing, heating and cooking (Tumuluru *et al.*, 2012). It has been reported that the extrusion process could improve the digestibility of protein and starch, while reducing the nutrient content at the same time (Singh *et al.*, 2007). In addition, such cooking processes can also positively influence the content of bioactives and their bioavailability in these products (Gu *et*

al., 2008; Masatcioglu *et al.*, 2013). Furthermore, hot extrusion conditions could affect the bioactive components of the final products; for example, temperature, feed moisture, screw speed and screw configuration (Brennan *et al.*, 2011), may have detrimental effects on the products' bioactive components (Kosinska-Cagnazzo *et al.*, 2017). Bread is one of the most popular staple foods in the world. Due to its nutritive value, low price and simplicity of use, bread has been treated as the basis of many civilisations' diets (Swieca *et al.*, 2014). Bread making is a complex process, which mostly includes mixing, fermentation, proving and baking (Reshmi *et al.*, 2017). Bread baking induces many modifications in the composition and properties of the original food matrix, and also results in changes in the nutritional benefits of the final product (De la Cueva *et al.*, 2017). Caramelisation and the Maillard reaction could also produce newly formed compounds that are responsible for different biological activities that improve the organoleptic properties of bread (Michalska *et al.*, 2008). Bread is made from refined wheat flour and is always regarded as having a low health potential; thus, it will be an interesting opportunity to require the incorporation of functional supplements (Msaddak *et al.*, 2017).

To date, very little information has been available on the potential contributions of mushrooms to the overall properties of snack products as a result of different cooking processes. In this study, black ear mushroom powder was incorporated into fresh pasta, hot extruded products and bread, to report on the potential use of this mushroom material as a functional ingredient in the food industry. The aim of the current research was to evaluate the effect of mushroom additions on the physical, textural and microstructural properties of snack products. Particular

attention was focused on starch gelatinisation, antioxidant capacities and the potential glycaemic modulating effects black ear mushroom powder may possess in terms of the additional nutrients it could provide to the final snack products.

9.2. Materials and methods

9.2.1. Materials

As described in 3.1.

9.2.2. Products processing

9.2.2.1. Pasta processing

The pasta processing was carried out as described in 3.2.1.

9.2.2.2. Dough and bread preparation

Dough and bread preparation was carried out as described in 3.2.2.

9.2.2.3. Extrusion processing

The extrusion processing was carried out as described in 3.2.3.

9.2.3. Near infrared spectroscopy

The near infrared spectroscopy analysis was determined as described in 3.7.

9.2.4. Physical characteristics

9.2.4.1. Pasta analysis

The pasta analysis was determined as described in 3.3.1.

9.2.4.2. Bread analysis

The bread analysis was determined as described in 3.3.2.

9.2.4.3. Hot extruded products analysis

The hot extruded products analysis was determined as described in 3.3.4. and 3.3.5.

9.2.4.4. Moisture content

The moisture content was determined as described in 3.5.5.

9.2.5. Textural characteristics

9.2.5.1. Pasta

The textural characteristics of pasta samples were determined as described in 3.4.1.

9.2.5.2. Dough and bread

The textural characteristics of dough and bread samples were determined as described in 3.4.2. and 3.4.3.

9.2.5.3. Hot extruded products

The textural characteristics of hot extruded samples were determined as described in 3.4.4.

9.2.6. Nutritional analysis

The nutritional analysis was determined as described in 3.5.1. and 3.5.2.

9.2.7. Differential scanning calorimetry (DSC)

The DSC analysis were determined as described in 3.8.

9.2.8. Total phenolic content and antioxidant capabilities

The total phenolic content and antioxidant capabilities analysis were carried out as described in 3.10.

9.2.9. The *in vitro* starch digestibility analysis

The *in vitro* starch digestibility analysis was carried out as described in 3.9.

9.2.10. Microscopy

The microscopy analysis was carried out as described in 3.11 at 500 times magnification level. The microstructure analysis of the both longitudinal surface and transverse cross section of raw pasta samples was evaluated. The raw pasta samples were freeze-dried previously to the analysis. In terms of bread samples, both the bread crust and crumb were freeze-dried and then evaluated.

9.2.11. Statistical analysis

Statistical analysis was carried out as described in 3.12.

9.3. Results and discussion

9.3.1. Nutrients in mushroom snack products

The nutritional composition of the control and black ear mushroom snack products is shown in Table 9.1. The protein contents of semolina and black ear mushroom powder were also determined and the data concerning the high-grade flour was obtained from the manufacturer. In descending order, the protein contents were: high-grade flour (11.5 %) > semolina flour (11.32 ± 0.28 %) > black ear mushroom powder (9.63 ± 0.17 %). The difference between semolina and mushroom powder were slight, thus the addition of black ear mushroom powder to the pasta did not alter the final protein content significantly, while increased such content of hot extruded products significantly, but such an addition decreased the protein content of

the bread products significantly and gradually. Interestingly, the protein contents of both the pasta and hot extruded product controls, which were made of only semolina and water, were higher than the semolina flour by itself. It has been reported that cooking processing could improve the protein contents of food and other functional properties, depending on the processing methods (Osungbade *et al.*, 2016). The fat contents of the high-grade wheat flour, semolina and black ear mushroom powder are 1.4 %, 1.17 ± 0.09 % and 0.88 ± 0.032 %, respectively. Addition of the black ear mushroom powder did not bring about significant trend in the fat content of the pasta and bread samples, while some samples contained higher fat content than control. In contrast, the fat content of black ear mushroom powder in the supplemented hot extruded products increased gradually and significantly compared to the control. Moreover, the free lipids of the raw material could bind with starch (especially amylose), while such formation tended to decrease under high temperatures (Tumuluru *et al.*, 2012). Black ear mushroom powder might play an extra positive role in the destruction of the matrix between the free lipids and other components during hot extrusion processing, resulting in the higher fat contents of the black ear mushroom hot extruded products.

According to Table 9.1, the incorporation of black ear mushroom powder decreased the starch content of all products significantly and gradually compared to the control. Furthermore, such incorporations also increased the IDF and TDF contents of all products progressively and significantly compared to the control. The results of SDF determination showed different trends among the three products. For instance, the addition of black ear mushroom powder to both the pasta and hot extruded products showed no significant effects on the SDF content

(except for the 5 % black ear mushroom hot extruded sample). Interestingly, extrusion cooking has been proven to enhance the TDF contents of foodstuffs. In terms of SDF, the extrusion process could develop additional components by transglucosidation, from cleaving the 1,4 carbon-oxygen bonds, and the formation of new anhydroglucose linkages. On the other hand, IDF could be increased during this process, whereby retrograded amylose (RS₃) was formed, which was insoluble at room temperature (Elleuch *et al.*, 2011). In terms of the bread samples, with the increase in mushroom powder content the SDF content showed a gradual reduction compared with the control. Regulation (EC) No. 1924/2006 about nutrition and health claims allowed claims for dietary fibre-containing foods: “rich in fibre” for those containing at least 6 g dietary fibre in 100 g product. Thus, in this study, all these black ear mushroom powder enriched products could be labelled as “rich in fibre” (Cappa and Alamprese, 2017).

9.3.2. Physical properties of mushroom snack products

When it came to pasta, black ear mushroom enriched products exhibited a significant increase in cooking losses, swelling index, water absorption index and moisture content (Table 9.2a). The cooking loss represented their resistance to disintegration during cooking, an essential factor for pasta quality. Black ear mushroom powder provided more fibre to the final pasta, which was responsible for weakening the starch matrix, thereby resulting in increased cooking losses (Phongthai *et al.*, 2017). A similar swelling index trend has been reported by Brennan and Tudorică (2007), where an increased fibre content could enhance the swelling index of

Table 9.1 The nutrient components of snack product

(a)

Sample name	Protein % DW	Fat % DW	Water (g/100g product)	Starch % DW	IDF % DW	SDF % DW	TDF % DW
Control cooked pasta	11.44 ± 0.24 ^a	2.93±0.12 ^b	3.5±0.17 ^d	69.95 ± 1.58 ^a	2.10 ± 0.024 ^c	1.07 ± 0.039 ^a	3.17 ± 0.015 ^c
5 % black ear cooked pasta	11.13 ± 0.15 ^a	3.23±0.058 ^a	4.6±0.17 ^c	69.32 ± 1.16 ^{ab}	7.74 ± 0.98 ^b	1.04 ± 0.22 ^a	8.78 ± 1.20 ^b
10 % black ear cooked pasta	11.19 ± 0.15 ^a	2.93±0.058 ^b	5.33±0.40 ^b	65.26 ± 1.49 ^{ab}	8.89 ± 0.20 ^b	1.27 ± 0.10 ^a	10.16 ± 0.095 ^b
15 % black ear cooked pasta	11.29 ± 0.063 ^a	3.23±0.058 ^a	6.07±0.15 ^a	63.47 ± 0.62 ^b	12.51 ± 0.65 ^a	1.35 ± 0.13 ^a	13.85 ± 0.78 ^a

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$).

(b)

Sample name	Protein % DW	Fat % DW	Water (g/100g product)	Starch % DW	IDF % DW	SDF % DW	TDF % DW
Control bread	13.44 ± 0.14 ^a	9.05 ± 0.29 ^a	13.7±0.1 ^c	66.56 ± 1.31 ^a	3.10 ± 0.24 ^c	3.70 ± 0.12 ^b	6.80 ± 0.12 ^c
5 % black ear bread	11.90 ± 0.31 ^b	6.53 ± 0.75 ^b	14.78±0.096 ^b	64.42 ± 1.09 ^a	6.00 ± 0.50 ^c	4.32 ± 0.072 ^a	10.32 ± 0.57 ^b
10 % black ear bread	10.33 ± 0.082 ^c	7.72 ± 0.46 ^{ab}	15.83±0.12 ^a	55.99 ± 1.08 ^b	10.12 ± 0.63 ^b	3.32 ± 0.089 ^c	13.44 ± 0.54 ^b
15 % black ear bread	10.82 ± 0.35 ^c	7.79 ± 0.57 ^a	15.75±0.13 ^a	54.25 ± 1.29 ^b	15.21 ± 1.28 ^a	2.53 ± 0.046 ^d	17.73 ± 1.32 ^a

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$).

(c)

Sample name	Protein % DW	Fat % DW	Water (g/100g product)	Starch % DW	IDF % DW	SDF % DW	TDF % DW
Control hot extruded	12.49 ± 0.14 ^b	0.40 ± 0.06 ^c	7.13±0.25 ^d	73.25 ± 0.83 ^a	2.66 ± 0.21 ^d	2.89 ± 0.049 ^a	5.55 ± 0.16 ^d
5 % black ear hot extruded	12.83 ± 0.27 ^{ab}	1.31 ± 0.19 ^b	8±0.1 ^c	72.63 ± 1.03 ^a	5.29 ± 0.19 ^c	2.24 ± 0.065 ^b	7.53 ± 0.12 ^c
10 % black ear hot extruded	13.08 ± 0.27 ^a	1.45 ± 0.17 ^{ab}	8.57±0.23 ^b	66.68 ± 0.16 ^b	8.36 ± 0.65 ^b	2.59 ± 0.28 ^{ab}	10.94 ± 0.93 ^b
15 % black ear hot extruded	13.09 ± 0.05 ^a	1.85 ± 0.22 ^a	9.17±0.15 ^a	65.45 ± 0.42 ^b	11.75 ± 0.21 ^a	2.63 ± 0.07 ^{ab}	14.39 ± 0.14 ^a

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$).

pasta that was related to the high water-binding capacity of the additional fibre fraction (Cleary and Brennan, 2006); this was in agreement with results of the water absorption index and moisture content in this study.

For the bread dough, during bread manufacture at the higher levels of black ear mushroom inclusions, there were some small clumps in the dough which may be explained by the additional fibre competing with starch and protein for water during dough formation; thus, leading to the starch and protein fractions of the wheat flour being more discrete and less incorporated into the matrix (Aravind *et al.*, 2012 b, c). Table 9.2c shows the physical characteristics of black ear mushroom powder bread, including the height, volume and moisture content. The height and specific volume of black ear mushroom bread decreased significantly and gradually with increasing mushroom content. The possible cause for the decrease in the volume could be the increased gelation potential of the mushroom dietary fibre that increased the rigidity of the doughs, resulting in lower a dough expansion and loaf volume (Pérez-Quirce *et al.*, 2017).

The effect of the inclusion of black ear mushroom powder on the physical characteristics of extruded snack products is shown in Table 9.2d. Snack products with mushroom inclusions exhibited significantly lower expansion rates compared to the control. Interestingly, there was a negative correlation between the expansion ratio and density properties of the extruded samples, as shown by Brennan *et al.* (2012 b). The results from this study supported this observation. This finding was related to the higher water-holding capacities of the additional

dietary fibre provided by black ear mushroom powder, which reduced the amount of water removed from the product as steam during the hot extrusion processing and resulted in a denser product (Brennan *et al.*, 2008 b). In addition, the WAI and WSI of the black ear enriched samples were observed to be higher than those of the control product. It has been reported that the WSI of extrudates was affected by the moisture content of the products post extrusion (Sompong *et al.*, 2011), while no significant differences were found in the moisture content between the control and mushroom supplemented samples. In general, consumers prefer extruded snacks that are highly expanded and with low density properties because of the desired crunchiness (Brennan *et al.*, 2013 b). Therefore, the addition of black ear mushroom powder had some negative effects on extruded snacks.

9.3.3. Textural properties of mushroom snack products

In terms of the textural characteristics of pasta, black ear mushroom pasta exhibited a less uniform appearance than the control. The firmness and elasticity were the main textural properties that determine pasta acceptability for consumers (Brennan and Tudorică, 2007). The elasticity of pasta decreased gradually with increasing mushroom supplementation levels, while the firmness of pasta indicated a converse trend (Table 9.2a). Firmness can be associated with attractive forces among the particles that were distributed vertically opposed disintegration (Cleary and Brennan, 2006). Thus, the increased firmness values might be explained by the contribution black ear mushroom powder made to the strength of the pasta's structure in the longitudinal direction. As mentioned above, there was more water in the

Table 9.2 The physical and textural properties of snack products

(a)

Sample	Cooking (g/100 g)	Loss	Swelling Index (g water/g dry pasta)	Water Absorption Index (g/100 g)	Force g	Area F-D 1:2 g:cm	Elastic Limit/Tensile Strength Force g	Elasticity Distance mm	Moisture cooked %
CP	2.51 ± 0.0008 ^c		1.83 ± 0.05 ^d	82.46 ± 3.71 ^c	54.07 ± 4.18 ^c	6.08 ± 0.63 ^c	37.47 ± 3.02 ^a	50.1 ± 7.07 ^a	64.68 ± 0.0067 ^c
5 % BEP	2.69 ± 0.0025 ^{bc}		1.96 ± 0.061 ^c	87.28 ± 4.16 ^c	234.85 ± 14.39 ^b	30.24 ± 0.87 ^b	30.89 ± 2.84 ^b	53.76 ± 5.28 ^a	66.23 ± 0.69 ^b
10 % BEP	3.29 ± 0.0018 ^b		2.12 ± 0.01 ^b	95.61 ± 1.16 ^b	245.74 ± 24.95 ^{ab}	29.42 ± 2.37 ^b	26.51 ± 3.27 ^c	38.22 ± 7.2 ^b	67.97 ± 0.11 ^a
15 % BEP	4.38 ± 0.0043 ^a		2.25 ± 0.034 ^a	104.38 ± 2.33 ^a	266.79 ± 28.74 ^a	33.88 ± 2.53 ^a	24.26 ± 4.04 ^c	26.91 ± 7.33 ^c	69.20 ± 0.33 ^a

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CP=Control pasta and BEP=Black ear mushroom pasta

(b)

Sample	Stickiness (Force) (g)	Work of Adhesion (Area F-T 1:2) (g.sec)	Dough Strength/Cohesiveness (mm) (Travel 1:2)	Resistance to Extension (Force) (g)	(Negative) Extensibility (Distance) (mm)	Dough Moisture %
CBD	33.31±3.52 ^a	1.94±0.32 ^a	1.22±0.0012 ^a	94.51±6.02 ^c	26.65±0.28 ^a	40.32±0.13 ^b
5 % BEBD	15.88±1.26 ^b	0.71±0.014 ^b	0.71±0.042 ^b	128.23±1.45 ^b	22.66±0.96 ^b	40.42±0.22 ^b
10 % BEBD	4.84±0.26 ^c	0.14±0.0058 ^c	0.42±0.02 ^c	116.26±3.85 ^b	18.20±1.64 ^c	40.19±0.11 ^b
15 % BEBD	3.98±0.54 ^c	0.12±0.024 ^c	0.38±0.02 ^c	153.97±12.43 ^a	17.34±0.63 ^c	41.12±0.14 ^a

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CN=Control bread dough and BEBD=Black ear mushroom bread dough

(c)

Sample	Firmness (Force) (g)	Hardness (Force) (g)	(Negative) Adhesiveness (Variable) (g.sec)	Springiness (mm)	Cohesiveness (ratio)	Gumminess (g)	Chewiness (g)	Resilience (ratio)	Height (mm)	Specific volume (mL/g)	Bread Moisture %
CB	405.16±71.19 ^c	303.04±6.65 ^c	1.01±0.31 ^b	0.96±0.0087 ^a	0.81±0.0038 ^a	253.81±10.52 ^c	239.73±10.07 ^c	0.46±0.01 ^a	60.33±0.98 ^a	3.08±0.035 ^a	27.84±2.34 ^a
5 % BEB	309.20±6.26 ^c	214.36±11.74 ^d	1.61±0.49 ^{ab}	0.89±0.013 ^b	0.82±0.0077 ^a	175.18±13.84 ^d	157.27±15.52 ^d	0.41±0.0061 ^b	59.34±3.64 ^a	3.04±0.041 ^a	30.28±1.74 ^a
10 % BEB	709.82±7.44 ^b	465.08±9.96 ^b	1.23±0.43 ^b	0.90±0.0039 ^b	0.79±0.011 ^b	369.04±17.55 ^b	323.29±10.26 ^b	0.41±0.016 ^c	51.53±0.65 ^b	2.27±0.054 ^b	29.65±3.39 ^a
15 % BEB	1092.57±45.27 ^a	775.02±23.58 ^a	2.82±0.63 ^a	0.83±0.020 ^c	0.77±0.0077 ^c	585.49±15.88 ^a	474.71±16.91 ^a	0.44±0.0022 ^a	48.02±2.43 ^b	1.99±0.054 ^c	29.75±1.41 ^a

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CB=Control bread and BEB=Black ear mushroom bread

(d)

Sample	% Expansion	ER	Lsp mm/g	Density kg/m ³	Density g/l	Moisture content %	Count Peaks+ F (g) 1:2	Force g	L*	a*	b*	ΔE	WAI	WSI
CHE	394.9 ± 31.02 ^a	15.69± 2.51 ^a	45.36± 2.50 ^c	203.3±27 .64 ^a	138.83±5 .13 ^b	8.24±0.05 ^a	44.58±3.26 ^a	7982.60±8 14.82 ^b	96.7±0.04 ^a	10.7±0.15 ^a	26.77±0.28 ^c	0	6.55 ± 0.41 ^b	11.06 ± 1.03 ^c
5 % BEHE	377.98±1 5.51 ^{ab}	14.31± 1.17 ^{ab}	54.35± 2.93 ^b	183.56±1 6.75 ^b	130.19±4 .07 ^b	8.12±0.20 ^a	43.08±1.62 ^a	6061.29±8 16.25 ^c	90.78±0.19 ^b	10.38±0.05 ^a	31.6±0.36 ^a	7.65	6.50 ± 0.068 ^b	12.26 ± 0.84 ^{bc}
10 % BEHE	357.75±2 3.46 ^c	12.85± 1.68 ^c	58.32± 3.82 ^a	192.08±2 2.80 ^{ab}	144.71±8 .63 ^{ab}	8.07±0.10 ^a	35.67±2.74 ^b	9502.80±1 054.74 ^a	89.71±0.48 ^c	9.69±0.10 ^b	31.25±0.40 ^a	8.36	6.63 ± 0.12 ^b	14.62 ± 1.10 ^{ab}
15 % BEHE	374.48±1 7.80 ^{bc}	14.05± 1.35 ^{bc}	51.58± 4.40 ^b	198.28±2 4.51 ^{ab}	160.51±6 .93 ^a	8.19±0.05 ^a	37.25±3.93 ^b	9826.19±1 366.79 ^a	86.99±0.34 ^d	9.41±0.20 ^b	29.28±0.24 ^b	10.11	7.20 ± 0.025 ^a	14.72 ± 0.61 ^a

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CHE=Control hot extruded product and BEHE=Black ear mushroom hot extruded product

mushroom pasta samples than in the control, which may also impact upon the mechanical properties of pasta that were attributed to the active role of water as a plasticiser of composite materials that increases the flow dynamics of the system (Tudorică *et al.*, 2002).

According to Table 9.2b, the addition of black ear mushroom powder decreased the stickiness of the bread dough drastically. The extensibility of bread dough also reduced gradually with increasing black ear mushroom powder content. Similarly, it has also been illustrated that the elasticity of the dough decreased by the incorporation of barley flour (Sullivan *et al.*, 2013). There is a positive correlation between the viscoelasticity of dough and the gluten content; thus, the decreased dough elasticity indicates that a lower gluten content was present in the supplemented bread dough, which could be explained by the dilution effect of the wheat gluten complex by the addition of black ear mushroom powder (Hussein *et al.*, 2013). Furthermore, the significantly decreased hardness of bread dough enriched with black ear mushroom powder may be related to the interactions between water and the additional components, which inhibited the swelling of starch granules and the water absorption of the gluten, then exerted effects on the formation of the gluten network (Peng *et al.*, 2017).

The textural attributes of bread included: hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience. These textural attributes of bread with, and without, black ear mushroom powder were analysed by TPA and the results recorded (Table 9.2c). In this study, the addition of black ear mushroom powder significantly increased the TDF content of the samples. In general, the incorporation of dietary fibres into bakery products aimed to

their prolong freshness because of their ability to retain water. As a result, economic losses could be reduced. However, at the same time, such additions could cause undesirable changes to the colour and texture of foods (Elleuch *et al.*, 2011). Incorporation of the black ear mushroom powder significantly enhanced the hardness of the bread, and this was probably due to the lower volume and greater gelling potential of the additional dietary fibre.

Table 9.3 illustrates the significant positive correlations between bread hardness and both IDF and TDF ($r = 0.918$ and 0.881 , respectively; $p \leq 0.001$). Table 9.3 further demonstrates the strong negative correlation between bread hardness and SDF ($r = -0.975$; $p \leq 0.001$), which may be correlated with the contributions from SDF in increasing the viscosity of the liquid phase in a food system (Guillon and Champ, 2000). As a rule, the addition of fibre reduced loaf volume and increased firmness of bread, which was consistent with the findings in this study (Elleuch *et al.*, 2011). Such a phenomenon indicated the lower amounts of air retained in the dough structure during proving and baking. Similarly, previous research has shown that there was a negative correlation between the hardness of bread and its specific volume (Pérez-Quirce *et al.*, 2017). The increased hardness of bread might also be attributed to the limitation of yeast growth in the mushroom-supplemented bread, which leads to decreased gas production during fermentation (Ammar *et al.*, 2016). The springiness and cohesiveness of the bread increased significantly for those breads supplemented with mushroom powder. In addition, increasing the size of mushroom substitution increased the gumminess and chewiness of the bread. All in all, the higher hardness, gumminess, chewiness and lower springiness, cohesiveness represented poor quality in the black ear mushroom powder bread.

These results agree with a previously reported study, which showed that high amounts of dietary fibre were associated with negative influences on the textural properties of bread (Bouaziz *et al.*, 2017). Furthermore, Table 9.2d shows the textural quality of hot extruded snacks. The hardness of the products increased with the increasing mushroom inclusion rate and a significant decrease could be observed between the samples in terms of the number of peaks during the compression of the samples.

Table 9.3 Pearson's Correlation Coefficient (r) of physicochemical and Nutritional Properties of Snack Products

(a) Pasta

	AUC	IDF % DW	SDF % DW	TDF % DW
IDF	-0.886***			
SDF	-0.820***	0.730*		
TDF	-0.893***	1***	0.723**	
TPC	-0.903***	0.976***	0.736**	0.978***

(b) Bread

	AUC	IDF % DW	SDF % DW	TDF % DW	TPC	Firmness	Hardness
IDF % DW	-0.936***						
SDF % DW	0.765**	-0.834***					
TDF % DW	-0.935***	0.996***	-0.781**				
TPC	-0.120	0.229	0.298	0.306			
Firmness	-0.897***	0.937***	-0.961***	0.904***	-0.111		
Hardness	-0.851***	0.918***	-0.975***	0.881***	-0.142	0.989***	
Dough Strength	0.805**	-0.879***	0.536	-0.907***	-0.583*	-0.700*	-0.651*

(c) Hot extruded products

	AUC	IDF % DW	SDF % DW	TDF % DW
IDF	-0.955***			
SDF	0.109	-0.136		
TDF	-0.954***	0.997***	-0.064	
TPC	-0.845***	0.858***	-0.253	0.846***

9.3.4. Thermal properties of mushroom snack products

The results of thermal analysis for starch for all samples with, and without, black ear

mushroom powder are reported in Table 9.4. The enthalpy of gelatinisation (ΔH) of the starch from all products supplemented with black ear mushroom powder were higher than that of the control samples. Even though the starch contents of black ear enriched products were significantly lower than the control, the higher ΔH values indicated their greater energy requirements for starch gelatinisation (Sandhu and Siroha, 2017). Except for the starch content, the enthalpy value of starch is also dependent on the amylopectin ratio, starch crystallites and starch granule size (Sandhu and Lim, 2008). The addition of black ear mushroom powder might alter these factors inherent in the starch through the different cooking processes, thereby limiting starch gelatinisation. It has been reported that increased ΔH may be due to the strengthening of hydrogen bonding by the hydrophobic alkenyl groups resulting in a reduction in starch swelling (Won *et al.*, 2017). Furthermore, the increased starch gelatinisation enthalpy from the incorporation of black ear mushroom powder might be due to the decreasing water activity, changes in the molecular structure of the water, a reduction in the plasticising effect of the solvent and the interaction of the additional components with starch (Zhou *et al.*, 2017). In this case, the fibre contents of black ear mushroom enriched products were significantly higher than in the control. Karkle *et al.* (2012) showed that fibre was able to limit the water availability for the starch alone, which also inhibited its gelatinisation.

At the same mushroom substitution level, the raw pasta had a higher ΔH value than the cooked pasta (Table 9.4). This indicated that the water and temperature during pasta cooking played a positive role in starch gelatinisation. According to Silva *et al.* (2016), gelatinisation was the first set of starch changes during heating and was characterised by irreversible disruption of

the molecular order of the starch; furthermore, the initial increase in granule size could also result in increased suspension viscosity. In addition, in terms of the cooked pasta and bread samples, during boiling and baking process, respectively, it has been shown that starch undergoes irreversible changes and lost its crystalline organisation. Meanwhile, the amount of water and heat available in the process plays important roles in such effects (Attanasio *et al.*, 2004). Previous research has indicated that the changes in starch granules made by thermal treatment contributed to the foods' properties, including its rheological properties, texture, moisture and starch digestibility (Noda *et al.*, 2008; Silva *et al.*, 2016). It has also been reported that fermentation which occurs during bread making is responsible for higher expansion rates in the starch, which indicate the degree of starch gel elasticity after gelatinisation (Liao and Wu, 2017). The work by Reyes *et al.* (2016) showed that there was a positive correlation between the gelatinisation enthalpy and fermentation time.

Compared with the other two products, the ΔH values of the hot extruded snacks were relatively lower and the value of the control sample was even below the sensitivity threshold of the equipment, which meant that starch granules were almost completely gelatinised or dextrinised during the process of extrusion. Similar results were found by Parada *et al.* (2011) who showed that the melting enthalpy was not observed in all extruded snacks with, or without, guar gum. These results were to be expected since, during hot extrusion, a high temperature and high shear processing technique, where starch transformation was achieved by both mechanical and thermal energy, and an increase in both promotes higher losses of its granular structure (Karkle *et al.*, 2012) and the fractionation of macromolecules at low

moisture levels (Parada *et al.*, 2011). Even though these products (except for the raw pasta) were subjected to heat treatment, the altered heating regimes resulted in different starch granule morphologies (Yin and Walker, 1995), which may be a factor in their final different ΔH values. Furthermore, the shear force occurring during extrusion of both the pasta and hot extruded snacks could tear apart the starch granules and allow the faster transfer of water physically into the interior of the starch molecules (Lai and Kokini, 1991). However, Wen *et al.* (1990) reported that the loss of crystallinity during extrusion was no longer caused by penetration of water but by mechanical disruption of the molecular bonds by the intense shear fields within the extruder.

9.3.5. Phenolic contents and antioxidant activities of mushroom snack products

Natural antioxidants may inhibit lipid peroxidation in food and improve food quality and safety (Duan *et al.*, 2006). TPC, DPPH and ORAC assays were conducted in this study. These analyses provided a quick assessment of the presence of these antioxidant components and whether or not they were enhanced or degraded. Fig. 9.1a shows the total phenolic contents of all samples with, or without, the addition of black ear mushroom powder. For the three different products, the addition of mushroom powder increased the TPC values significantly compared to the control. Antioxidant substances, such as phenolic compounds, can block the harmful actions of free radicals, which could scavenge the free radicals and detoxify the organisms (Papoutsis *et al.*, 2016). In terms of both pasta and hot extruded products, Table 9.3 indicates strong positive correlations between TPC and both IDF and TDF ($p \leq 0.001$). However, when it

came to the bread, no significant correlation was observed between the TPC and dietary fibre content. In general, dietary fibre matrices could carry commonly present antioxidant compounds embedded in them (Sungsinchai *et al.*, 2017). One can speculate that the results suggest that different cooking processes had significantly different effects on the properties of the phenolic-dietary fibre network. Figs 9.1b and 1c represent the antioxidant capacities of all samples according to the DPPH and ORAC results, which showed similar trends as the TPC results. As well as the phenolics, researchers have also observed the antioxidant properties of *Auricularia auricula* polysaccharide (Fan *et al.*, 2007; Yoon *et al.*, 2003).

Previous research has indicated that cooking enhanced the hydrolysis of components to release the phenolic compounds thus making them more available for extraction (Burgos *et al.*, 2013). The work of Slavin *et al.* (2013) indicated that high temperature treatment produced higher values of soluble free phenolic acids in soybeans, and that temperature encourages cleavage into the free phenolic form. However, food processing has been reported to have some advantages not only improving the flavour and palatability of foods but also in enhancing the bioavailability of nutrients by inactivating anti-nutritional factors (Xu and Chang, 2008).

In a study where cooked pasta was used to analyse TPC, it was observed that the phenolics removed from common beans during cooking and discarding the cooking water could be around 40 to 50 % (Xu and Chang, 2008). Such a loss of phenolics could be due to leaching of the phenols into the soaking and cooking water, as well as the breakdown of phenolic compounds during processing. In addition, the increase the black ear mushroom powder

Table 9.4 Thermal properties (DSC measurements) for mushroom snack products

Sample name	T onset °C	T gelatinisation °C	T endset °C	ΔH J/g	ΔTr °C
Control raw pasta	58.52±1.69	65.02±1.36	68.36±0.79	3.31±0.77	9.85±0.90
5 % black ear raw pasta	56.88±1.65	64.11±1.96	70.05±1.20	4.72±0.53	13.17±2.84
10 % black ear raw pasta	54.26±2.39	64.96±1.23	70.56±2.27	4.95±0.36	16.30±4.66
15 % black ear raw pasta	52.62±1.74	62.96±0.54	70.33±1.94	5.96±1.49	17.71±0.21

Sample name	T onset °C	T gelatinisation °C	T endset °C	ΔH J/g	ΔTr °C
Control cooked pasta	57.87±0.71	67.9±0.47	72.07±0.50	0.71±0.09	14.2±1.22
5 % black ear cooked pasta	59.31±1.56	67.97±2.15	74.1±0.88	1.76±0.26	14.80±2.44
10 % black ear cooked pasta	57.96±0.30	66.53±1.3	74.06±0.52	1.7±0.25	16.1±0.82
15 % black ear cooked pasta	57.53±0.18	64.2±0.17	74.46±1.18	2.83±0.23	16.93±1.36

Sample name	T onset °C	T gelatinisation °C	T endset °C	ΔH J/g	ΔTr °C
5 % black ear bread	57.71±0.88	63.84±0.92	70.1±1.98	0.33±0.04	12.39±1.10
10 % black ear bread	58.69±0.48	63.4±0.35	71.06±0.64	0.90±0.07	12.37±1.12
15 % black ear bread	54.47±0.11	60.27±0.52	68.52±0.73	1.62±0.06	14.05±0.62

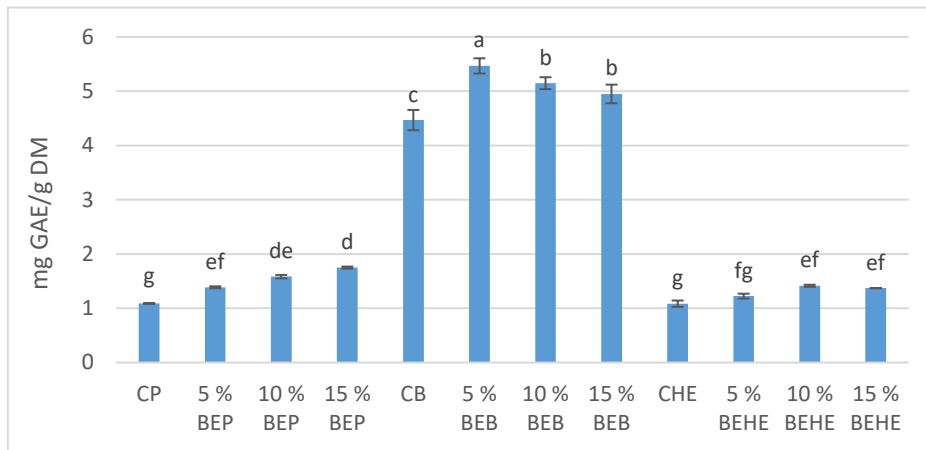
Sample name	T onset °C	T gelatinisation °C	T endset °C	ΔH J/g	ΔTr °C
5 % black ear hot extruded	58.43±0.35	62.20±1.39	70.69±0.64	0.036±0.008	12.26±1.00
10 % black ear hot extruded	59.39±1.20	61.67±1.68	68.31±1.64	0.027±0.006	8.93±0.45
15 % black ear hot extruded	57.11±3.46	62.37±2.54	68.48±0.57	0.10±0.005	11.37±2.88

Mean ± standard deviation.

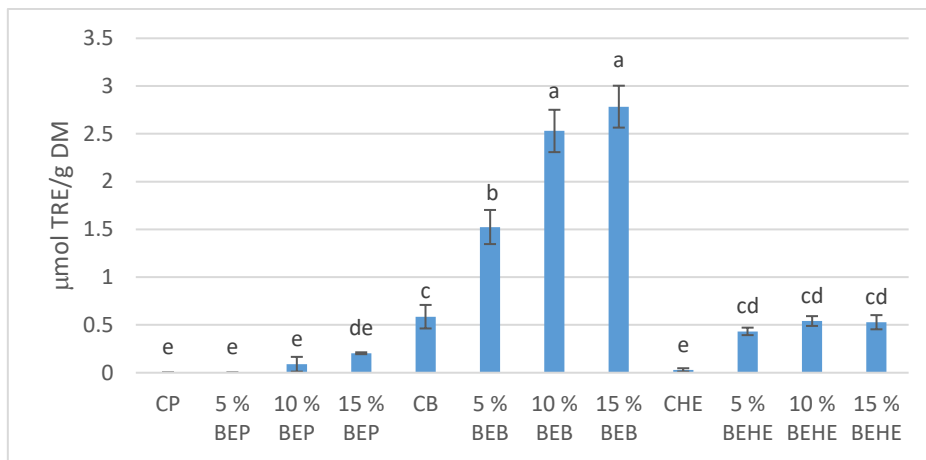
brought to pasta may be attributed to differences in both the content and distribution of phenolic compounds in the final products that resulted from the different effects of boiling on the final TPC. The exact chemical nature of the reduction in TPC and the effect of black ear mushroom inclusion is not fully understood, while the reasons may be chemical transformation, decomposition of phenolics and the formation of phenolic-protein complex under thermal and pressure conditions (Xu and Chang, 2008).

It was noticeable that the bread samples had significantly higher antioxidant capacities than both the cooked pasta and hot extruded products, and the increase after mushroom powder supplementation was relatively higher than that of the pasta and the hot extrudates. The baking process has been reported to be able to increase the content of total phenolic compounds by affecting the solubility of the bound forms of phenolic acids (Žilić *et al.*, 2016). The higher TPC values of the bread samples could be attributed to the release of free phenolic acids from the bound forms and the formation of Maillard reaction products. Researchers have indicated a decrease the TPC values of black soybean crackers (Slavin *et al.*, 2013). The work of Moore *et al.* (2009) suggested that baking may initially destroy the available phenolics, but the release of phenolics may occur during extended or higher temperature baking to make up for this initial loss.

(a)



(b)



(c)

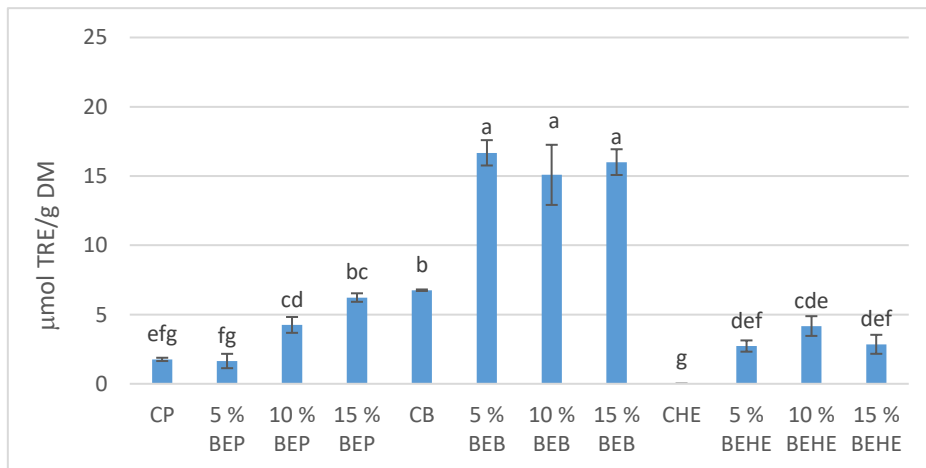


Figure 9.1 Values for total phenolic component and antioxidant capacities of black ear mushroom enriched cereal products

Total phenolic component (TPC) (a); antioxidant capacities: the DPPH. scavenging activities (b); and the ORAC assay results (c). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$). Abbreviations: CP=Control pasta; BEP=Black ear pasta; CB=Control bread; BEB=Black ear bread; CHE=Control hot extruded product and BEHE=Black ear mushroom hot extruded product.

9.3.6. *In vitro* starch digestibility of mushroom snack products

Figs. 9.2 (a), (b) and (c) indicate the amount of reducing sugars released over 120 min during *in vitro* starch enzymatic digestion carried out to mimic the behaviour of cereal snack products when eaten. There were significantly fewer reducing sugars released from the black ear mushroom powder enriched samples than from the control products. The highest substitution level (15 %) resulted in the strongest decrease. Figure 9.2 (d) supports these findings where the effects of incorporating black ear mushroom powder into pasta, bread and hot extruded snack products on standardised AUC values are shown in comparison to the control products. There was a clear decrease in AUC reducing sugars levels of the black ear mushroom powder enriched samples compared with the control. Table 9.1 shows that the starch content of black ear mushroom enriched samples decreased significantly and gradually with the increased substitution levels. The incorporation of black ear mushroom powder influenced the amylose content in the samples, which have been reported to be positively correlated with the amount of resistant starch formation, especially in cooked foods, after cooling (retrograded starch can form) (Svihus *et al.*, 2005). Interestingly, the glucose chains in amylose have been proven to be bound to each other by hydrogen bonds. As a result, they are less available to enzyme attack compared with amylopectin, which has many branched chains (Singh *et al.*, 2010). Furthermore, the work of Aravind *et al.* (2011) revealed a negative relationship between the amylose content and the extent of starch gelatinisation. In this study, Table 9.4 indicates a relative increase in the ΔH values of samples with black ear mushroom powder compared with the control, suggesting less starch gelatinisation. Gelatinisation markedly enhances the susceptibility for amylytic degradation, which may be attributed to the loss of its crystalline

structure (Perez and Oliva-Teles, 2002). These factors may play roles in the decreased starch digestibility with increased black ear mushroom powder contents. In addition, the interactions between starch, protein, lipid and fibre could also influence starch digestion. In terms of the protein content, the addition of black ear mushroom powder into the pasta and hot extruded products showed no significant differences with the control, while such additions into bread resulted in a significant decrease. It should be taken into account that protein digestion usually precedes starch digestion. As a result, the protein layers should be mostly degraded before starch digestion takes place, representing the importance of protein content on starch digestibility (Svihus *et al.*, 2005). Fardet *et al.* (1999) found that gluten was able to reduce starch digestibility by increasing the degree of starch encapsulation. Similarly, the addition of non-gluten protein fractions to flour sample led to increased starch digestion and higher glycaemic index (Aravind *et al.*, 2011). In addition, black ear mushroom fat may also be a factor in altering the final glycaemic index of the samples, as it has been shown that the formation of fatty acids-amylose complexes was able to reduce the digestion rate of starch granules (Svihus *et al.*, 2005). Black ear mushroom powder has a lower fat content than both high grade wheat flour and semolina, although the mushroom enriched hot extruded snack products had higher fat contents than the control, this suggests there were more free fatty acids after the destruction of the matrix between fatty acids and other components under high thermal treatments. This phenomenon was consistent with the significantly higher AUC values of hot extrudates in the other two products. Furthermore, incorporation of black ear mushroom powder significantly increased the IDF and TDF contents of the final products (Table 9.1). Significant positive correlations existed between AUC and both IDF and TDF ($p \leq 0.001$) for all

three products (Table 9.3). It has been suggested that the addition of dietary fibre was able to compete for water with starch during cooking processing, while limiting water movement, starch gelatinisation and susceptibility to enzymes through encapsulating starch granules (Brennan and Tudorică, 2008). Furthermore, dietary fibre can develop a protective matrix with protein around the starch granules limiting the digestive enzymes' activity (Tudorică *et al.*, 2002). Hence, the higher fibre contents of the mushroom additions played an important role in decreasing the glucose released from the samples containing black ear mushroom powder. Correlations were observed between AUC and the SDF of cooked pasta ($r = -0.820$; $p \leq 0.001$); bread ($r = 0.765$; $p \leq 0.01$) and hot extruded product ($r = 0.109$; $p > 0.05$), which may be explained by the different cooking processing methods that resulted in changes in the SDF properties, including the amounts and physical and structural characteristics (Sayanjali *et al.*, 2017). For example, thermal treatments have been shown to be able to change the ratio between the IDF and SDF, the amount of TDF and the physicochemical properties (Elleuch *et al.*, 2011). Zhang *et al.* (2009) reported that the solubility and swelling properties of oat SDF could be reduced after undergoing high thermal and high screw speeds during processing. In general, SDF are characterised by their capacity to reduce the glycaemic response (Elleuch *et al.*, 2011), which was in agreement with the results of pasta in this study. However, the processing of bread and hot extruded products limited this healthy effect of the SDF, especially in bread processing. Therefore, the mechanisms of the effect of cooking processing on the SDF properties of black ear mushrooms' SDF properties within different food matrixes needed further study.

Figure 9.2 (d) shows another interesting finding in that the AUC values of these three products were in a clear order (hot extruded sample > bread > pasta) which was consistent with the temperature order these three products underwent. This could be due to the different food processing methods, which altered the starch structure and influenced the starch resistibility against digestion (Juansang *et al.*, 2012). Especially for the thermal treatment, the conditions could influence the starch gelatinisation and retrogradation, two of the most important thermal behaviours related to starchy foods. In terms of the gelatinisation, this resulted in a breaking up the starch structure by disrupting the inter- and intra-molecular hydrogen bonds between the starch chains; thus, making starch more accessible by enzymes; thereby, increasing the glycaemic index (Chung *et al.*, 2006). Starch granules that underwent hot extrusion cooking were heated under high temperatures and high pressures and shear forces. As a result, the slowly digested starch fraction of the samples could be partially, or even fully, destroyed, although the water content during this cooking process was quite low (Licata *et al.*, 2014). The slow digestion properties of the black ear enriched hot extruded samples may be explained by some additional granular structures that could survive hot extrusion processing and then develop secondary structures, such as amylose-lipid complexes (Robin *et al.*, 2016). Similarly, Pu *et al.* (2013) reported that the gelatinisation of high-amylose starch could be assisted by processing at high temperatures and a higher pressure. While, to our knowledge, little research has been carried out to study the effect of fermentation on starch digestibility, the fermentation process was able to erode the surface of starch granules and even lead to obvious cracks, depending on the conditions applied (Liao and Wu, 2017), which may increase the susceptibility of the surface of the starch to enzymes. While they also illustrated that, after

fermentation treatment, starch could still keep the original functional groups and not produce new compounds. Furthermore, it has been hypothesised that bulk fermentation enhanced the retrogradation of starch by the realignment of amylose and amylopectin chains and, as a result, this would increase resistant starch development in the final products, thereby reducing starch digestibility (Minervini *et al.*, 2010). The work of Buddrick *et al.* (2015) also showed that the formation of resistant starch in bread depended on the fermentation technique, time and temperature, and the different bread making conditions, as well as the oil added to the bread formulation. Furthermore, Table 9.3 demonstrates the negative correlations between TPC and AUC values within both pasta and hot extruded products ($p \leq 0.001$). However, no significant relationships were found between TPC and the AUC of the bread samples. A high phenolic fraction has been proven to be associated with enzyme inhibition and starch molecule interactions, thus impairing starch digestibility, increasing resistant starch and decreasing the glycaemic responses of foods (Moraes *et al.*, 2015). While, in this study, such functionality of the phenolic compounds was strongly limited after the bread making processing compared with other two processing methods. Besides, it was obvious that the AUC values of the pasta samples were lowest of the three products so, this must be taken into consideration, a such lower release of glucose contents may result from the loss of starch and other components from the pasta because of cooking losses.

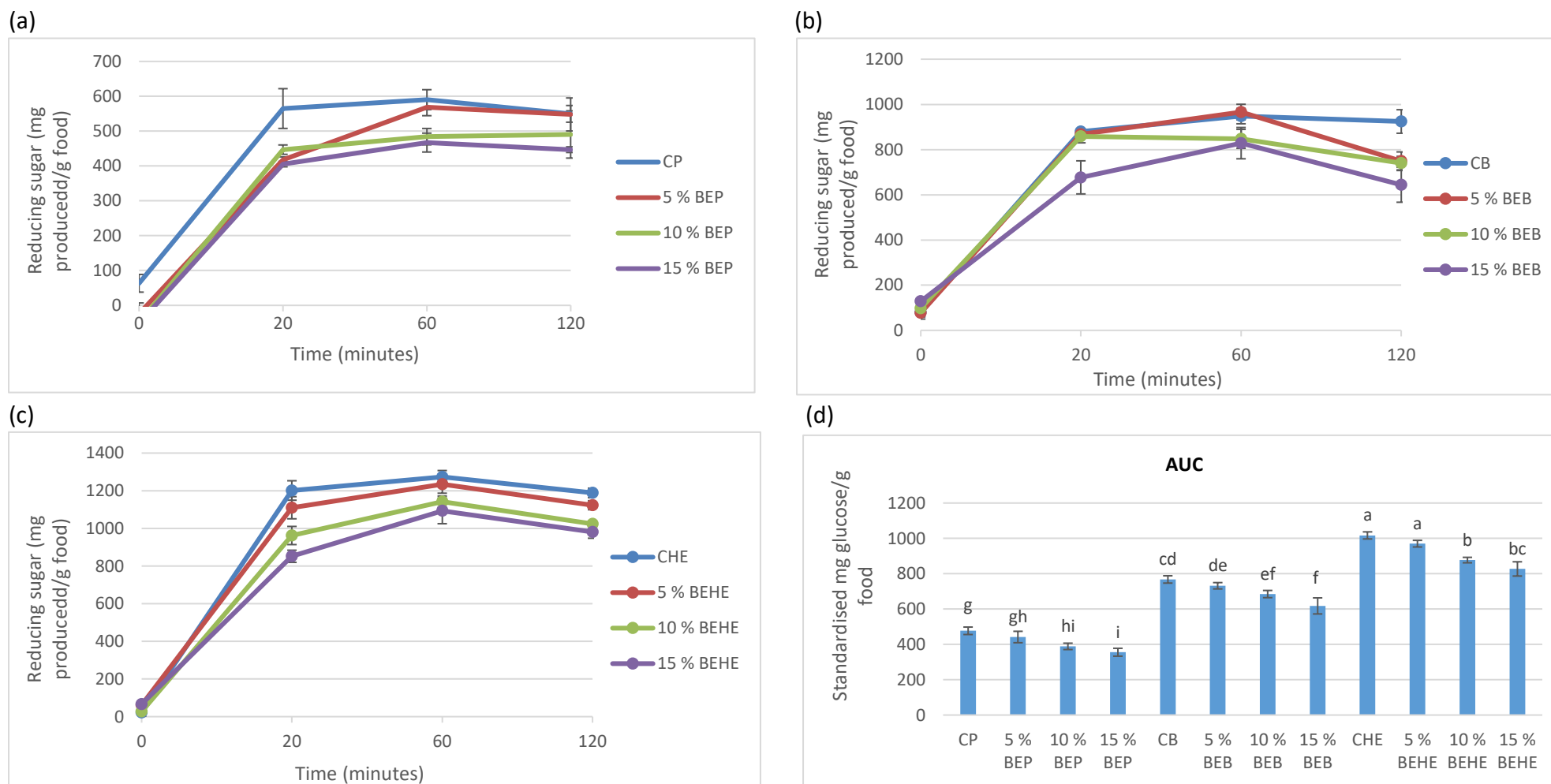


Figure 9.2 Levels of reducing sugars released during *in vitro* digestion of black ear mushroom enriched cereal products

Comparing the control to 5 %, 10 %, 15 % black ear mushroom cooked pasta (a); black ear mushroom bread (b); and black ear mushroom hot extruded products (c). Values for area under the curve (AUC) (d). Abbreviations: CP=Control pasta; BEP=Black ear pasta; CB=Control bread; BEB=Black ear bread; CHE=Control hot extruded product and BEHE=Black ear mushroom hot extruded product.

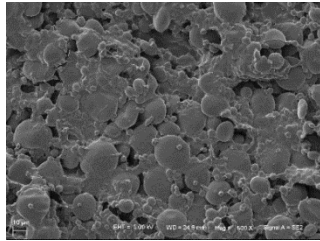
9.3.7. Microscopy of mushroom snack products

Representative scanning electron micrographs of the raw pasta, bread and hot extruded products are provided in Figures 9.3 (a) (d) (c) respectively. Figure 9.3 (a) demonstrated that there were mainly two different sizes and shapes of starch granules of uncooked pasta samples that included large lenticular and small granular shapes; this was consistent with the findings of Hager *et al.* (2013). It was also observed that there was a relatively high proportion of starch in the raw pasta samples, which still kept their granular forms, compared with the other two products. This may be explained by the cold extrusion process raw pasta was treated to that led to a limited water system without intense heat. Images showed that the addition of black ear mushroom powder did not provide any distinguishable changes to the longitudinal surface structure of the pasta, even though the 15 % substitution sample seemed to be less dense and rougher. The transverse cross section of the control pasta showed a more cloud like appearance and, comparatively, the black ear mushroom enriched pasta had a rougher surface with numbers of tiny air holes and cracks. This finding may be attributed to the lack of viscoelastic gluten protein after the addition of mushroom powder.

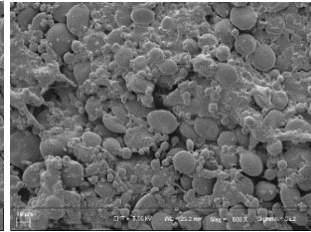
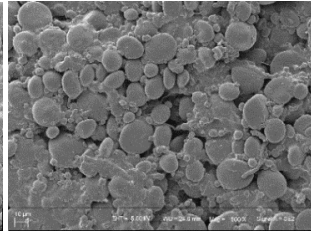
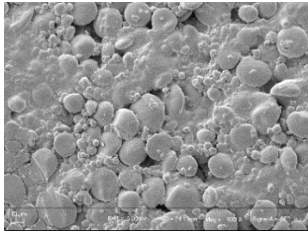
The structural changes of bread mainly comprised solidification and expansion. To be specific, the granular structure of native and fermented starch contained significant differences in their shapes, where the native granules were round and polygonal shaped with relatively smooth surfaces (Liao and Wu, 2017). Figure 9.3 (b) represented the microstructure of the bread crust and crumb with, or without, black ear mushroom powder. The network-like structure in the

bread crumb was predominantly attributed to starch gelatinisation (Martínez and Gómez, 2017). However, the shape of starch granules was maintained significantly well in the bread crust, forming a compact external layer, which was mainly due to the low degree of starch gelatinisation present. It has been reported that water in the bread crust evaporated quickly, and then not enough water was available could be used for the gelatinisation of the starch (Martínez and Gómez, 2017). Furthermore, the starch granules in the structure of the 15 % black ear bread crust appeared to be more fused with neighbouring granules; thus, becoming part of a continuous network. However, the effect of mushroom powder inclusion on the microstructure of the bread crumb was significant. The crumbs of the 10 % and 15 % black ear mushroom inclusion breads were characterised by an open structure with large holes that were related to air bubbles entrapped in the crumb during baking (Brennan and Tudorică, 2008). Moreover, no intact starch granules were visible in any of the crumbs, indicating a high proportion of starch gelatinisation after the whole bread making process. Images of hot extruded snacks with, or without, black ear mushroom powder are shown in Figure 9.3. SEM observations revealed that the microstructures of all these samples existed as visible agglomerates, while still being quite homogeneous. Meanwhile, it was obvious that when the black ear mushroom powder was added to the formulation, the interactions between the additional and original compounds generated different microstructures.

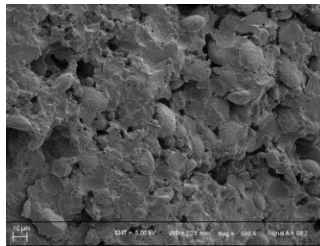
(a)



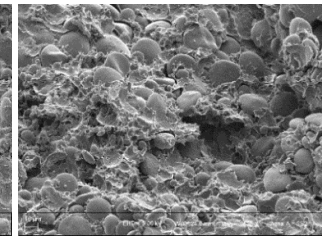
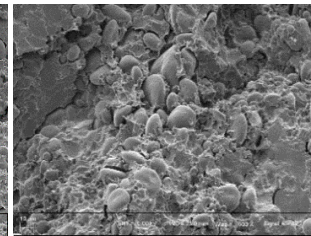
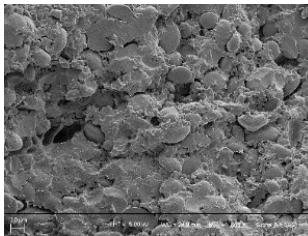
CPL



BEPL

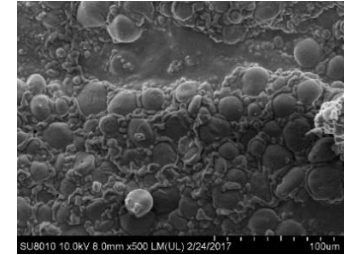


CPC

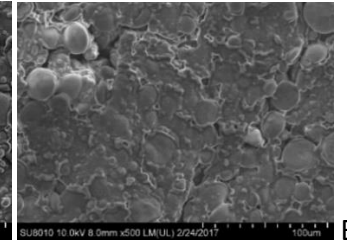
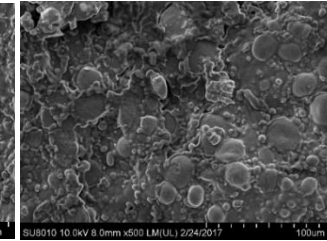
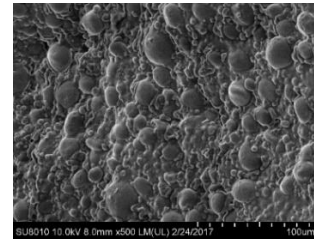


BEPC

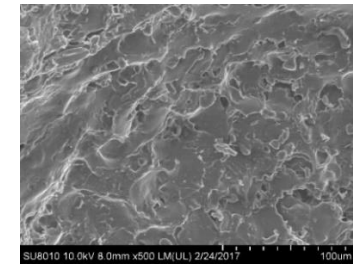
(b)



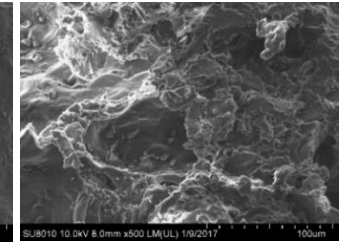
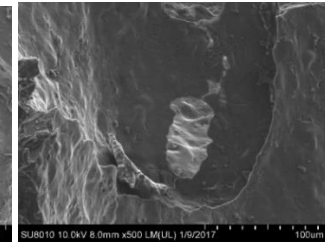
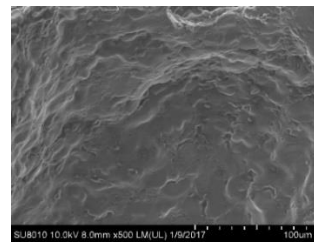
CBT



BEBT



CBB



BEBB

(C)

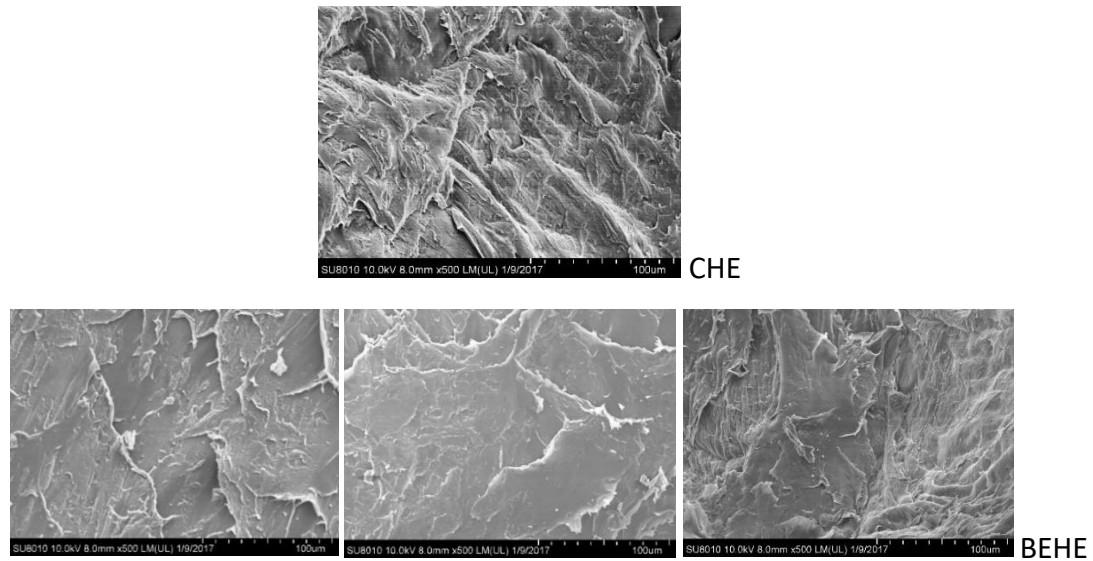


Figure 9.3 Scanning electron micrographs of black ear mushroom enriched cereal products Raw pasta (a), bread (b) and hot extruded products (c) at 500× magnification. Abbreviations: control pasta longitudinal surface (CPL); black ear mushroom pasta longitudinal surface (BEPL); control pasta transverse cross section (CPC); black ear mushroom pasta transverse cross section (BEPC); control bread crust (CBT); black ear bread crust (BEBT); control bread crumb (CBB); black ear mushroom bread crumb (BEBB); control hot extruded product (CHE) and black ear mushroom hot extruded products (BEHE). From left to right: 5 %; 10 % and 15 % substituent level.

9.4. Conclusion

In this study, we incorporated black ear mushroom powder into fresh pasta, bread and hot extruded products. All the mushroom enriched samples represented different physical and textural properties compared with the control, in terms of most parameters, while the addition of black ear mushroom powder also had negative effects on the final products. Moreover, we also observed the limited starch gelatinisation and significantly increased phenolic contents after such incorporations. This was very meaningful considering that the effects of black ear mushroom powder additions depended, to some extent, on the cooking process. In conclusion, black ear mushroom powder could be included into cereal products, including pasta, bread

and hot extruded products, to promote the health values of final products and expand the application of edible mushrooms in Western countries.

Chapter 10

General discussion and conclusions

10.1. Aims and hypothesis

Four different species of common edible mushroom powder (white button, shiitake, porcini and black ear mushroom) were incorporated into three different cereal products (fresh semolina pasta, bread and hot extruded snack), then novel products were successfully developed. In order to determine the effect of mushroom powder addition on the physical, chemical and nutritional properties of these final novel food products, a series of parameters were analysed, including physical and textural properties; nutrient content; *in vitro* starch digestion; antioxidant capacities. Three different cooking processes, of cold extrusion (fresh pasta); hot extrusion (hot extruded snack) and fermented cooking (bread), were utilised thus we also evaluated the influences of food processing on the benefits of mushroom addition to the final samples.

Answers to the objectives of research proposal:

1) Novel cereal products were developed and the contributions that mushroom powder made to final food quality and human nutrition were identified, after evaluating a series of properties, including physical, chemical and nutritional characteristics.

2) The nutrients and bioactive ingredients in mushroom powder were analysed together with their incorporation into final products, such effects were critically discussed the effects of food processing on the final properties of snack products.

3) The energy content, starch gelatinisation, glycaemic response, antioxidant properties and microscopy of these mushroom enriched RTE snack products were determined and interactions evaluated to determine synergistic and antagonistic effect.

4) Due to limits of time the sensory analysis of products was not conducted. Further research is required to evaluate the acceptability these novel products to consumers.

Answers to the hypothesis of research proposal:

1) incorporation of mushroom powder made negative effects on the physical and textural properties of cereal products, such as increased pasta cooking loss; increased firmness of pasta and decreased bread dough extension.

2) addition of mushroom powder increased the total dietary fibre content and total phenolic content of RTE snack products, and increased the antioxidant capacities of these final products.

3) food processing played a role in the characteristics of the RTE snack products after mushroom powder addition.

4) mushroom incorporation limited the starch gelatinisation and digestibility of these novel products, as a result, reduced their glycaemic index.

10.2. Summary

The results of this study illustrated that edible mushroom powder could be incorporated into cereal RTE-snack products (fresh pasta, bread and hot extruded snack) in order to develop novel functional products with mushroom bioactive ingredients. Even though addition of mushroom powder into cereal products created some negative effects on the physical and textural properties of the products. The results support that mushroom powder cause an important impact in reducing the total starch content, and fortifying the TPC, IDF and TDF of products. Inclusion of mushroom powder could not only add nutritional benefits into the final products, but also may functionally act as a modification agent by reducing the hydrolysis of starch during digestion and enhancing the antioxidant capacities of cereal products. This work also presents mushroom incorporation as a viable way of inhibiting starch from gelatinisation and altering the microstructure of samples.

10.3. Cross discussion between papers

The WAI of the four mushroom enriched pasta types showed three different tendencies with the increase of substituent levels compared with control (Table 4.1 and 9.2a). Generally, the differences in WAI between the different species of mushroom powder addition in this study

could be related to the structural difference or to the different quantities and particle size of individual nutrients (such as dietary fibre) (Foschia *et al.*, 2015 a). Addition of porcini mushroom decreased the WAI of pasta significantly and gradually, which may be attributed to the higher degree of gelatinisation of starch granules in the porcini mushroom pasta surface, preventing water absorption (Zhang *et al.*, 2013 b), as can be seen in their higher ΔH values (Table 5.4) and in the longitudinal surface SEM images (Fig. 5.3). Even though the ΔH values of black ear mushroom enriched pasta were relatively higher than that of white button and shiitake mushroom enriched samples (Table 9.4), such addition did not cause extra starch gelatinisation in the pasta longitudinal section (Fig. 9.3a), as a result, their WAI increased significantly and gradually compared with control. These phenomenon could also be due to the relatively higher total dietary fibre content of black ear mushroom pasta (Table 9.1) and lower such content of porcini mushroom samples (Table 5.1). The higher dietary fibre content in this material resulted in a strong water-absorbing capacity, causing a significant increase in WAI (Foschia *et al.*, 2015 a). The effects of different mushroom powder incorporation on the gluten matrix of pasta played an important role in the starch granule availability to imbibition (Zhang *et al.*, 2013 b). Evaluating the results for the hot extruded products, black ear mushroom inclusion increased the WAI significantly which may be explained by the increased dietary fibre content of black ear enriched extrudates (Table 9.1). Dietary fibre could disrupt the pasta matrix to promote water absorption and expose starch granules to swelling and rupture (Foschia *et al.*, 2015 a), which is in agreement with the increased WSI of black ear mushroom extruded samples. However, the addition of the other three mushroom species decreased the WAI of hot extruded samples significantly.

Similarly, the WAI of chestnut mushroom coproduct enriched hot extruded samples was observed to be lower than those of control (Brennan *et al.*, 2012 b). A possible explanation is that the high proportion of IDF increased water-holding ability of the samples and water retention postflashing off of moisture at the extruder die face, as a result, reducing WAI of the products (Brennan *et al.*, 2012 b). This would also explain the higher density values of white button and porcini mushroom samples (Table 8.4). The exact mechanisms for these completely opposite effects of different mushroom on the WAI of hot extruded products still need to be studied further.

The effect of inclusion of different mushroom powder on the textural characteristics of different cereal snack products is shown in Fig. 4.2, Table 6.2, Table 6.3 and Fig. 8.2.

Incorporation of all mushroom material created a similar influence on the textural properties of pasta and bread dough, such as increased the firmness of cooked pasta, decreased the extensibility of cooked pasta (shiitake mushroom showed no significant influence) and bread dough. Interestingly, different mushroom powder did not behave in a similar way on the hardness of bread and hot extruded products. This observation could be explained possibly that high thermal cooking processing altered the effects of mushroom powder on the textural characteristics of cereal products, to be specific, the ingredients of mushroom powder were influenced under high temperature processing, then leading to different trends of textural properties with the increase of mushroom content.

According to the DSC results shown in Tables 5.4; 7.1; 8.6 and 9.4, the magnitude of the changes in starch gelatinisation behaviour of black ear mushroom was bigger than other three mushroom species for a given amount of incorporation. Addition of black ear mushroom led to higher dietary fibre contents of final cereal products, which acted as hydrophilic components to uptake water and retarded swelling, hence limited the gelatinisation of the starch granules (Aravind *et al.*, 2012 b).

Previous studies have found that the bioactivities of fermented foods are usually stronger than those of their unfermented counterparts (Huang *et al.*, 2017; Jayabalan *et al.*, 2014). In addition to the total phenolic content of these three different products, bread samples always exhibited the highest results when compared with cooked pasta and hot extruded products. Such an observation could illustrate that the bioactive performance of food materials is affected by certain types of processes, such as hydration, thermal treatment and fermentation. It has been indicated that the fermentation process benefited an increase in the total phenolic content (Fernandes *et al.*, 2017). Most likely, the bioactive components produced during fermentation were able to catalyse the hydrolysis of the phenolic compounds, resulting in an enhanced total phenolic content. Furthermore, mushroom could produce the hydrolytic and oxidative enzymes which is necessary to degrade individual components of the growth substrate during cultivation, such as β -glucosidase (Cai *et al.*, 1999). It also has been illustrated that the β -glucosidase enzyme played an positive role in the hydrolysis of the phenolic components, and its activity was significantly increased after

fermentation treatment (Fernandes *et al.*, 2017). This observation may also be attributed to the activity of these hydrolytic enzymes which can soften and break down the cell wall matrix of samples, hence rendering the bound and conjugated phenolics in the samples more susceptible to extraction (Huang *et al.*, 2017). Furthermore, it has been reported that the bioaccessibility of the phenolics in the yeast fermented wheat bran was significantly upgraded when subject to gastrointestinal digestion (Anson *et al.*, 2009). Additionally, the results of DPPH and ORAC representing the antioxidant capacities of all the three cereal products followed similar trend with that of total phenolic content as mentioned above that bread samples obtained the highest activity than the other two (Fig. 5.2; 7.4; 8.3 and 9.1). This result was in agreement with previous reports that fermentation process increased the antioxidant activity of seeds and oat (Huang *et al.*, 2017; Cai *et al.*, 2012).

Starch digestion is one of the essential metabolic responses following meal ingestion, meanwhile lots of intrinsic and extrinsic food factors play roles in influencing both the duration and extent of starch digestion (Capriles *et al.*, 2008). In this study, the AUC value was used as an important indicator to the amount of reducing sugars released over 120 min *in vitro* starch digestion. Hagenimana *et al.* (2006) reported that the starch-digestion rate depended mainly on processing conditions. Cooking processing exposes the food starch matrix and promotes gelatinisation. As a result, the susceptibility to enzymatic digestion is increased, which was in agreement with the higher ΔH values of raw pasta than that of cooked samples in this study (Table 5.4 and 9.4). Figures 5.1, 7.2 and 8.4 illustrated that

there was a clear order of the AUC values of these three products: hot extruded snack; bread and cooked pasta in descending order. The results of DSC indicated that the extent of starch gelatinisation of these three cereal products followed the similar order. According to the work of Holm *et al.* (1988), plasma glucose and insulin responses as the rate of *in vitro* hydrolysis with α -amylase were strongly correlated positively to the degree of starch gelatinisation. So the degree of starch gelatinisation is an important determinant for the rate of starch hydrolysis *in vitro*. Additionally, it was proved that both resistant starch type (RS) 1 (physically inaccessible starch) and RS type 2 (native granular starch) were affected by thermal processing (Capriles *et al.*, 2008). For cooked pasta, starchy foods cooked with an adequate amount of water could lead to RS contents lose due to their cooking loss and starch gelatinisation, which may explain the lowest AUC values of cooked pasta samples among all the three products (Cummings and Englyst, 1995). Baking treatment has been found to increase the RS, which could be partly RS type 1 and 2 and also retrograded amylose-RS type 3 (Capriles *et al.*, 2008). When it comes to hot extruded products, the observation of the relatively higher AUC values of the products were supported by the work of Wang *et al.* (1993) that extrusion greatly increased wheat bran and whole flour starch susceptibility to enzymes. Similarly numerous studies have indicated that extruded cereal products should be regarded as high glycaemic index foods which in most cases exceed that of bread (Brennan, 2005; Brennan *et al.*, 2008 a). Furthermore, even though amylose complexes are generally developed by adding a complexing agent to a hot aqueous solution of starch, this can be achieved momentarily with an extruder at low moisture contents (Bhatnagar and Hanna, 1994). It is evident to form the amylose-lipid complex during

extrusion and the extent of such complex development is dependent upon both starch and lipid type present in food matrix (Singh *et al.*, 2007). Interestingly, formation of these complexes lead to the decreased water-solubility and susceptibility of the starch granules to digestion (Bhatnagar and Hanna, 1994). So it may explain the limit effect of mushroom materials on the inherent high glycaemic reponse of hot extruded products.

The different cooking processings used in the experiments also illutsrated different influences on the correlations between indexes. For instance, significant negative correlations (Table 7.3 and 8.3) were found to exist between AUC and both IDF and TDF ($p \leq 0.001$) of bread and hot extruded products, whereas the negative correlations of pasta samples (Table 5.2) were less close ($p \leq 0.05$). While no significant difference was observed between the correlations between AUC and SDF of all the three products. No significant correlation between TPC and AUC of bread was observed in Table 7.3, however, such correlation of both pasta and hot extruded snack was strongly negatively ($p \leq 0.001$). In conclusion, all the three cereal products suffered from a complex multi-step, multi-functional and thermal/mechanical process. Further investigations on granular and molecular levels would help to understand the mechanisms underlying these changes as well as the influence of process variables. However this observartion seems to indicate that fibre can reduce the effect of thermal processing on starch disruptuon and loss of starch structural integrity

10.4. Limitations and recommendations for future research

This study showed that mushroom powder obtained a clear benefit in terms of enhancing the nutritional properties of cereal products. The mushroom powder enriched products had significant reduction in glucose release during the *in vitro* digestion and increase in the antioxidant capacities compared to the control, which illustrates that there is a great potential of using edible mushroom materials in manipulating the postprandial glucose response of cereal products. *In vivo* analysis is necessary to confirm these functional benefits. Additionally, further work is required to analyse the role of each mushroom components played in cereal products quality, such as mushroom polysaccharides, protein, fat, fibre and antioxidant components.

During the production of fresh mushrooms, the waste material, including the hyphae and basal material remaining in the compost, account for up to more than 20 % of the crop weight which lead to significant disposal expenses (Brennan *et al.*, 2012 b). Thus it is considerably interesting to incorporate these mushroom coproducts into RTE-snack products in order to reduce the disposal costs substantially and develop an added-value coproduct as a low-cost fibre and other bioactive components source. While it is very time consuming and labour intensive to clean and separate the useful materials from residual compost, which is one of the biggest challenges. Additionally, further work is required to assess the acceptability of these novel cereal products to determine how their properties are perceived

by consumers, meanwhile to determine the relationship between instrumental textural analysis and sensory perception.

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