Technical and nutritional properties of vegetable enriched pasta utilising juice and pomace from spinach, red cabbage, beetroot and carrot

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Jinghong Wang

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Declaration

Some aspects of this thesis have been published or submitted in *peer-review* journals and presented at conferences.

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Wang, J., Brennan, M. A., Brennan, C. S., & Serventi, L. (2022). Predictive Glycaemic Response of Pasta Enriched with Juice, Puree, and Pomace from Red Cabbage and Spinach. Nutrients, 14(21), 4575.

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

Technical and nutritional properties of vegetable enriched pasta utilising juice and pomace from spinach, red cabbage, beetroot, and carrot

by

Jinghong Wang

Abstract

Vegetable pasta may deliver health benefits by increasing vegetable intake. Previous studies only applied one form of vegetable to pasta formula to investigate their effect. For example, Padalino, Costa, Del Nobile, and Conte (2019) used powdered glass wort (*Salicornia europaea*) to improve the nutritional value of pasta. In contrast, SIPOS et al. (2017) evaluated the nutritional value and sensory quality of pasta enriched with beetroot juice and beetroot puree. Few comparisons were found between different forms at the same addition level. Therefore, this study investigated the replacement of semolina with juice, puree, and pomace of spinach, red cabbage, beetroot and carrot. The effect of replacement on technical and nutritional value was evaluated.

The cooking loss of pasta made with spinach juice and spinach puree at 1 % substitution was the same as the control, while all other samples showed higher cooking loss. Spinach pasta was characterised by higher breaking force but lower breaking distance in the tensile test than the control, while all other pasta had a lower breaking force and breaking distance. Spinach pasta was generally firmer than the control. Red cabbage juice pasta was less firm than other forms of fortified pasta at 1 g/100 g substitution level. Spinach, red cabbage, beetroot and carrot juice are better colourants than puree or pomace as they change the colour of the pasta more dramatically at the same substitution level.

The *in vitro* starch digestibility test show that spinach pomace 10%, red cabbage pomace 10%, beetroot pomace 10%, carrot pomace 10%, spinach juice 1%, spinach puree 2%, and beetroot puree 4% significantly reduced the area under the curve of the *in vitro* starch digestion. This reduction was due to a combined effect of decreased starch content, increased dietary fibre content and inhibition of α-amylase caused by vegetable material addition. Total phenolic content (TPC) and antioxidant capacity increased significantly on raw, cooked and digested samples of vegetable-fortified pasta compared to the control. The β-carotene content of spinach and carrot pasta samples (raw, cooked, and digested) was also higher than that of the control. At the same substitution level, the juice was more efficient in improving the antioxidant capacity of resultant pasta compared to puree or

pomace. Mineral contents of vegetable-fortified pasta (raw and cooked) were also higher than the control. In general, at the same lower substitution level, juice and puree-enriched pasta samples generally exhibited better technical quality than pomace-enriched ones. The study exhibits that incorporating vegetable juice or puree to produce pasta results in a superior nutritional profile with a slight compromise of technical quality.

Keywords: vegetable pasta, glycaemic index, quality, texture, antioxidant, physicochemical, dietary fibre, α-amylase inhibition, β-carotene, minerals, colour

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Chapter 1

Introduction

1.1 Background

Pasta, traditionally made from durum semolina, is a popular staple food as it is palatable, easy to prepare, and affordable (Petitot, Abecassis, & Micard, 2009; Ziółkiewicz et al., 2023). Health and nutrition have become crucial factors for consumers' choice of food products. Pasta is a basic meal in everyday life. However, pasta is low in dietary fibre, vitamins, and minerals (Gull, Prasad, & Kumar, 2015; Peressini, Cavarape, Brennan, Gao, & Brennan, 2020). Incorporating pasta with healthy ingredients could be a good idea (Rachman, A. Brennan, Morton, & Brennan, 2020). Vegetables contain a variety of health-benefit nutrients such as fibres, vitamins, polyphenols, carotenoids, glucosinolates, and minerals that pasta lacks (Oliviero & Fogliano, 2016). However, customers tend to have ingrained eating habits that frequently fail to meet the 400 g dairy intake amounts of fruit and vegetables that are recommended by World Health Organisation even though they have aware of the health promotion role of fruit and vegetables (Capacci et al., 2012; Marinelli, Padalino, Conte, Del Nobile, & Briviba, 2018). In that case, adding fruit and vegetable compounds into basic food such as pasta and bread may be one good option to help people meet the suggested daily intake of fruit and vegetables. However, pasta used to be considered a "fattening food" due to the high starch content, the glycaemic response after pasta consumption is moderate compared to other staple foods such as bread and rice (Peressini et al., 2020). Zou et al. (2019) have shown that absorption of starch from pasta in the intestinal tract is slow and incomplete compared to other starch-based foods such as white bread. The addition of vegetable ingredients could be an option to lower the glycaemic response of pasta products further.

Nutritional improvement by vegetable enrichment was partially based on the high antioxidant capacity of vegetable ingredients (González et al., 2021; Oliviero & Fogliano, 2016). These antioxidant capacities are derived from phenolic compounds, vitamin C (ascorbic acid), vitamin E (tocopherols), carotenoids, phytosterols, isoflavones and organosulphur (Abuajah, Ogbonna, & Osuji, 2015). The health benefits of these antioxidants generally involve scavenging free radicals, reducing carcinogenesis in cells, binding toxins and carcinogens in the intestinal tract, lowering cholesterol absorption, and enhancing the immune system (Abuajah et al., 2015). Some vegetable ingredients deliver dietary fibre to pasta. Dietary fibre may also play a role in entrapping harmful toxins in the intestinal tract, lowering serum LDL-cholesterol without affecting HDL-cholesterol, and decreasing fat absorption (Abuajah et al., 2015). Theuwissen and Mensink (2008) reported that dietary fibre could lower glycaemic index, reduce the risk of cardiovascular disease, and act as a prebiotic for beneficial microbiota inside the intestinal tract. Minerals also contribute to human health. Traditional pasta lacks some essential minerals (e.g. iron and zinc), while some vegetables are rich in those minerals. Therefore, those vegetables can complementarily enhance pasta's nutritional value. For example, spinach is rich in iron (Hedges & Lister, 2007; Yuan et al., 2019), carrots are high in zinc (Sharma, Karki, Thakur, & Attri, 2012), and beetroot is considered a good supplement vegetable for calcium and potassium (Chhikara, Kushwaha, Sharma, Gat, & Panghal, 2019). Thus, enriching pasta with vegetable components could deliver these health benefits to the consumer.

Vegetable enrichment may have a quality impact on the cooking and sensory quality of pasta. Vegetable-fortified pasta generally lowers the cooking time, increases cooking loss, and changes water absorption (Abdel-Moemin, 2016; Bustos, Vignola, Paesani, & León, 2020; Padalino, Mastromatteo, Lecce, Cozzolino, & Del Nobile, 2013; Sun-Waterhouse, Jin, & Waterhouse, 2013). Textural properties are also altered. Vegetable-enriched pasta is typically characterised by low firmness, higher adhesiveness and lower elasticity (Abdel-Moemin, 2016; Carini, Curti, Spotti, & Vittadini, 2012; Padalino

et al., 2017; Sun-Waterhouse et al., 2013). These changes lead to lower overall quality and acceptability compared to traditional durum wheat pasta (Bouacida, Ben Amira, Ben Haj Koubaier, Blecker, & Bouzouita, 2017; Marinelli et al., 2018; Minarovičová et al., 2018; Sant'Anna, Christiano, Marczak, Tessaro, & Thys, 2014). There are several reasons for the quality decrease. Firstly, the substitution of durum wheat with vegetable ingredients dilutes the gluten content in pasta, thus weakening the gluten network (Abdel-Moemin, 2016). Secondly, fibre from vegetable ingredients can disrupt the gluten matrix, resulting in a weakened structure of pasta (Marinelli et al., 2018). Thirdly, the added ingredients may contribute to unpleasant flavour and taste, decreasing the overall sensory acceptability (Sun-Waterhouse et al., 2013). However, some vegetable content may improve the cooking and sensory quality of pasta in some quality aspects. For example, Sobota, Wirkijowska, and Zarzycki (2020) found that kale powder pasta (6% - 8% substitution level) had a better sensory quality than the control, which consumers prefer. The authors suggested that the physicochemical properties contribute to that better sensory quality.

Different forms of vegetable ingredients can have a different impact on either the cooking quality or sensory quality of pasta. Kowalczewski et al. (2015) used fresh potato juice and dried powder from potato juice to fortify pasta. The authors found that fresh potato juice pasta had a lower cooking loss than control durum wheat pasta while dried potato juice pasta had a much higher cooking loss, which indicates poor quality. Carini et al. (2012) compared soy ingredients (a combination of soy flour and defatted soy milk), whole soy flour and soy milk when combined with pasta. The author found that soy milk pasta has comparable extensibility and cooking loss compared to the control. In contrast, soy ingredients (a combination of soy milk and soy flour) and soy flour pasta were found to decrease in extensibility and increase in cooking loss dramatically. The juice forms of vegetables seem to provide a better quality of pasta than the dried flour form of vegetables. The reason for that might be the juice form of vegetables have a lower solid or fibre content that may disrupt the gluten matrix. However, no

compelling evidence showed that vegetable juice is superior material to maintain the pasta quality as those studies used relative less juice (in dry weight) compared to powder when added to pasta.

1.2 Aim

The aim of this research is to investigate the applicability of fortification methods in enhancing the physical and nutritional properties of vegetable pasta. Specifically, the study will focus on selecting suitable forms of vegetables to produce vegetable-enriched pasta with desirable technical and nutritional characteristics.

1.3 Objectives

1. To compare the culinary quality of fortified pasta with different forms of vegetables.

2. To compare the nutritional compounds of vegetable pasta using different forms of vegetables.

3. Using *in vitro* digestion to evaluate the bioavailability of nutrients from vegetable pasta.

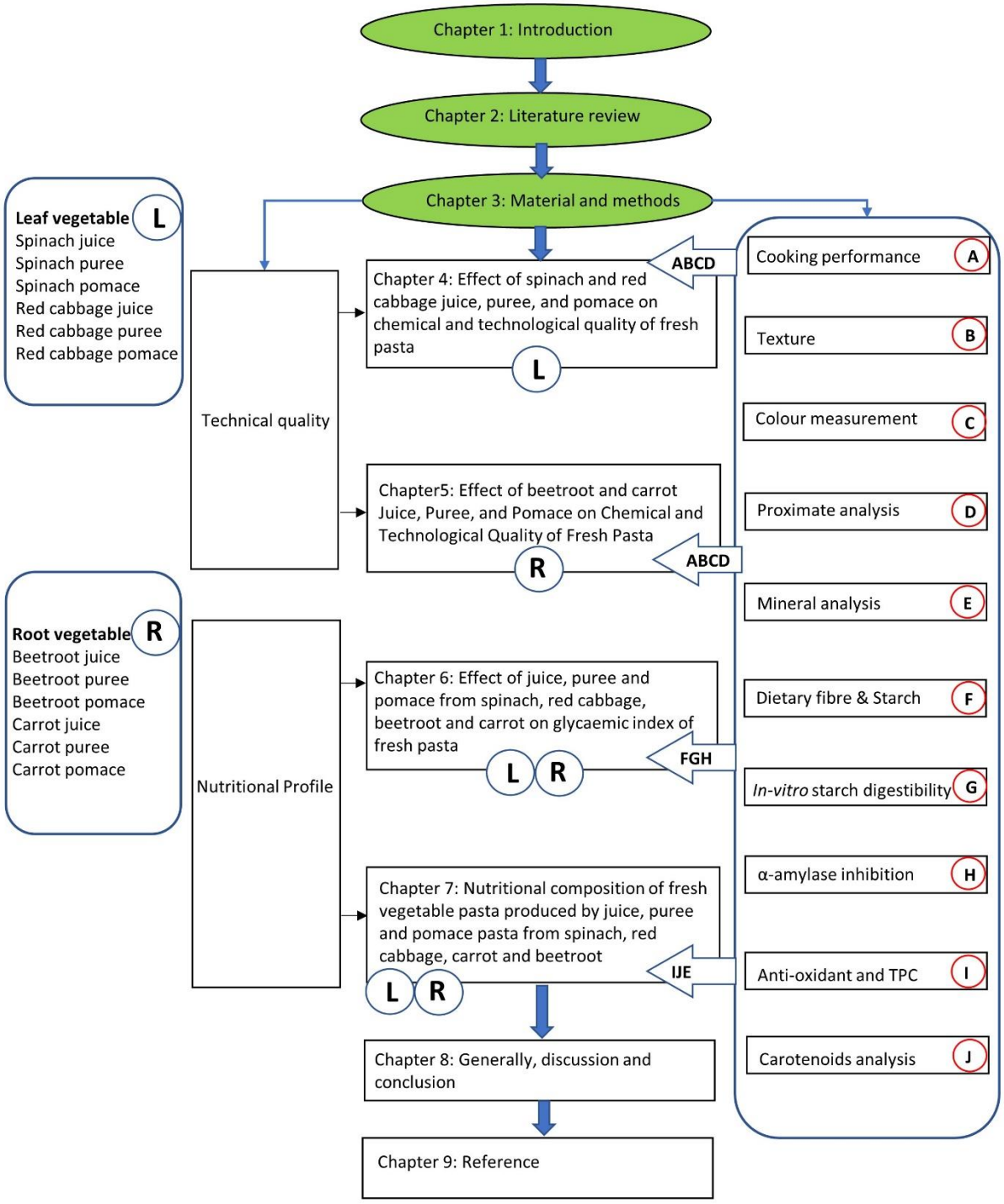
1.4 Hypotheses

1. Vegetable pasta will have lower cooking qualities when compared to traditional durum wheat pasta.

2. Vegetable pasta will have a better overall nutritional value than traditional pasta.

3. The phytochemical composition of different types of vegetables will lead to varying effects on the technical and nutritional quality of pasta when substituted, despite having the same substitution level.

1.5 Thesis Structure



Chapter 2

Impact of functional vegetable ingredients and processing on the technical and nutritional quality of pasta

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2.1 Introduction

Pasta is a staple food around the world (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 2015b). It is popular with consumers due to its low cost, and long shelf life (Petitot et al., 2009). Pasta consumption is increasing globally (Bustos, Perez, & Leon, 2015). Therefore, it is a good vehicle to deliver functional ingredients which contain extra nutrients lacking in the diet. These functional ingredients may include cereal products, dietary fibre, legumes, meats, fruit, and vegetables. Vegetable ingredients are of interest to the food industry not only because of the consumer awareness of vegetable consumption for health promotion effect but also because vegetables are high in availability (Marinelli et al., 2018). However, delivering such functional ingredients could impact pasta's sensory and cooking quality; thus, a decreased consumer acceptability may occur (Bustos et al., 2015). Much research has been conducted to study the nutritional and quality changes that occur with the addition of functional ingredients. Some of the research suggests that the nutritional value can be improved while minimising negative affect on the pasta quality. This article reviews pasta fortified by various functional ingredients and their effects.

2.2 Manufacture of pasta

2.2.1 Raw material

Traditional pasta is made of durum wheat semolina, which is produced by milling *Triticum turgidum* subspecies - durum (Peressini, Tat, & Sensidoni, 2019). However, durum wheat only contributes 6-8% of the world total wheat production, and more than half of durum is cultivated in the Mediterranean region (Dexter, Matsuo, & Morgan, 1981). Hence, the availability of durum wheat is limited. Therefore, quite a lot of pasta producers around the world partially or totally substituted the durum wheat with widespread bread wheat (milled from *Triticum aestivum*), although the final quality of pasta may be inferior to durum wheat pasta (Peressini et al., 2019). Some food hydrocolloids could be used as mean

additives to improve pasta quality. For example, Peressini et al. (2019) reported that the bread wheat pasta enriched with 0.5% propylene glycol alginate or 1% sodium alginate had similar qualities to durum wheat pasta.

Health and nutrition have become crucial concerns for consumers' choice of food product. Thus, pasta producers and researchers focus on additional functional ingredients to fortify pasta products. Many scholars focused on enriching pasta with various functional ingredients as raw materials to improve its functionality. Those ingredients include legumes (Fuad & Prabhasankar, 2010; Mahmoud, Nassef, & Basuny, 2012; Wood, 2009), whole grain cereals, vegetable compounds (Bustos et al., 2015; Chauhan, Vaidya, Gupta, & Pandit, 2017), extracted or isolated dietary fibres (Aravind, Sissons, Egan, & Fellows, 2012; Aravind, Sissons, Fellows, Blazek, & Gilbert, 2012; Bustos et al., 2015; Bustos, Pérez, & León, 2011; Tudorică, Kuri, & Brennan, 2002) and even meats (Liu et al., 2016).

2.2.2 Mixing

There are three stages to produce pasta – mixing of ingredients, cold extrusion and finally drying which is shown in **figure 2.1** below:

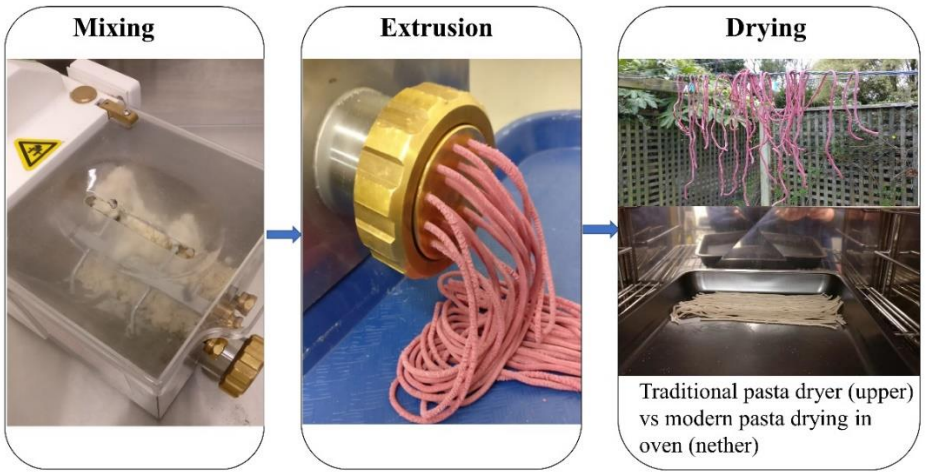

Figure 2.1 Pasta making procedure

In the mixing procedure, flour and other raw materials are mixed with water to form an unleavened dough (Li, Zhu, Guo, Brijs, & Zhou, 2014). Water is added to flour with stirring to assure homogeny until water diffuses into the centre of flour particles to form a dough (Bustos et al., 2015). The amount of water (mL) added to every 100-gram semolina is hydration level (%), which is an important factor in making non-brake nature and the smoother outer finish of the dough (Gopalakrishnan, Menon, Padmaja, Sajeev, & Moorthy, 2011). The optimal hydration point is the point where pasta can achieve its best possible processing parameter and quality (Murray, Kiszonas, & Morris, 2017). However, different studies show various optimal hydration levels, for example, Matsuo, Bradley, and Irvine (1972) reported an optimal hydration level of 33.5%, while Martin, Irvine, and Anderson (1946) showed that 31% is the optimal hydration level. The different optimal hydration levels in different studies may be due to the different particle sizes of semolina, raw material varieties, and successful processing parameters (Murray et al., 2017). In this case, the pasta fortified with other functional ingredients may also have different optimal hydration levels. Typically, for traditional durum wheat pasta, the hydration levels of dough are preferred at 27%-33% (Murray et al., 2017). The proper mixing operation is to allow a homogeneous plasticisation of flour with water (Kratzer, 2007). Insufficient water addition results in incorrect hydration level, while exceeding water results in big lump formation, which causes difficulties in succeeded extrusion (Bustos et al., 2015). Vacuum dough mixing is preferred as it is beneficial for the development of the gluten network due to the higher protein polymerisation (Liu et al., 2017). Vacuum dough mixing was also reported to prevent the oxidation of vulnerable pigments in pasta, such as carotenoids (Fu, Schlichting, Pozniak, & Singh, 2013).

2.2.3 Extrusion and drying

After mixing, the dough is shaped and kneaded through a die under high pressure, called extrusion (Bustos et al., 2015). Under mechanical pressure, the energy is applied to the dough, and the glutenins

and gliadins interact strongly and form intra-molecular and inter-molecular disulfide bonds. Thus, a cross-linked three-dimensional gluten matrix is formed (Petitot et al., 2009). The final quality of pasta is depended on the gluten network (Abecassis, Abbou, Chaurand, Morel, & Vernoux, 1994). The heat is generated by friction and pressure, so the extrusion rate and pressure should be carefully controlled. Insufficient heat can result in an amorphous gluten matrix, while overheating may cause disruption of the protein matrix and protein coagulation (Murray et al., 2017). During industry practice, the water-jacked barrel may be installed to reduce heat during the extrusion procedure (Murray et al., 2017). Pollini, Panto, Nespoli, Sissons, and Abecassi (2012) reported that different types of extrusion die could have an impact on pasta quality. The authors found that Teflon-coated bronze die can create a very smooth, uniform surface of pasta compared to traditional bronze die. After extrusion, pasta is dried to increase shelf life, although fresh pasta without drying was made and eaten historically (Murray et al., 2017). Original air drying was developed in Southern Italy to longer the shelf life. However, air drying is influenced by weather conditions; thus, modern pasta driers, which can control the temperature, relative humidity, air flow and drying time, were developed to remove the water content (Murray et al., 2017). The freshly extruded pasta without drying has about 30% of moisture content, and after drying, only around 12% of water left, which is similar to commercial flour (Bustos et al., 2015). The modern pasta industry tends to use high-temperature drying as the quality of the final products is superior to low-temperature drying or traditional air-drying (Baiano, Conte, & Del Nobile, 2006; West, Seetharaman, & Duizer, 2013).

2.3 Quality parameter of pasta

2.3.1 Technical quality

Technical quality, which is an essential factor in providing consumers al dente experience, mainly includes cooking loss (CL), optimal cooking time (OCT), swelling index (SI), water absorption index (WAI), extensibility, firmness, and colour (Peressini et al., 2019). Optimal cooking time is defined as the time once the cooking water penetrates the pasta core while cooking. In other words, it is time that the un-swollen pasta centre disappears (Heneen & Brismar, 2003). Cooking loss is the amount of solids loss from pasta structure to cooking water at the optimal cooking time (Bustos et al., 2015). A value of up to 8% of CL is considered to be acceptable for good-quality pasta (Diamante, Peressini, Simonato, & Anese, 2019). The swelling index is the gram of water absorbed per gram of dry pasta, and the water absorption index is the percentage of weight increase compared to uncooked pasta (Padalino et al., 2013). Swelling index and water absorption index are indicators of water absorbed by the starch and protein during cooking (Padalino et al., 2013). Durum wheat pasta typically has a SI value of around 1.8-1.9 (Bustos et al., 2015). The force and length needed to snap the pasta were expressed as the extensibility (Sun-Waterhouse et al., 2013). Firmness is the force needed to shear the cooked pasta (Bustos et al., 2015). The colour of pasta is one significant factor in consumer preference (Bustos et al., 2011). Generally, good quality pasta is considered low in swelling index, water absorption index, cooking loss and high in extensibility and firmness (Peressini et al., 2019).

2.3.2 Sensory quality

The sensory quality of pasta plays an important role in its overall quality (Murray et al., 2017). Some aspects of sensorial quality, like firmness, stickiness, adhesiveness, elasticity and colour, can be measured and standardised by both instrument analysis and panellists (Micale, Giallanza, Enea, & La

Scalia, 2018; Niu, Hou, Kindelspire, Krishnan, & Zhao, 2017). In comparison, some aspects like flavour, chewiness, and aroma can only be tested by panellists (Niu et al., 2017). Well-trained panellists can test the sample and provide compelling overall acceptability and results of consumer preference (Murray et al., 2017).

2.3.3 Nutritional quality

Traditional pasta is rich in carbohydrates especially starch (Li et al., 2014). In this case, pasta has been considered fattening food (Kill, 2001). However, some researches show that starch absorption of pasta in the intestinal tract is slow and incomplete compared to other starch-based foods such as white bread (Zou et al., 2019). Therefore, pasta only has a moderate glycaemic index (Chillo, Ranawana, Pratt, & Henry, 2011; Kristensen et al., 2010; Singh & Sharma, 2017). Papanikolaou (2020) reported that pasta intake reduced adult women's body mass index (BMI). Rosi et al. (2020) found that a high-pasta-intake Mediterranean diet significantly reduced obesity candidates' body weight. Furthermore, Granfeldt and Björck (1991) found that starch in pasta has a lower insulinaemic response in humans compared to other starch-based foods. Many authors suggested that the slow and incomplete digestion is due to the strong gluten network of pasta, which entraps the starch, preventing the starch from being digested by α-amylase (Slaughter, Ellis, & Butterworth, 2001; Zou et al., 2019; Zou, Sissons, Gidley, Gilbert, & Warren, 2015). However, some authors have found that gluten network entrapment is not the only reason for the slow digestion of pasta, as the gluten network cannot completely surround the starch granule after cooking, α-amylase can penetrate porous after-cooked gluten matrix (Fardet, Baldwin, et al., 1998; Fardet, Hoebler, et al., 1998). These authors explain the high molecular weight of starch and tortuosity of the protein matrix, which is obtained by pasta's compact structure that comes from processing, naturally resistant to digestion. In recent years, some other studies have been conducted to explain the reason (Zou et al., 2019; Zou et al., 2015). Research from Zou et al. (2019) showed that proteinaceous α-

amylase inhibitor, which is present in durum wheat, also plays a significant role in the slower digestion of starch. As it is heat resistant and presents in the pasta supernatant after cooking, it immobilises the α-amylase and prevents it from penetrating the gluten network to digest starch (Zou et al., 2019). Besides the starch content contributing most of the energy value of pasta, a significant amount of energy is derived from the protein content as the protein content contributes 11-15% of its total energy (Kill, 2001; Mridula, Gupta, Bhadwal, & Khaira, 2016). The specific nutritional value of cooked traditional pasta is shown in **table 2.1** below:

Table 2.1 Nutritional Contents of 100g Cooked Pasta fresh weight basis (Kill, 2001)

Calories Kcal	Protein g	Fat g	Carbohydrate g	Dietary Fibre g
104	3.6	0.7	22.2	1.2
Minerals				
Calcium mg	Iron mg	Magnesium mg	Phosphorus mg	Potassium mg
7	0.5	15	44	24
Zinc mg	Sodium mg	Manganese mg	Copper mg	
0.5	Trace	0.3	0.1	
Vitamins				
Folacin ug	Vitamin B_6 ug	Niacin mg	Thiamin mg	Riboflavin mg
4	0.02	0.5	0.01	0.01
Ascorbic acid mg	Riboflavin mg	Pantothenic acid mg	Vitamin B_{12}	Cholesterol mg
0	0.01	Trace	0	0

2.4 Functional ingredients used to fortify pasta

Consumers trends to purchase health-promote foods at a premium price (Micale et al., 2018). But they also do not tend to compromise on their convenience and sensory quality (Niu et al., 2017). In other words, they preferred food products they were familiar with, with additional health benefits. Lots of

functional cereal-based confectionary products are currently available in the market with good flavour and bioactive ingredients. Unfortunately, such products are high in sugar and fat; more importantly, some preservatives and additives were added to maintain the quality and shelf life; these make their health-promote effects questionable.

On the contrary, pasta is considered free from cholesterol with moderate glycaemic; furthermore, it is widely consumed worldwide as a versatile base meal. Hence, it is a reliable vehicle for delivering nutrients to consumers (Desai et al., 2021). Although pasta is rich in carbohydrates, it is low in dietary fibre, vitamins, phenolic compounds, and minerals (Gull, Prasad, & Kumar, 2018). This provided the opportunity to fortify pasta complementarily, to supply it with fibre, vitamins, proteins, and minerals by adding functional ingredients. These ingredients may include cereal products, extracted/isolated fibre, resistant starches (RS), legumes, fruits, vegetables, and even meats. Some of these ingredients were also studied to develop special functional pasta, such as gluten-free pasta. However, these functional ingredients have varied impacts on the quality and acceptability of pasta, and some of the impacts are negative (Rachman, Brennan, Morton, & Brennan, 2019). The bioactive compounds and the functionality of different ingredients currently used to fortify pasta are shown in **Table 2.2**

Table 2.2 Summary of functional ingredients used to fortify pasta

Ingredients	Type	Bioactive ingredients	functional description	Reference
Whole durum	flour	Vitamins, minerals, fibre, antioxidants, phytosterols, tocopherols	Improve antioxidant status, improve cardiovascular health	Aravind, Sissons, Egan, et al. (2012); Baiano et al. (2008); Chillo, Laverse, Falcone, Protopapa, and Del Nobile (2008)

Whole wheat	flour	Phenolic acids, dietary fibre, vitamins, minerals, stanols, sphingolipids, phytates	Improve antioxidant activity, reduction of risks of cardiovascular disease, type II diabetes and cancer	Fares, Platani, Baiano, and Menga (2010); Vignola, Bustos, and Pérez (2018); West et al. (2013)
Extracted Fibres	flour	Dietary fibre (guar gum, pea fibre, inulin, locust bean gum, xanthan gum, bamboo fibre, β-glucan, resistance starch	Glycaemic control, constipation prevention, short-chain fatty acid production, improve beneficial microbiota, enhance methanogenesis, reduce blood cholesterol	Aravind, Sissons, Fellows, et al. (2012); Bustos, Perez, and León (2013); Chillo et al. (2011); Cleary and Brennan (2006); Tudorică et al. (2002)
Buckwheat	flour	Polyphenols, vitamins B1 and B2, rutin, globulin, albumin	Improve antioxidant activity, balance protein consumption	Biney and Beta (2014); Carcea, Narducci, Turfani, and Giannini (2017)
Millet	flour	Phenolic compounds, minerals, lipids, and good quality proteins	Improve antioxidant activity	Gull et al. (2018); Yadav, Sharma, Chikara, Anand, and Bansal (2014)
Sorghum	flour	Resistance starch, phenolic compounds	Short-chain fatty acid production, stimulates healthy gut microflora, reduce glycemia and insulin response, improve antioxidants capacity	Khan, Yousif, Johnson, and Gamlath (2013)
Oat	flour	Polyphenols, stanols, sphingolipid, dietary fibre	Improve antioxidant activity, Lower GI, Blood cholesterol attenuation	Bustos et al. (2013); Carcea et al. (2017); Hager, Czerny, Bez, Zannini, and Arendt (2013); Kaur, Sharma, Nagi, and Dar (2012)
Barley	flour	Polyphenols, stanols, sphingolipid, dietary fibre	Improve antioxidant activity, Lower GI; Blood cholesterol attenuation	Carcea et al. (2017); Chillo et al. (2011); Kaur et al. (2012)

Beans	flour, milk	Protein, furosine, phenolic content, fibres, vitamins	Increase amino acid consumption, improve antioxidant capacity, reduce the risk of heart disease, reduce the risk of colon cancer	Carcea et al. (2017); Petitot, Boyer, Minier, and Micard (2010); Shogren, Hareland, and Wu (2006)
Peas	flour	Polyphenols, dietary fibre, proteins, vitamins	Lowering blood cholesterol, reducing the risk of colon cancer, reduced glycemic and insulinemic postprandial responses, proteins act as precursors of biologically active peptides	Carcea et al. (2017); Marinangeli, Kassis, and Jones (2009); Osorio-Díaz, Agama-Acevedo, Mendoza-Vinalay, Tovar, and Bello-Pérez (2008); Petitot et al. (2010)
Carrot	flour, juice, puree	Carotenoid, vitamin E, omega-3 fatty acids (from leaves), fibre content	Improved antioxidant status	Boroski et al. (2011); Carini et al. (2012); Jalgaonkar, Jha, and Mahawar (2018); Rekha, Chauhan, Prabhasankar, Ramteke, and Rao (2013); Yadav et al. (2014)
Potato	powder, juice	Phenolics, Flavonoids, Folate, kukoamines,	A potential on the treatment of gastrointestinal tract disease, improve antioxidant activity, cytotoxic to cancer cells, have anti-inflammatory properties	Kowalczewski et al. (2015)
Beetroot	puree, juice	Boron, betalains, folic acid, carotenoids, minerals, nitrate	Improve immune system; anti-cancer; strong antioxidants	Mridula, Gupta, Bhadwal, Khaira, and Tyagi (2016); Rekha et al. (2013)
Spinach	puree, paste	Iron, folic acid, lutein	Provide attractive colour; mineral enrichment, free radical scavenging	Padalino et al. (2013); Shyam, Mishra, Vaidya,

				and Sharma (2017); Yadav et al. (2014)
Tomato	powder, flour, puree, paste	Lycopene, β-carotene, Vitamin C, Vitamin E, flavonoids, phenolic compounds	High antioxidant, scavenge reactive oxygen species	Padalino et al. (2013); Pasqualone et al. (2016); Rekha et al. (2013); Yadav et al. (2014)
Capsicum	juice, powder, flour	Vitamin C, carotenes, phenols, capsaicinoids, xanthophylls and flavonoids	High antioxidant capacity, against intestinal disorders, degenerative disease & dysentery, promote mental health	Mridula, Gupta, Bhadwal, and Khaira (2016); Padalino et al. (2013)
Mushroom	powder, flour	Protein, dietary fibre, polyunsaturated fatty acid, polysaccharide, phenolic compounds; ergosterol; minerals, vitamins B1, B2, C, folates and niacin	Antioxidant ability; antifungal ability; antiviral ability; antitumor effect; hypocholesterolemic effect	Chauhan et al. (2017); Lu et al. (2018); Lu, Brennan, Serventi, Mason, and Brennan (2016)
Grape	marc powder, skin pomace powder	Phenolic acids, tannins, fibres, vitamins, carotenoids, anthocyanins	Antioxidant, cardiovascular protection activities, anti-inflammatory	Gaita, Alexa, Moigradean, and Poiana (2018); Marinelli et al. (2018); Sant'Anna et al. (2014); Teixeira et al. (2014)
Turnip	puree, paste	Vitamin C, riboflavin, dietary fibre, minerals	High antioxidant capacity, reduce high blood pressure, reduce the risk of diabetes	Parveen, Hussain, and Someshwar Rao (2014); Yadav et al. (2014)
Oregano	powder	Essential oil	Antioxidant, retard lipidic oxidation	Boroski et al. (2011)

Moringa leaves	powder	Flavonoids, quercetin, vitamin E, iron	Antioxidant, medical use	Jalgaonkar et al. (2018)

2.4.1 Addition of cereals & pseudocereals

The industry has created pasta that partially or totally replaced durum wheat with other cereals, and pseudocereals to create novel pasta (Carcea et al., 2017). Some cereals are rich in phytochemicals and other nutrients. For example, barley is high in dietary fibre (10%-20%) (Hatcher, Lagasse, Dexter, Rossnagel, & Izydorczyk, 2005), and also contains tocopherols and tocotrienols, which contribute to high antioxidant activity and reduction of serum LDL-cholesterol (Dörmann, 2007). Oats, maise, and teff can lower blood lipids and reduce blood glucose response (Hager et al., 2013). Wholegrain wheat contains high amounts of dietary fibre and bioactive micro-nutrients such as lutein and ferulic acid compared to refined wheat (Kristensen et al., 2010). Some researchers have used extracted dietary fibre to create functional pasta (Tudorică et al., 2002). Dietary fibre can be divided into two categories: soluble fibre, which is easily fermented in the large intestine, and insoluble fibre, which is slowly fermented during digestion (Foschia, Peressini, Sensidoni, & Brennan, 2013). Dietary fibre consumption is related to reduced bowel transit time, constipation prevention, and beneficial microbiota stimulation (Tudorică et al., 2002).

Hence, cooperating these ingredients with pasta can deliver these benefits to the consumer. For example, buckwheat-enriched pasta has a more balanced amino acid content and higher antioxidant capacity than control pasta (Biney & Beta, 2014). Baiano et al. (2006) used whole durum meal to substitute refined durum wheat and found a threefold increase in total dietary fibre content (9.88g/100 g). Costabile et al. (2008) showed that wholemeal pasta reduced the desire to eat and increased satiety.

This was due to the high-fibre content promoting satiety by decreasing the energy density of products (Costabile et al., 2008; Karl & Saltzman, 2012).

However, Kristensen et al. (2010) reported that fibre content did not affect satiety and glycemia when compared to refined wheat pasta with whole wheat pasta. This may be because the dietary fibre type in the whole wheat pasta was not viscous and did not induce increased viscosity or gel formation when hydrated, which is considered to delay gastric emptying and reduce prandial blood glucose (Wanders et al., 2014). This conflict of results indicates further appetite research on wholemeal pasta should be carried out to provide clearer results. Especially as this study was limited to whole wheat pasta, the appetite regulation function of other whole grain cereal pasta, as well as extracted dietary fibre, still needs to be studied.

Some cereals also contain anti-nutritional factors such as phytic acid and phytate (Stevenson, Phillips, O'Sullivan, & Walton, 2012; Tazrart, Zaidi, Lamacchia, & Haros, 2016). Phytic acid, present in cereal bran, decreases the bioavailability of iron, magnesium, zinc and calcium and further accelerates these minerals' loss from the body (Stevenson et al., 2012). Thus, fortification with minerals could supplement the loss and might not be as beneficial as predicted.

2.4.2 Legume addition

Legumes contain many essential nutrients, including carbohydrates, proteins, especially essential amino acids, minerals, fibres, and vitamins (Foschia, Horstmann, Arendt, & Zannini, 2017). Lots of these nutrients are deficient in pasta; therefore, legumes are ingredients to supplement these nutrients in the diet. The most consumed legumes in the World are beans, faba bean, lentil, pea, chickpea (Bouchenak & Lamri-Senhadji, 2013). In general, the legume content is considered low in fat except for that soya beans, chickpea and lupine, which contain higher lipid content (Jukanti, Gaur, Gowda, & Chibbar, 2012).

Legumes are rich in protein content. The protein content of legumes depends on their species. Typically, the protein content is around 17%-25% (dry weight) in peas and 38%-40% in lupins and soybean (Gueguen & Cerletti, 1994). Legumes also contain some essential amino acids that are lacking in cereals, such as lysine and threonine, the first and second limiting amino acids in durum wheat. (Petitot et al., 2010). Shogren et al. (2006) have substituted 25% of durum semolina with defatted soy flour, resulting in a 67% improvement in total protein, 84% enhancement of threonine, and 127% increase of lysine, respectively. Fortification of pasta with legumes can help meet the dietary intake requirement of amino acids, especially for 2-5-year-old children (Bouchenak & Lamri-Senhadji, 2013).

Many legumes also contain dietary fibre and resistant starch, which reduce glycaemic response and provide health benefits (Bouchenak & Lamri-Senhadji, 2013). For example, substituting 20% durum wheat with chickpea flour significantly reduced the glycaemic index value of pasta from 80.68 to 70.7 (Osorio-Díaz et al., 2008). However, not all addition of legumes positively affects GI. Sęczyk, Świeca, and Gawlik-Dziki (2016) use 5% carob flour to fortify durum pasta. The result shows a higher expected glycaemic index than durum pasta (83.9 vs 72.2). No significant predictive GI value was reported when 30% of whole durum wheat was substituted by yellow pea flour (Marinangeli et al., 2009). This indicated that the legumes' influence on GI value is dependent on the components of the legumes themselves. Oliviero and Fogliano (2016) reported that more bioavailable starch is the mean reason for increased GI. Sęczyk et al. (2016) summarised that the GI value of legume-enriched pasta is determined by the integration of protein network, physio-chemical characteristics of starch, contents of dietary fibre, lipids, and polyphenols. Legume-enriched pasta is generally high in polyphenol content and antioxidant capacity, which is related to protective effects on many pathological conditions such as metabolic disorders and CVD (Prakash & Gupta, 2011). For example, durum pasta incorporated with 5% carob flour increased Total Phenolics Contents (TPC) from 3.51 mg GAE/G\g DW to 12.12 mg GAE/G\g DW. Also, the

total antioxidant capacity increased from 0.07 mg TE/g DW to 1.35 mg TE/g DW according to ABTS measurement (Sęczyk et al., 2016).

2.4.3 Vegetable enriched pasta

Vegetables contain variances of phytochemicals like fibres, vitamins, polyphenols, carotenoids, glucosinolates, as well as minerals (Oliviero & Fogliano, 2016). However, customers trends to have ingrained eating habits that always fail to meet the 400 g dairy intake amounts of fruit and vegetables that are recommended by the World Health Organization even though they have aware of the health promotion role of fruit and vegetables (Capacci et al., 2012; Marinelli et al., 2018). This may partially be because some bitterness and unpleasant vegetable flavour hindered the consumer from eating it (Meengs, Roe, & Rolls, 2012). In that case, adding fruit and vegetable compounds into staple food such as pasta and bread may be one good option to help customers meet the suggested daily intake. Furthermore, the addition of vegetables to pasta can also contribute to artisan reasons to deliver a beautiful meal with a special flavour (Oliviero & Fogliano, 2016).

From table 2.3, most vegetable enrichment is based on the high antioxidant capacity of vegetable ingredients. These antioxidant capacities are generally derived from phenolic compounds, Vitamin C (ascorbic acid), vitamin E (tocopherols), carotenoids, phytosterols, isoflavones and organosulphur (Abuajah et al., 2015). These antioxidants commonly involve scavenging free radicals, reducing carcinogenesis in cells, binding toxins and carcinogens in the intestinal tract, lowering cholesterol absorption, and enhancing the immune system (Abuajah et al., 2015). Dietary fibre from vegetables plays roles in entrapping harmful toxins in the intestinal tract, lowering serum LDL-cholesterol without affecting HDL-cholesterol, decreasing fat absorption, lowering glycaemic index, reducing the risk of cardiovascular disease, and acting as prebiotics for beneficial microbiota in the intestinal tract (Abuajah et al., 2015; Theuwissen & Mensink, 2008). Minerals also play an important role in human health, while traditional pasta lacks some essential minerals, as Table 2.1 shows. Some vegetables are rich in those

essential minerals and can be used to improve pasta's nutritional value. For example, spinach is rich in iron (Hedges & Lister, 2007), carrots contain sufficient Zinc (Sharma et al., 2012), and beetroot is considered a good supplement vegetable for calcium and potassium (Chhikara et al., 2019). In these cases, enriching pasta with vegetable components may deliver these compounds to the consumer.

For example, Lu et al. (2018) conducted an *in vitro* study and reported that white button and porcini mushrooms reduced the ready-digestible reducing sugar significantly compared to control durum pasta, and the predicted glycaemic response of pasta was decreased. The authors suggested that the effect may be due to β–glucan and other dietary fibre fractions in mushrooms. Also, mushroom powder contains more protein than semolina and may improve the integration of the protein matrix, thus retard the encapsulated starch from the digestion enzyme. Another example is from Padalino et al. (2017), which used tomato peel powder as a functional ingredient and showed a significant increase in soluble fibre and the insoluble fibre content of resultant pasta compared to durum pasta. Moreover, the available carbohydrates decreased from 68 g/100 g of durum control pasta to 60 g/100 g when the 15% of semolina was substituted, which suggested a potential to reduce glycaemic response (Padalino et al., 2017). The same authors also found a dramatic increase in bioactive lycopene, one kind of carotenoid with strong antioxidant capacity. Lycopene is also considered to inhibit the formation of peroxides and protect low-density lipoproteins (Shashirekha, Mallikarjuna, & Rajarathnam, 2015).

Many authors use small amounts of vegetable compounds to substitute the wheat/durum semolina to achieve overall acceptability with improved nutritional value. For example, Gaita et al. (2018) used 3%, 6%, and 9% grape pomace skins substitute wheat flour, and the result shows that total antioxidants capacity increased by 44.3%, 96.3%, 141.2%, respectively, according to FRAP assay measurements. Lu et al. (2018) also provided another example, and the authors used white button mushrooms, shiitake, and porcini to enrich durum pasta. The results showed that the dietary fibre content increased significantly even at the 5% substitution level, which is the lowest level used in the study, from 3.17 g/100 g of

durum pasta to 6.04 g/100 g of 5% white button mushroom enriched pasta. In some studies, the low substitution level results in an acceptable quality decrease or even better sensory quality. For example, Gaita et al. (2018) reported that 3% and 6% of durum wheat substituted by grape pomace skin results in higher sensory scores than control pasta. These indicated that a low substitution level might be one desired way to develop vegetable-fortified pasta.

2.5 Quality impact of functional ingredients

Pasta enriched with functional ingredients is frequently reported to have inferior cooking and sensory quality compared to that traditional durum pasta (Vignola et al., 2018). These quality decreases are generally involved in the change in optimal cooking time (OCT), higher cooking loss (CL), change of water absorption (WA) and swelling index (SI), change of texture properties (firmness, elasticity, adhesiveness), change of colour and taste. These finally result in decreased overall acceptability of functional ingredients enriched pasta.

Fibre content, delivered by functional ingredients fortification, can disrupt the gluten matrix, weakening the pasta structure (Marinelli et al., 2018). The technical quality of pasta depends on the ability of the starch-protein matrix to retain its integrity and is related to consumer acceptability (Rakhesh, Fellows, & Sissons, 2015). Fibre contents, especially soluble fibre, compete with starch to bind with protein resulting in weak starch-protein interactions (Sissons, Ames, Hare, & Clarke, 2005). Research shows that the starch-protein matrix prevents cooking water from contacting inner starch by competing for water with it, and increasing fibre content alters the starch-protein matrix, thus accelerating water absorption and reducing optimal cooking time (Vignola et al., 2018). This can also explain the observation that the higher addition of dietary fibre results in a greater reduction of optimal cooking time (Rakhesh et al., 2015). The addition of dietary fibre causes quite different results for water absorption and swelling index. Aravind, Sissons, Fellows, et al. (2012) found that the addition of bran and germ content resulted in less water absorption. However, Kaur et al. (2012) reported that water absorption increased

significantly when durum flour was substituted by wheat bran, oat bran & rice bran. This conflict of results may be due to the different fibres having different natures of the water affinity. Also, Foschia et al. (2013) found that inulin (both long-chain and short-chain) and psyllium-fortified pasta significantly increased in water absorption. This is because both inulin and psyllium have high water binding ability (Kaur et al., 2012). Gull et al. (2015) added guar gum to pasta and found increased water absorption due to guar gum's strong water binding and absorption capacity. This further indicates that fibre's water-binding capacity can significantly influence pasta's water absorption as well as swelling index. The influence of fibre content on the water absorption of pasta may be determined by the fibre's physical-chemical properties (Foschia et al., 2013). Cooking loss, a very important quality factor of pasta, is significantly influenced by fibre addition. For example, Aravind, Sissons, Fellows, et al. (2012) reported that cooking loss of bran-enriched pasta increased from 5.67% to 7.64% when the substitution level increased from 0% to 30%. Aravind, Sissons, Fellows, et al. (2012) used scanning electron microscopy (SEM) observed holes and less integrated structure of that inulin-enriched pasta. The authors found that fibre contents impacted the protein matrix. Moreover, Lu et al. (2018) used the same technique and reported an irregular, and uneven structure of mushroom-enriched pasta and the authors suggested that fibre in mushroom impact the protein matrix of pasta. Carini et al. (2012) measured the water status in carrot-enriched pasta, reported that sugars and soluble fibres in carrots stalled the gluten proteins interaction with water and caused an altered water distribution during dough development, thus results in an incorrect hydration level and weaken gluten network. Thus, poor texture and higher cooking loss was observed in resultant pasta.

The added ingredients may contribute to a change of colour, unpleasant flavour and taste that decrease the overall acceptability (Sun-Waterhouse et al., 2013). Jalgaonkar et al. (2018) reported that the addition of 5%-8% moringa leaves powder to pasta result in leafy flavour and bitter taste, which decrease its sensory acceptability. Marti et al. (2017) summarised that wholegrain-enriched pasta

generally has a dark colour, bitter taste, off-flavour and off-odours develop during storage. These undesired characteristics are probably due to the Maillard reaction between the reducing sugars, which are produced due to the reaction between the damaged starch and the amylase present in wholemeal pasta and protein (de Noni & Pagani, 2010). The processing parameters, such as drying temperature and drying time, also have an influence on the Maillard reaction during pasta processing (de Noni & Pagani, 2010). As the ingredients change, the processing needs to be adjusted to minimise the Maillard reaction and its impact on quality. Thus, the optimisation of processing parameters needs to be studied further. Interestingly, in the south of Italy, people use toasted whole durum wheat to produce pasta with brown colour and aromatic flavour, and the local populace prefers this pasta (de Noni & Pagani, 2010). This indicates that functional product acceptability might differ in different regions; thus, the design and production may vary depending on the region.

The cooking quality of fortified pasta is depended on the substitution level, where, in general, a low substitution level results in a lower quality impact (Bustos et al., 2015). For example, Kaur et al. (2012) reported that cooking loss of wheat bran and barley bran pasta increased linearly with substitution level when over 15% of durum wheat was substituted by them. In that research, oat bran shows the lowest cooking loss increase (from 0.76% to 1.62%), while rice bran has the highest cooking loss increase (from 0.76% to 2.48%). These quality impacts were partially due to the dilution of gluten and the resultant weakening of the gluten matrix (Torres, Frias, Granito, Guerra, & Vidal-Valverde, 2007). Sęczyk et al. (2016) found that 1-5% carbo flour supplement pasta does not significantly impact pasta's overall sensory quality while improving the nutritional value of pasta. Another example is from Shogren et al. (2006), who reported that up to 35% substitution of durum flour with soy flour results in acceptable sensorial pasta with no significant difference in flavour and texture, while 50% substitution results in undesired beany and bitter flavour and excessive cohesiveness. Steglich, Bernin, Moldin, Topgaard, and Langton (2015) found that the fibre's influence on the texture of the pasta is based on its substitution

level—a higher concentration of fibre results in lower texture quality. Bustos et al. (2015) suggested that the functional ingredients substitution level should generally be lower than 10% to minimise the cooking quality impact and achieve sensorial acceptability.

The addition of functional ingredients can improve the technical quality of the resultant pasta in some aspects. For example, some fibres have been shown to reduce the cooking loss of pasta, such as resistant starch II & resistant starch IV was reported to lower cooking loss with increased substitution levels (Bustos et al., 2013). Wholemeal pasta is generally characterised by more firmness and chewiness, which consumer desires, compared to wheat pasta (Vignola et al., 2018). Wholemeal flour contains more lipids and fat than refined flour, as the germ is rich in these constituents (Marquart, 2008). The lipid tends to bind to starch granules and safeguard a firm starch gel, leading to a firm lipid-starch structure and a firmer product (Vignola et al., 2018). Liu et al. (2016) used beef meat emulsion added to pasta; it was found that the addition of meat emulsion provided a more homogenous structure, which deeply embeds the starch granule, compared to the durum pasta according to scanned electronic microscope (SEM) analysis. As a result, the overall acceptability increased from 4.13 to 6.91, the nutritional value such as essential amino acid improved with decreased relative GI (from 51.5 of control to 31.9 of 45% substitution), as well as texture quality improved significantly (Liu et al., 2016). The authors suggested that meat proteins interact with insoluble gluten networks, forming a more stable matrix structure that contributes to improved texture, cooking quality, and decreased relative GI.

2.6 Role of additives on pasta enriched with functional ingredients

Although functional ingredients enriched pasta comes up with superior nutritional quality compared to traditional pasta, the technical and sensory quality impact of ingredients addition set obstacles for the consumer to consume it as daily food (Li et al., 2014). In this case, additives may be helpful to minimise the quality impact and even provide better quality than traditional pasta. Edible gums and other additives can be used to improve the cooking and sensory quality of pasta (Li et al., 2014). For example,

Gull et al. (2018) used pearl millet with additive-carboxy methylcellulose added to pasta, and the resultant pasta had a lower cooking loss than durum wheat pasta control. The author explained that the probable reason was that the carboxymethyl cellulose improved the gluten matrix, thus helping to embed the starch and decrease the cooking loss. Xanthan gum was reported to reduce the cooking loss, firmness and adhesiveness and increase the water absorption of pasta when 1-3% of xanthan gum was added to 15% detoxified Matri flour-enriched pasta (Ahmad et al., 2018). The authors explained that decreased cooking loss is probably due to the xanthan form network around the starch granules, thereby hindering water swelling and amylose diffusion. Increased water absorption is because of xanthan gum's ability to absorb water. Reduced firmness and adhesiveness may be owing to the xanthan gum can form a stronger network with gluten to encapsulate the starch granule (Ahmad et al., 2018). Padalino et al. (2017) respectively made use of 2% of Carbossi-methylcellulose (CMC), guar gum (GUAR), xanthan gum (XAN) and agar (AG) to improve the quality of tomato by-product fortified wholemeal pasta. The result showed that the overall sensory quality was slightly improved, although the improvement was not statistically significant in some aspects. In that study, CMC and GUAR improved the overall sensory quality more obverse. The authors explain that the improvement is because such hydrocolloids have a strong affinity to starch, resulting in a more homogenous and stable polymeric network, which is important for entrapping starch granules and improving pasta quality. Peressini et al. (2019) tested several kinds of hydrocolloids to enhance the quality of the pasta that was made from common wheat. The authors suggested that adding 0.5% propylene glycol alginate (PGA) and 1% sodium alginate (AL) to pasta results in the best quality pasta regarding overall acceptability. It is because such hydrocolloids contribute to forming a physically cross-linked network around the starch granules, thus increasing the overall quality (Peressini et al., 2019). The authors also suggested that overuse of hydrocolloids may result in exceeding firm pasta, which lowers the acceptability of the pasta product. Some emulsifiers, such as distilled glyceryl monostearate (GMS), may be used as lubricants for easier

extrusion process because they can less nozzle wear and provide a less adhesive surface of rice pasta (Lai, 2002). Some protein can also improve the quality of pasta. For example, microbial transglutaminase, one of the enzymes produced by *Streptoverticulum spp*., can be used to improve wheat protein structure and dough structure due to its ability to the formation of inter- and intra-molecular lysine cross-links (Tseng & Lai, 2002). Marinelli et al. (2018) made use of 0.6% transglutaminase to enhance the quality of 15% red grape marc flour fortified pasta; the overall sensory acceptance increased from 5.25 to 6.00 even the resultant pasta's quality is still poorer than control durum pasta with the overall quality scored at 7.30. Properly use additives can improve the overall cooking and sensory quality of pasta, while consumers may concern about the safety of the additives; thus, the nature additives are suggested (Li et al., 2014).

2.7 Different forms of ingredients impact on technical and Nutritional quality of pasta

Interestingly, different forms of functional ingredients can have various effects on the technical and sensory quality of fortified pasta. Tazrart, Zaidi, et al. (2016) used broad bean flour (Vicia faba) to enrich durum pasta and found decreased optimal cooking time and water absorption, increased cooking loss, and an overall decreased cooking quality. The authors explained that decreased optimal cooking time and increased cooking loss were due to fibre's disruption of the gluten network, while reduced water absorption may be owing to the lower starch content of the resultant pasta. However, such a situation may be different when liquid forms of legume ingredients like soybean milk, which contain relatively fewer solids particles, were used to enrich pasta. For example, He, Guo, and Zhu (2019) added soya milk to make the dough, which was used to produce frozen-cooked noodles, resulting in a much higher sensory quality than control frozen noodles. The sensory quality of soy milk frozen-cooked noodles is close to high-quality fresh noodles. The authors explained the improvement from soy milk addition is due to a more strength gluten network (He et al., 2019). It is suggested that unfolded soy protein

enhances the interaction with wheat protein, which is due to the formation of the oil-lecithin emulsion during soybean milk preparation (Azizi, Rajabzadeh, & Riahi, 2003). This may be proved by Carini et al. (2012), who used defatted soy milk added to pasta, which cannot form an oil-lecithin emulsion because the oil was removed. Therefore, the addition had a much less positive or negative effect on pasta quality. He et al. (2019) also show more cooking stability & firmness, less cooking loss and good acceptability of the final frozen-cooked noodle product. Although soybean milk is used to fortify frozen-cooked noodles, the superior dough quality indicated that liquid ingredients might be one preferred option for pasta fortification. This may be because such liquid ingredients minimise the interference of insoluble fibre and thus may be one good option to fortify pasta. However, nutritional improvement of such methods still needs to be further researched.

Carini et al. (2012) used whole soy flour, defatted soy milk, and soy ingredients (a combination of soy flour and defatted soy milk) to make fortified fresh pasta. For cooking quality, defatted soy milk pasta is close and even better than control pasta according to firmness, extensibility and cooking loss, while soy flour and soy ingredients enriched pasta have much lower quality in these parameters. However, as for nutritional value, defatted soy milk only improved the protein content of pasta from 9.0% to 9.5%, while soy flour improved this value to more than 16% (Carini et al., 2012). This indicates that soy protein in soy milk is much lower than in soy flour. Thus its ability to affect the proper gluten development, which is due to soy protein's great affinity for water (Traynham, Myers, Carriquiry, & Johnson, 2007), is less than soy flour, and the quality impact is lower. Another reason may be that soy milk contains much lower fibre than soy flour; thereby, the fibre's impact on gluten structure can be minimised. Carini et al. (2012) also compared the quality impact of carrot juice pasta with carrot flour pasta. For extensibility and cooking loss, carrot juice pasta showed great performance, which is close to the control, while carrot flour-enriched pasta showed a very high cooking loss, which exceeds the acceptable level of 8%. The

authors suggested that carrot flour and whole soy flour significantly weaken the gluten network while such an effect is much lower in defatted soy milk and carrot juice.

Another interesting result is from Kowalczewski et al. (2015), who used potato juice, a by-product during potato starch production, to fortify pasta. The authors used fresh potato juice and dried potato juice added to pasta formula based on the dry mass, and the results showed that fresh potato juice pasta had a lower cooking loss even than control durum wheat pasta while dried potato juice pasta had a much higher cooking loss. The author explained that fresh potato juice contains the native form of potato proteins, which may lose or denaturation during drying, promoting the formation of the strong protein matrix that better encapsulates the starch fraction and decrease the cooking loss. What is more, the overall sensory rating of fresh juice-enriched pasta is not significantly different from the control durum wheat pasta, while the dried potato juice-enriched one had dramatically lower sensory quality. This observation was then further interpreted by SEM analysis of pasta surface, which shows that fresh potato juice-enriched pasta has a less smooth surface. This surface is likely to hinder amylose release from starch granules and contributes to lower cooking loss and good texture (Kowalczewski et al., 2015). The research plausibly indicated that even under the same dry mass, fresh juice of vegetables maybe still a better option than dried juice powder for the quality of enriched pasta.

Rekha et al. (2013) found that the addition of carrot, spinach, tomato and beetroot puree decreased cooking loss and improved the appearance and texture of the final pasta product. The addition also retained considerable levels of carotene, lycopene, and betalains content and delivered these nutrients to the final products. The authors also used SEM to observe the microstructure of the resultant pasta. The result showed that starch granules and vegetable matter were better bound by the protein matrix compared to wheat pasta control. These results are in accordance with Yadav et al. (2014), who used 2% of dry solids vegetable paste of carrot, tomato, spinach & turnip added to Pearl millet and wheat flour to produce pasta. Results showed that the addition of vegetable paste leads to better quality (lower

cooking loss, improved firmness, decreased adhesiveness except for carrot paste, an excellent overall sensory acceptability). Furthermore, Pasqualone et al. (2016) reported lyophilised tomato matrix (one kind of dried puree) fortified pasta with a 1.5%-2.5% substitution level has a more attractive colour, better chewiness, and more springiness with no significant cook loss increase. Although, in that research, the preparation of lyophilised tomatoes is relatively complex and costly. The authors found that tomato powder was well embedded in the protein network, which may contribute to the good quality. However, varieties of research using vegetable powder showed different results with puree-fortified research. Kowalczewski et al. (2015) show carrot powder significantly decreases the sensory and culinary quality of pasta. Padalino et al. (2017) show that 10-15 % tomato peel flour significantly decreased the overall sensory quality of fortified spaghetti, and authors had to use hydrocolloids like Carbossi-methylcellulose and guar gum to improve the quality to an acceptable level. Shyam et al. (2017) substituted 10% of wheat flour with spinach leaf flour and observed a higher cooking loss, water absorption and volume increase.

Although these researchers used different substitution levels, it may indicate that puree/paste of vegetables may be more suitable to be added into pasta due to its superior quality of the final product. Or the fortification can be applied by a combination of vegetable puree/liquid and other functional dried functional ingredients; it may be designed to use dried functional ingredients to deliver the main nutrients and liquid/puree state ingredients to maintain the quality. Previous studies showed that juice or puree forms of vegetables provided better overall technical quality than dried flour when they were used to fortify pasta. However, this statement can be challenged because of the inconsistency of those studies. For example, Carini et al. (2012) compared soy milk enriched pasta (72.3% semolina + 27.7% soy milk) to soy flour enriched pasta (41.5% semolina + 24.6% soy flour + 3.1% gluten + 30.8% water) and found a much better overall technical quality of soy milk enriched pasta (higher force at rupture, higher extensibility and lower cooking loss). The comparison is not compelling as the actual substitution level of

soy flour-enriched pasta is much higher than soy milk enriched one. Similar situations were also found in many other studies (Rekha et al., 2013; Yadav et al., 2014). At the same substitution level, it is unclear which form of vegetable is suitable to add to pasta with desirable technical quality and nutritional properties. Further research on pasta fortified with different forms of vegetables might be conducted to provide a comprehensive understanding of their impact on pasta's cooking, sensory and nutritional quality.

2.8 Conclusion

Incorporating functional ingredients into pasta has shown promising results in terms of improving the nutritional value and being able to maintain consumer acceptance. Vegetables contribute bioactive ingredients and enhance the antioxidant capacity of pasta. The increased fibre content, which many of these functional ingredients have, also can reduce the glycaemic response in consumers, thus providing a two-pronged approach to improving health. Foods that may be used as functional ingredients should be considered carefully, and these ingredients often contain a large amount of bonded-phenolic and less water-soluble nutrients, which will improve retention during cooking. Low substitution levels of flour are preferred as high levels of fortification can have deleterious effects on the cooking and sensory quality. Using puree/liquid functional ingredients to fortify pasta has been shown to deliver a superior quality end product compared to traditional flour substitution with powder. Therefore, the possibility of using a combination of puree/liquid state and powder to enrich pasta should be considered a potential method to produce high-quality and highly nutritious pasta.

Chapter 3

Materials and Methods

3.1 Materials

Fresh beetroot, carrot, red cabbage, and spinach (Pams, Foodstuffs South Island, Christchurch, New Zealand) were bought from the local market (New World, Lincoln). Semolina (Sun Valley Foods Ltd., Hamilton, New Zealand, labelled protein = 10.7 g/100 g, fibre = 2.1 g/100 g, sodium = 10 mg/100 g) was obtained from the local Countdown Supermarket (Christchurch, New Zealand)

3.2 Vegetable Material Preparation

3.2.1 Beetroot and carrot

The beetroot and carrot were washed, the inedible head was removed with a sharp knife, and it was peeled using a knife. The peeled beetroot was cut into cubes of 20-30 mm. A juicer (shown in **Figure 3.1 left**; Model: Oscar Neo DA 1000; Nature's Wonderland Ltd., Brisbane, Australia) was then used to produce the juice. The beetroot juice and pomace were collected in two separate containers. The juice was transferred to a glass jar with a lid and stored at -28 °C until use. The pomace was spread out on a baking tray and put into an oven ((Model: OEB 6.10, Manitowoc, Germany) to be dried at 60 °C for 7 h. The dried pomace was then ground into powder using a coffee grinder (Breville BCG200, Breville group, Alexandria NSW, Australia) for 10 s. The beetroot pomace powder was then collected in a zip lock plastic bag, sealed, and stored (**Figure 3.1 right**) at room temperature (approximately 20 °C). The beetroot puree was produced by mixing fresh pomace and juice together using a blender (Nutri-bullet NBO7200−1210DG; Capitalbrands Ltd., Boston, MA, USA), then the puree was collected in a glass jar with a cap and stored at -28 °C.

Figure 3.1 Juicer (left) and beetroot pomace sealed in the bag (right)

3.2.2 Spinach

The spinach was washed, and the roots were removed using a sharp knife. Then the stem and leaves were put into the juicer (Model: Oscar Neo DA 1000; Nature's Wonderland Ltd., Brisbane, Australia), and the pomace and juice were collected separately. The spinach juice was placed into a glass jar with a lid and stored at -28 °C until use. The pomace was spread out on a tray and put into an oven to be dried at 60 °C for 7 h. The dried pomace was then ground into powder using a coffee grinder (Breville BCG200, Breville group, Alexandria NSW, Australia) for 10 s, and the pomace powder produced was stored in a sealed zip lock plastic bag at room temperature (approximately 20 °C). The spinach puree was produced by mixing juice and fresh pomace together using a blender (Nutri-bullet NBO7200−1210DG; Capitalbrands Ltd., Boston, MA, USA), and then the spinach puree was collected in a glass jar with a cap and stored at -28 °C.

3.2.3 Red Cabbage

The inedible parts (stem and its surrounding hard portion) of the red cabbage were removed using a knife, and the leaves of the red cabbage were washed and cut into small pieces before the leaves were

put into the juicer (Model: Oscar Neo DA 1000; Nature's Wonderland Ltd., Brisbane, Australia). After juicing, the red cabbage juice was placed into a glass jar with a lid and stored at -28 °C. The pomace was spread out on a backed tray and put into an oven to be dried at 60 °C for 7 h. The dried pomace was then ground into powder using a coffee grinder (Breville BCG200, Breville group, Alexandria NSW, Australia) for 10 s, and then stored in a sealed zip lock plastic bag at room temperature (approximately 20 °C). The red cabbage puree was prepared by mixing juice and fresh pomace using a blender (Nutri-bullet NBO7200−1210DG; Capitalbrands Ltd., Boston, MA, USA). The puree was stored at -28 °C in a capped glass jar.

3.3 Pasta preparation

3.3.1 Control Pasta

Fresh pasta was produced using a pasta machine (MPF15N235M; Firmar, Villa Verucchio, Ravenna, Italy) with spaghetti die (12 holes and each hole is 2.25 mm diameter of die hole). Control pasta was produced by adding 30 g of 40 °C type water to 100 g of semolina flour and then mixing for 20 min according to the manufacturer's guidelines. Extruded fresh pasta was stored in a zip lock plastic bag at -18 °C until further analysis.

3.3.2 Vegetable puree & juice enriched pasta

Juice and puree both have a low solid content, so achieving a high solid substitution level is difficult to achieve. Too much water addition may cause the formation of large lumps, thus resulting in difficulties in succeeded extrusion (Bustos et al., 2015). Furthermore, according to the study of Wood (2009), suspensions in liquid and puree may cause particle aggregation and a heterogeneous dough with larger lumps, which can also cause difficulty in the extrusion procedure. In that case, the maximum

substitution level (Upper limit) of puree and juice forms of different vegetables, which can be used to produce pasta without the formation of large lumps, can be measured and compared to provide a reference level. Based on such situation, the maximum juice/puree's addition level is based on all the hydration water is from juice/puree. It can be calculated by the below equations:

$$y + \boldsymbol{a}x/100 = 100 \qquad (1)$$

$$x(100 - \boldsymbol{a}) = 100 \times \boldsymbol{h} \qquad (2)$$

$$\boldsymbol{s} = 100 - y \qquad (3)$$

x is the weight (gram) of juice/puree content to achieve the maximum addition level, y is the weight (g) of durum semolina that will be used to produce pasta. $\boldsymbol{a}$ g/100 g is solid content of juice or puree. Where $\boldsymbol{h}$ is hydration level =30 g/100 g

Based on equation (1), (2) & (3), Minimum solid content $\boldsymbol{a}$ g/100 g to achieve setting substitution level $\boldsymbol{s}$ can be calculated as:

$$\boldsymbol{a} = \frac{100\boldsymbol{s}}{\boldsymbol{s}+\boldsymbol{h}} \qquad (4)$$

The maximum substitution level of juice or puree $\boldsymbol{m}$ can be calculated as:

$$\boldsymbol{m} = x - \boldsymbol{h} \qquad (5)$$

Demonstration of those equations can be checked in **Appendix A**.

3.3.2.1 Solid content measurement of vegetable juice and puree

In order to get a proper substitution level of durum wheat using juice and puree, the solid content of juice and puree should be measured. The solid content of the juice and the puree was measured using

an oven-drying method. The juice and puree were homogenised well by a blender (Nutri-bullet NBO7200−1210DG; Capitalbrands Ltd., Boston, MA, USA). Then samples were weighed in a pre-weighed metal dish using an analytical balance (ARC120; OHAUS Corp., Parsippany, NJ, USA). Then the dish was placed in an oven at 105 °C -110 °C overnight. Dried vegetable samples in the metal dish were cooled to room temperature (approximately 20 °C) in a desiccator for 1 h before reweighing. The solid content could be calculated as below:

$$Solid\ content\ (g/100g) = \frac{residue\ weight\ g}{sample\ weight\ g} \times 100 \qquad (6)$$

The solid content of raw material is shown in **Table 3.1**. Based on **Table 3.1** and **equation (5)**, the maximum substitution level (dry weight basis) of juice and puree to produce pasta is shown in **Table 3.2**. It shows that the maximum substitution level of juice/puree-enriched pasta is low (less than 5g/100 g).

Table 3.1 Solid content of vegetable raw material

Material	Solid content g/100 g
Beetroot juice	9.31 ± 0.13
Beetroot puree	11.98 ± 0.13
Beetroot pomace	86.45 ± 0.08
Carrot juice	7.03 ± 0.01
Carrot puree	10.07 ± 0.33
Carrot pomace	85.56 ± 0.02
Spinach juice	4.65 ± 0.04
Spinach puree	8.60 ± 0.07
Spinach pomace	85.02 ± 0.11
Red cabbage juice	4.15 ± 0.01
Red cabbage puree	8.64 ± 0.02
Red cabbage pomace	86.85 ± 0.05

Values = mean ± standard deviation, n=3

Table 3.2 The maximum substitution level to make puree/ juice-enriched pasta

Material	Maximum substitution level to produce pasta (g/100 g dry weight basis)
Beetroot juice	3.08
Beetroot puree	4.08
Carrot juice	2.27
Carrot puree	3.36
Spinach juice	1.46
Spinach puree	2.82
Red cabbage Juice	1.29
Red cabbage puree	2.84

3.3.2.2 Produce juice and puree pasta

Juice and puree (**from 3.2**) were defrosted at room temperature (approximately 20 °C) for 2 h. Then juice, puree and tap water were heated in a water bath to 40 °C. Before adding the juice or puree into the pasta machine, the juice and puree were homogenised in a blender (Nutri-bullet NBO7200−1210DG; Capitalbrands Ltd., Boston, MA, USA) for 1 min. The juice/puree enriched pasta was prepared by adding heated juice/puree and water (specific formula see **table 3.3**) to semolina in a lab-scale pasta machine (Model: MPF15N235M; Firmer, Ravenna, Italy) and mixing for 20 min according to its manual. Then the pasta was extruded by the machine with 12 holes of spaghetti die (2.25 mm diameter per hole). Extruded fresh pasta was stored in a sealed zip lock plastic bag at -18 °C until further analysis.

3.3.3 Pomace enriched pasta

Vegetable pomace was added into semolina in the lab-scale pasta machine according to the recipe (**Table 3.3**) and mixed in the machine for 10 m. Then a certain amount (**Table 3.3**) of 40 °C type water was added to the pasta machine and mixed for 20 min according to the manufacturer's guidelines. Then the pasta was extruded by the machine with 12 holes of spaghetti die (2.25 mm diameter one hole). Extruded fresh pasta was stored in a sealed zip lock plastic bag at -18 °C until further analysis.

3.3.4 Pasta formulation

Based on **3.3.2.1**, the upper limit of juice and puree of root vegetables (beetroot and carrot) was used to produce pasta, and those pasta samples were compared with control and pomace-enriched pasta. For puree and juice of leaf vegetables (spinach and red cabbage), 1 g/100 g for juice and 2 g/100 g for puree were chosen to produce pasta as these substitution levels can be achieved by both spinach and red cabbage material. All of those recipes shared the same set hydration level (30 g water per 100 g dry material). A comprehensive formula was designed to examine how vegetable material could influence vegetable pasta's technical and nutritional quality. Specific recipes are shown in **Table 3.3abcd** below:

Table 3.3 Formulation of vegetable pasta

Table 3.3 (a) Formulation of spinach pasta

Pasta type	Semolina g	Water g	Vegetable amount g	Dry matter from vegetable g
Control	100.00	30.00	0.00	0.00
Spinach juice 1%	99.00	21.49	9.51	1.00
Spinach puree 1%	99.00	11.63	19.37	1.00
Spinach puree 2%	98.00	23.27	8.73	2.00
Spinach pomace 1%	99.00	30.00	1.00	1.00*
Spinach pomace 2%	98.00	30.00	2.00	2.00*
Spinach pomace 10%	90.00	30.00	10.00	10*

* The study focuses on the analysis of pasta, and due to the low water content of vegetable pomace (close to semolina), the water content of pomace was not considered a significant factor in this study

Table 3.3 (b) Formulation of red cabbage pasta

Pasta type	Semolina g	Water g	Vegetable amount g	Dry matter from vegetable g
Control	100.00	30.00	0.00	0.00
Red cabbage juice 1%	99.00	24.07	6.93	1.00
Red cabbage puree 1%	99.00	23.15	8.85	1.00
Red cabbage puree 2%	98.00	23.15	8.85	2.00
Red cabbage pomace 1%	99.00	30.00	1.00	1.00*
Red cabbage pomace 2%	98.00	30.00	2.00	2.00*
Red cabbage pomace 10%	90.00	30.00	10.00	10*

* The study focuses on the analysis of pasta, and due to the low water content of vegetable pomace (close to semolina), the water content of pomace was not considered a significant factor in this study

Table 3.3 (c) Formulation of beetroot pasta

Pasta type	Semolina g	Water g	Vegetable amount g	Dry matter from vegetable g
Control	100.00	30.00	0.00	0.00
Beetroot juice 3.08%	96.92	0.00	33.08	3.08
Beetroot puree 3.08%	96.92	7.38	25.70	3.08
Beetroot puree 4.08%	95.92	0.00	34.08	4.08
Beetroot pomace 3.08%	96.92	30.00	3.08	3.08*
Beetroot pomace 4.08%	95.92	30.00	4.08	4.08*
Beetroot pomace 10%	90.00	30.00	10.00	10*

* The study focuses on the analysis of pasta, and due to the low water content of vegetable pomace (close to semolina), the water content of pomace was not considered a significant factor in this study

Table 3.3 (d) Formulation of carrot pasta

Pasta Type	Semolina g	Water g	Vegetable amount g	Dry matter from vegetable g
Control	100.00	30.00	0.00	0.00
Carrot juice 2.27%	97.73	0.00	32.27	2.27
Carrot puree 2.27%	97.73	9.73	22.54	2.27
Carrot puree 3.36%	96.64	0.00	33.36	3.36
Carrot pomace 2.27%	97.73	30.00	2.27	2.27*
Carrot pomace 3.36%	96.64	30.00	3.36	3.36*
Carrot pomace 10%	90.00	30.00	10.00	10*

* The study focuses on the analysis of pasta, and due to the low water content of vegetable pomace (close to semolina), the water content of pomace was not considered a significant factor in this study

3.4 Cooking performance

3.4.1 Optimal cooking time

According to the procedure from AACC (2010), 20 g of pasta strands were cut to 5 cm, and then cooked in 600 mL of boiling water. One strand was taken every 30 s during cooking and pressed between two glass slides. Once the white starch core disappeared, the time was recorded as optimal cooking time.

3.4.2 Cooking loss

10 g of pasta was cooked in 600 mL of boiling water at both optimal cooking time and 7 min (optimal cooking time of control pasta). Cooking loss was measured according to AACC (2010). Cooking water was collected by a stainless-steel vessel and dried in an air oven at 105 °C until a constant weight was reached. The residue was weighed and reported as gram residues per 100 g of raw material.

3.4.3 Swelling index and water absorption index, and water content of uncooked and cooked pasta

Swelling index and water absorption index were evaluated according to Bustos et al. (2015) with slight modifications. Pasta (10 g) was cooked to the optimal cooking time and 7 min, respectively. The cooked pasta was weighed after wash and stain, and recorded as P_c. Then the cooked pasta was dried at 105 °C until a constant weight was reached, recorded as P_{cd}. Swelling index and water absorption index can be calculated as following Equation 6 and Equation 7 below:

$$\text{Swelling index}=(P_c-P_{cd})/P_{cd} \quad (7)$$

$$\text{Water absorption index}=(P_c-P_u)/P_u*100 \quad (8)$$

Where P_u is the weight of uncooked pasta, P_c is the weight of cooked pasta, P_{cd} is the weight of dried, cooked pasta.

3.5 Texture properties

The firmness, breaking distance, and breaking force were measured by a texture analyser (TA.XT2; Stable Microsystems, Godalming, UK) with a 5 kg load cell. The pasta was cooked to the optimal cooking time and 7 min (optimal cooking time of the control), respectively. Firmness test is according to AACC (2010) with some modifications. Five strands of cooked pasta were placed on a flat metal plate. A noodle blade was used to compress the cooked pasta strands. The test parameters were set as test speed = 0.2 mm/s, post test speed = 10 mm/s, and distance of 5 mm. Tension test settings were according to Foschia, Peressini, Sensidoni, Brennan, and Brennan (2015a). The A/SPR spaghetti/noodle rig (the tension test setting: Pre-test speed, 3 mm/s; test speed, 3 mm/s; initial distance, 10 mm; final Distance 120 mm) was used in the test. Data are represented as the mean of 9 measurements from triplicate cooking batches (each cooking triplicate measurement).

3.6 Colour

A portable colorimeter (Minolta Chroma Meter CR210; Minolta Camera, Osaka, Japan) was used to measure cooked (to OCT) and uncooked pasta. Each pasta was measured nine times from the triplicate cooking batches, and the result was expressed as L* (bright-ness range from 100 to 0), a* (redness-greenness range from 128 to -128), b* (yellow-ness-blueness range from 128 to -128). The instrument was calibrated using a standard white tile (L*=98.03, a*=-0.23, b* = 0.25).

3.7 Proximate composition analysis

3.7.1 Moisture content

The moisture content of cooked and raw pasta was measured according to AACC (2000). Pasta was cooked to the optimal cooking time. Then, uncooked or cooked pasta was weighed in a pre-weighed metal dish using an analytical balance (ARC120; OHAUS Corp., Parsippany, NJ, USA). Then the dish was placed in an oven at 105 °C -110 °C overnight. Dried pasta samples in the metal dish were cooled to room temperature in a desiccator for 1 h. Then the dish was weighed in the same analytical balance. The solid content can be calculated as below:

$$Moisture\ content\ (g/100g) = 100 - \frac{residue\ weight\ g}{sample\ weight\ g} \times 100 \qquad (9)$$

3.7.2 Ash content

Ash content of raw materials, uncooked pasta, and cooked pasta (cooked to optimal cooking time) were measured according to AOAC (2005). Vegetable juice, vegetable puree, uncooked pasta and cooked pasta (OCT) were freeze-dried (Model E.D. 5.3, Cuddon LTD., Aukland, NZ) to a constant weight and ground to powder by a coffee grinder (Breville BCG200, Breville group, Alexandria NSW, Australia). Then, the ground samples (5 g) were placed into pre-weighed crucibles and placed in a furnace at 550 °C overnight. The crucibles were reweighed after 1 h cool down in a desiccator. Ash content was calculated as below:

$$Ash\ content\ \left(\frac{g}{100g}\ or\ \%\right) = \frac{ash\ weight\ g}{sample\ weight\ g} \times 100$$

3.7.3 Protein content

The protein content was determined by Dumas total N methods. Dumas was chosen due to it is less time-consuming, and generally more accurate than traditional Kjeldahl method (Müller, 2017). It is because Dumas method is highly automated and involves less procedure of chemical reaction that may introduce error. Also, Dumas methods avoid the use of hazardous chemicals, which is safer and environmentally friendly (Müller, 2017).

The freeze-dried ground sample (200 mg) was loaded into the Dumas machine (Elemental analyser Vario MAX CN, Frankfurt, Germany) to determine the total nitrogen. A conversion factor of 6.25 was applied to both pasta and raw materials to convert N to protein %. It should be noted that the conversion factor for different vegetables might vary as total N included non-amino acid N like nitrate and N from nucleic acids (Simonne, Simonne, Eitenmiller, Mills, & Cresman III, 1997). Thus, the protein results are approximate, especially for the raw material.

3.8 Mineral analysis

Weighed 0.2 g of freeze-dried ground sample in a microwave vessel, then 2 mL of Nitric acid (69%, trace element grade) and 2 mL of hydrogen peroxide were added to the vessel and well mixed. The vessels were put into a microwave digestor (GEM MARS Xpress, GEM Corporation, North Carolina, USA) to undergo two steps of digestion: ramped to 90 °C over 15 min and held for 5 min, then ramped to 185 °C over 10 min and then held for 15 min. After digestion, the mineral contents were then measured by an inductively coupled plasma optical emission spectrophotometer (The Varian 5110 ICP-OES, Varian Australia Pty Ltd, Melbourne, Victoria, Australia). The calibration standards and internal standards (Merck ICP standard solutions (Ca, K, Mg, P, Na, Al, Cu, Fe, and Zn) were serially diluted, creating at least five standards for each element. The setting of the spectrophotometer is: Axial Torch, Power 1.20 kW,

Plasma gas flow 15.0 L/min, Aux 1.5 L/min, Nebulizer 0.9 L/min, Sea spray nebuliser and cyclonic spray chamber.

3.9 Dietary fibre and starch

3.9.1 Dietary fibre

The soluble dietary fibre, insoluble dietary fibre, and total dietary fibre of freeze-dried uncooked and cooked pasta were measured in duplicate using dietary fibre assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Samples were analysis based on AOAC official method (AOAC, 2005), 1 gram of freeze-dried ground sample was weighed in 400 mL tall form beakers with a magnetic stirrer. 40 mL of MES-TRIS blend buffer (0.05 M, pH 8.2) were then added to the beaker and stirred until samples were dispersed. 50 µL of heat-stable α-amylase solution (3000 U/mL, Megazyme) were added to the beaker, and the beaker was covered by aluminium foil squares and incubated in a shaking water bath (100 °C) for 30 min with continuous agitation. Then the beaker was cool to 60 °C using another 60 °C water bath, following scraped by a spatula and rinsed with 10 mL water. 100 µL of protease was added to the mixture with agitation and foil cover for another 30 min. Then 5 mL of 0.561 N HCl solution was added into the beaker while stirring. After adjusting the pH to 4.1-4.8, 200 µL of amyloglucosidase solution was added with stirring. The mixture was incubated for another 30 min with agitation at 60 °C water bath. The mixture in the beaker was transferred into a sintered glass crucible with celite (0.1 mg). Then suction was applied, and the residue was washed twice with 10 mL 70 °C water. The filtrate was moved to a 600 mL beaker for soluble dietary fibre measurement while the residue was washed twice with 10 mL of 95% ethanol and another twice with 10 mL acetone to determine insoluble dietary fibre. 4 mL 60 °C 95% ethanol was added to the filtrate, and place it in room temperature for 60 min to allow the soluble dietary fibre to precipitate. Then the mixture was filtered by sintered glass crucible with celite

(0.1 mg). Then suction was applied, and the residue was washed twice with 78 % ethanol (15 mL), 95 % ethanol (15 mL) and acetone (15 mL), respectively. All crucibles were dried at 105 °C overnight and weighed. The dried fibre was then analysed for protein and ash, as described above. The total dietary fibre was calculated by summing the insoluble dietary fibre and soluble dietary fibre.

3.9.2 Starch content

Megazyme starch analysis kits (Megazyme International Ireland Ltd, Wicklow, Ireland) were used to determine the total starch content of vegetable pasta according to the AOAC Official Method 966.11. Two sets (one for sample and one for blank) of 100 mg of ground samples were weighed into corning culture tubes. Then 10 mL of 100 mM sodium acetate buffer (pH5.0) was added to the sample with stirring then followed by thermal stable α-amylase (3000U, Megazyme International Ireland Ltd, Wicklow, Ireland), while for blank tube α-amylase was replaced by sodium acetate buffer. Then the samples and blanks were put into a 100 °C water bath with intermittent shaking for around 20 min and then cooled down in another 50 °C water bath for approximately 10 min. After that, 0.1 mL of aliquot amyloglucosidases (3300U, Megazyme International Ireland Ltd, Wicklow, Ireland) was then added to sample tubes then mix, while 0.1 mL of sodium acetate buffer was added to the blanks. Samples and blanks were incubated in a 50 °C water bath for another 30 min. Then samples were cooled at room temperature with intermittent mixing for 15 min. Transfer 2 mL of each solution to microfuge tubes and centrifuge the tubes at 3000 rpm (1811 g) for 15 min. A 1 mL aliquot of the supernatant was added to tubes (12mm X 120 mm) followed by 4 mL of sodium acetate buffer and mixed. A 0.1 mL aliquot of samples of each sample was transferred in triplicate to the bottoms of the test tube (single 0.1 mL aliquot for blank). Then 3 mL of glucose determination reagent (GOPOD) was added to the samples and incubated at 50 °C water bath for 20 min. The absorbance of the solution was measured against the blank at 510 nm. Starch content was calculated as below:

$$Total\ starch\ = \Delta A\ \times F\ \times \frac{EV}{0.1} \times D\ \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} = \Delta A\ \times F\ \times EV\ \times \frac{D}{W} \times 0.90$$

$$= \Delta A\ \times\ F \times \frac{9.18}{W}$$

EV = Sample extraction volume [10.2 mL]

0.1 = volume of sample analysed

D = dilution factor = 1

$\frac{1}{1000}$ = conversion from µg to mg

$\frac{100}{W}$ = conversion to 100mg sample; W = sample weight in mg

$\frac{162}{180}$ = factor to convert from free glucose, as determined to anhydroglucose, as occurs in starch

Where ΔA is the absorbance of the sample solution against the reagent blank. F is 100 (µg of D-glucose) divided by the D-glucose standard absorbance. W is the sample weight (mg).

3.10 *In vitro* starch digestibility

An *in vitro* digestion was carried out according to Peressini et al. (2020) with slight modification. Frozen pasta (5 g) was defrosted for 120 min at room temperature and cooked to OCT in boiling tap water (250 mL). The pasta was drained for 1 min and cut into 2 mm strands.

The cooked pasta (2.5 g) was weighed in a 60 mL plastic biopsy pot that was placed on a pre-heated magnetic heat stirring block (IKAAG RT 15, IKA-WERKE Gmbit & Co., Staufen, Germany). The sample in the biopsy pot was then stirred continuously with 30 mL distilled water, 0.8 mL 1 M HCl and 1 mL of 10 % pepsin (Sigma Aldrich, USA) at 37 °C for 30 min to mimic gastric digestion. An aliquot (1 mL) was taken

(time 0 min) and added to the Falcon plastic tube with 4 mL of ethanol. Then, 2 mL 1M $NaHCO_3$, 5 mL 0.1 M Sodium maleate buffer (pH 6) and 5 mL 2.5% pancreatin were added to the digestion pot. An aliquot (1 mL) was taken at 30 min, 60 min and 120 min and added to a Falcon plastic tube with 4 mL of ethanol. The biopsy pots were stored at -19 °C for the antioxidant analysis described at **3.12** and carotenoid analysis described at **3.13**.

The falcon tubes containing the aliquot (1 mL) samples were centrifuged at 1000 RPM (201 g) for 5 min. An aliquot (50 µL) of each was placed in a glass test tube alongside the reagent blank (50 µL water), 5 mg/mL glucose standard solution (50 µL), 10 mg/mL glucose standard solution (50 µL). Freshly prepared enzyme solution (250 µL, 1% invertase and 1% amyloglucosidase in acetate buffer pH 5.2) was added to the test tubes. The mixture stayed at room temperature for 20 min before 750 µL of 0.04 M DNS (dinitro-salicylic acid) reagent was added to each tube. The tubes were then covered with aluminium foil and placed in a boiling water bath for 15 min. The tubes were then cooled in cold water bath before adding 4 mL of RO water and absorbance was read at 530 nm against RO water. The reducing sugar was calculated as mg/g sample. The starch digestion was expressed as either reducing sugar produced against time or area under the curve (AUC).

3.11 α-amylase inhibition

3.11.1 Sample extraction

Raw pasta and cooked pasta (cooked to OCT), vegetable juice and puree samples were freeze-dried. 2.5 g of freeze-dried pasta and vegetable alongside with vegetable pomace samples and acarbose powder (Sigma Aldrich, USA, used as positive control) were extracted by stirring overnight at 20 °C in 25 mL of 70% methanol solution. The mixtures were then centrifuged at 2500 RPM (2305 g) for 10 min. The supernatant was collected and kept at −18 °C until analysis.

3.11.2 Determination of α-Amylase Inhibition

The α-amylase inhibition activity of the extract was determined according to the procedure from Sultana et al. (2020) with slight modification. Sample extract (1 mL) was taken, and a series of step-down dilutions were made using sodium phosphate buffer (0.02 M Na2HPO4 and 0.02 M NaH_2PO_4 pH 6.9 with 0.006 M NaCl). Those samples were diluted to 10 µg/mL to 100 mg/mL, depending on the sample type. An aliquot (100 µL) of dilution was then mixed with 100 µL of pancreatic α-amylase solution (3000 U, Megazyme Inter-national Ireland Ltd., Wicklow, Ireland). The mixture was then incubated at 37 °C for 10 min. A 100 µL aliquot of 10 mg/mL starch solution (dissolved in the same buffer) was then added and incubated at 37 °C for 10 min. Then 400 µL of 0.04 M DNS reagent was added to terminate the reaction, and the mixture was incubated in a boiling water bath (with a cover to avoid light) for 30 min. The test tube (with the mixture) was cooled down in a water bath, and 2.7 mL of distilled water was added to the test tube. After mixing, the absorbance was read at 540 nm. The phosphate buffer was used as blank, and acarbose was used as the positive control. The result was expressed to the percentage of inhibition at each concentration and then converted to IC_{50} (the concentration inhibiting 50% of enzyme activity)

3.12 Antioxidant and Total Phenolic compounds

3.12.1 Sample extraction

Raw pasta and cooked pasta (cooked to OCT) were freeze-dried. Freeze-dried pasta (2.5 g) was weighed in 60 mL plastic biopsy pot. The samples then stirred overnight at 20 °C with 25 mL of 70% methanol solution. The mixtures were then centrifuged at 2500 RPM (2305 g) for 10 min. The supernatant was collected and kept at −18 °C until analysis. For digesta described in **3.10**. Defrosted digesta were well mixed, then 20 mL of the digesta mixture was put into a 25 mL centrifuge tube and centrifuged at 2500

RPM (2305 g) for 10 min. The supernatant was made volume to 25 mL with 70% methanol and stored at −18 °C until analysis.

3.12.2 Ferric Reducing/Antioxidant Power (FRAP)

Ferric Reducing /Antioxidant Power (FRAP) was tested based on the procedure from Rachman, A Brennan, Morton, and Brennan (2020). The working solution of the FRAP reagent was prepared by mixing 300 µM Acetate buffer (pH 3.6), TPTZ solution (10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl) and $FeCl_3$ (20 mM) at a ratio of 10:1:1 (v/v/v). 250 µL of standard of iron (II, Iron sulphate heptahydrate, $FeSO_4 7H_2O$) or sample extract was added to 2.5 mL of FRAP reagent, and the absorbance at 593 nm was recorded using VWR V-1200 Spectrophotometer (VWR International Co., Pennsylvania, USA) immediately and after 2 h incubation at 37 °C. The results were expressed as mmol Fe^{2+}/100g sample.

3.12.3 ABTS radical scavenging capacity.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging capacity was determined following Rachman, A Brennan, et al. (2020). The ABTS working solution was prepared by mixing ABTS stock solution (7 mM) with 2.45 nM potassium persulfate ($K_2S_2O_8$) and allowing the reaction in a dark environment for 16 h. On the day of analysis, the ABTS solution was diluted with PBS (pH 7.4) to achieve an absorbance of 0.70 (± 0.02) at 734 nm. Each sample (300 µL) and Trolox standard (0 – 200 µmol) were added to 2.7 mL ABTS solution and incubated at room temperature for 6 min. Then absorbance at 734 nm was recorded as µmol Trolox equivalents (TE) per 100 g dry matter.

3.12.4 Total Phenolic content

The total phenolic contents were measured according to the method used by Lu et al. (2018). The sample extracts (500 µL) along with gallic acid standards (0 – 150 ug/mL) were mixed with 2.5 mL Folin-

Ciocalteau reagent (0.2 M) and 2 mL sodium carbonate (7.5g / 100 mL). The mixtures were stored for 2 h in a dark environment, and the absorbance was read at 760 nm. The results were expressed as mg gallic acid equivalents per 100 g of dry matter.

3.13 β-Carotene Analysis of carrot and spinach pasta

3.13.1 Sample extraction for β-Carotene Analysis

The aqueous phase of digesta was isolated by centrifugation (3000 RPM, 3320 g) for 10 min. of Aqueous phase digesta (6 mL), or of freeze-dried raw and cooked pasta (2 g), or of spinach and carrot raw material (2 g; pomace, freeze-dried puree and juice of spinach and carrot) were extracted with 4 mL solution of methanol: water: acetone (2:1:1 v/v/v) and vortex for 1 min. The upper acetone phase was transferred to a centrifuge tube and 2 mL acetone was added to the previous sample tube repeat extract twice. 2 mL of hexane was then added to the centrifuge tube and vortex for 1 min, following a centrifuge (3000 RPM, 3320 g) for 2 min. The hexane extraction was repeated two more times, and the supernatant was collected and rotary-evaporated at 30 °C. The dried extract was mixed with 2 mL of methanol and filtered through a 0.45 μm filter for HPLC analysis.

3.13.2 HPLC Analysis of β-Carotene

Agilent 1100 series (Agilent Technologies, Walbronn, Germany), was used to analyse β-carotene. It was equipped with a binary pump and an auto-sampler with a thermostat (kept the temperature at 25 °C in a column oven compartment). Chromatographic analysis was performed using a column (EXL-1110-1546U, ACE 3μ C18-PFP 150 mm × 4.6 mm, Advanced Chromatography Technologies, Aberdeen, Scotland). HPLC conditions were as follows: solvent A (Methanol 100%), solvent C (Ethyl-acetate 100%), separation of β-carotene achieved by a step gradient shown in **Table 3.4** below with a flow rate 0.8

mL/min based on van Leeuwe, Villerius, Roggeveld, Visser, and Stefels (2006). Qualification was carried out by an external standard (Sigma C4582). Analysis was carried out in duplicate test.

Table 3.4 HPLC Gradient

Time (min)	A%	C%
0	68	32
3	68	32
7	30	70
9	30	70
10	68	32
15	68	32

Solvent A, Methanol 100%, Solvent C, Ethyl-acetate 100%

3.14 Statistical analysis

All experiments were performed in triplicate (same batch of pasta with multiple independent cooking procedures) except when stated otherwise. All results were statistically analysed using SPSS (version 16, IBM, San Jose, CA, USA). A one-way ANOVA was carried out, the standard deviation was calculated, and the difference was evaluated using the Tukey test. For the data that violates the assumption of Tukey test (homogeneity of variances test, $p < 0.05$), the Anova-Duncan test was used instead.

Chapter 4

Effect of spinach and red cabbage juice, puree and pomace on chemical and technological quality of fresh pasta

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4.1 Introduction

Pasta is staple cereal food around the world (Vitale et al., 2020). It is considered a good vehicle for delivering functional ingredients (Oliviero & Fogliano, 2016). Vegetables contain many health-promoting phytochemicals that traditional pasta lacks (Marinelli et al., 2018). Those phytochemicals include dietary fibre, vitamins, polyphenols, carotenoids, glucosinolates, and minerals. Even if the consumer was aware of the health benefits of consuming vegetables, their ingrained eating habit prevented them from a sufficient vegetable intake (Marinelli et al., 2018). Hence, it may be a suitable option to incorporate vegetables into staple foods such as pasta or bread.

Vegetable pasta has been studied by many researchers (Mridula, Gupta, Bhadwal, Khaira, et al., 2016; Sun-Waterhouse et al., 2013). An inferior cooking and sensory quality of vegetable pasta have been frequently reported compared to traditional pasta (Jalgaonkar et al., 2018; Petitot et al., 2010; Rayas-Duarte, Mock, & Satterlee, 1996). Increased cooking loss (CL), decreased firmness, and elasticity were reported by the researcher (Oliviero & Fogliano, 2016). However, typical vegetable addition is based on powdered vegetables. The authors substitute parts of semolina for vegetable powder (Jalgaonkar et al., 2018; Sahni & Shere, 2016; Sant'Anna et al., 2014). It has been reported that vegetable powder produced by the traditional oven-dry method could suffer from nutrition loss (Karam, Petit, Zimmer, Baudelaire Djantou, & Scher, 2016). The particle size of the powder was also associated with quality impact (Padalino et al., 2015). Some studies linked vegetable pasta to by-product utilisation. Gull et al. (2015) added carrot pomace powder to the pasta formula, and Simonato, Trevisan, Tolve, Favati, and Pasini (2019) used 5%-10% olive pomace to fortify pasta.

A few studies were reported to use other forms of vegetables to add to pasta. For example, Carini et al. (2012) have managed to add carrot juice to pasta. The author found that carrot juice pasta had similar extensibility and cooking loss compared to the control, while carrot flour enriched pasta had a very high

CL (more than 8 g/100 g) and lower extensibility, which indicates inferior quality. However, researchers ignored that the actual substitution level (based on the dry matter) for carrot flour pasta is much higher than for carrot juice pasta. In that case, it is not an like to like comparison. Rekha et al. (2013) made use of carrot, spinach, tomato and beetroot puree to fortify pasta. The authors found a decreased cooking loss, and improved texture of the resultant pasta. Such findings are unsatisfactory because the study lacks comparison with powder form enriched pasta, and the description of puree-semolina mixing procedure is blurred. Juice and puree addition also have their limitation when combined with pasta. Juice contains low solids (typically from 2% to 15%). Achieving high substitution based on the dry matter may be impossible for juice and puree. This is due to wrong hydration and overwhelming water addition, which may cause big lump formation, and could result in difficulties in successful extrusion (Bustos et al., 2015). The water content of juice and puree makes them more difficult to store and transport. It may cause an increased cost for the food industry.

This project aims to compare the key chemical composition, cooking performance, and texture quality of vegetable-fortified pasta which uses different forms (juice, puree, and pomace) of vegetables. Two kinds of leaf vegetables, spinach (*Spinacia oleracea* L.) and red cabbage (*Brassica oleracea* convar. capitata var. capitata f. rubra), were selected for this study. Spinach is cheap and widely available. It is reported to have antioxidant and antidiabetic effects (Vyas, 2017). Spinach is also widely accepted by the food industry to produce commercially available green pasta. Red cabbage is nutritious as it is high in fibre and antioxidant phytochemicals (Iborra-Bernad, Tárrega, García-Segovia, & Martínez-Monzó, 2014). Red cabbage materials in this study may provide a novel purple colour pasta.

4.2 Material and methods

4.2.1 Raw materials

As described in section 3.1

4.2.2 Vegetable preparation

As described in sections 3.2.3 and 3.2.4

4.2.3 Pasta preparation

As described in section 3.3

4.2.4 Proximate analysis

Moisture, ash and protein were described in section 3.7. Starch content was described in section 3.9.2

4.2.5 Cooking performance

As described in section 3.4

4.2.6 Texture measurement

As described in section 3.5

4.2.7 Colour measurement

As described in section 3.6

4.2.8 Statistical analysis

As described in section 3.14

4.3 Results and discussion

4.3.1 Proximate composition of spinach and red cabbage

The protein, moisture, and ash content of spinach and red cabbage pasta are shown in **Table 4.1**. The protein content in pasta is essential as it is key of pasta structure. In pasta, the protein gluten can be described as the backbone, with starch granules trapped in it, it plays a crucial function in pasta structure (Bonomi et al., 2012). It is widely accepted that this structure is mainly maintained by disulphide bonds with the help of other non-covalent interactions such as hydrogen bonds and ionic bonds (Ooms & Delcour, 2019; Perssini, Sensidoni, Pollini, & De Cindio, 2000; Sicignano, Di Monaco, Masi, & Cavella, 2015). It is suggested that protein-rich material may result in protein-protein interaction and form a more cohesive structure, thus helping the gluten form a homogeneous pasta structure (Jayawardena, Morton, Brennan, & Bekhit, 2019). **Table 4.1 (a)** shows that the spinach raw material is rich in protein. Ranked by protein content, the spinach pasta is as follows spinach juice > spinach puree > spinach pomace based on the dry weight. As a result, all the uncooked spinach pasta shows a significantly higher ($p < 0.05$) protein content than the control. Lisiewska, Kmiecik, Gębczyński, and Sobczyńska (2011) reported that raw spinach contains 36 ± 12 mg/100 g cysteine content, around 1.5% of its total amino acid composition. Cysteine can provide sulfhydryl groups to form disulphide bonds during dough formation (Filip & Vidrih, 2015). It indicates that protein from spinach may positively impact the formation of gluten network and pasta quality. A higher protein content was observed in the cooked pasta compared to the raw. A similar trend was found by Manthey and Hall III (2007), who used buckwheat bran flour to enrich pasta. It is possibly due to the leaching of starch into the cooking water,

increasing the proportion of protein content in the pasta. Although for spinach juice 1%, the uncooked pasta shows significantly higher protein content than the control, the cooked one shows no difference ($p > 0.05$). This may be because the juice sample contains more soluble protein that may be lost during cooking. The cooked red cabbage pasta has a significantly lower protein content than the control except for red cabbage puree 2%, possibly because red cabbage raw material has a lower protein content (red cabbage pomace contains 11.06 g/100 g compared to spinach pomace of 23.91 g/100 g).

The total starch content of vegetable-enriched pasta decreased with increased vegetable substitution. Cooking did not show any significant difference in total starch composition ($p < 0.05$) of vegetable enriched pasta. The ash content of foods is mainly inorganic metal compounds (Reilly, 1980). Perssini et al. (2000) found that the sodium chloride content increases the strength and solid-like semolina-flour dough behaviour via optimisation of ionic strength. McCann and Day (2013) found that salt delays the formation of the gluten network by reducing the rate of gluten hydration. Tang et al. (2019) found that salt content can increase the strength of the disulphide bond in flour Raman gluten dough as less free SH groups are detected. Thus, the ash content may influence pasta quality. **Table 4.1** shows spinach raw material characteristics with higher ash content in every form compared with red cabbage raw material. The addition of vegetable material increased the ash content of vegetable pasta significantly ($p < 0.05$) in every sample. This result is similar to that of Prabhasankar et al. (2009), who used Japanese seaweed to fortify pasta. The ash content indicates a higher mineral content in those samples. Cooking causes a decreased ash content of vegetable pasta. It may be because some metal in ash is present in a water-soluble form and is lost during cooking. Desai, Brennan, and Brennan (2018) found similar trends showing that cooked fish powder fortified pasta had a lower ash content than before cooking.

Table 4.1 Proximate chemical analysis of spinach and red cabbage pasta

	Protein g/100 g dry matter		Total Starch g/100 g dry matter		Moisture g/100 g Material		Ash g/100 g dry matter	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
(a) Spinach pasta and spinach raw material								
Raw material								
Semolina	12.58 ± 0.11	N/A	71.42 ± 0.51	N/A	10.95 ± 0.10	N/A	0.95 ± 0.04	N/A
Spinach juice	38.56 ± 0.06	N/A	N/A	N/A	95.35 ± 0.04	N/A	21.82 ± 0.02	N/A
Spinach puree	31.17 ± 0.07	N/A	N/A	N/A	91.41 ± 0.07	N/A	17.40 ± 0.05	N/A
Spinach pomace	23.91 ± 0.13	N/A	N/A	N/A	12.95 ± 0.02	N/A	13.29 ± 0.01	N/A
Spinach pasta								
Control	12.49 ± 0.01 ^{d,B}	12.96 ± 0.01 ^{de,A}	70.35 ± 0.39 ^{a,A}	70.24 ± 0.26 ^{a,A}	36.16 ± 0.70 ^{ab}	64.58 ± 0.23 ^{a}	0.68 ± 0.01 ^{g,A}	0.44 ± 0.01 ^{f,B}
Spinach juice 1%	12.76 ± 0.01 ^{c,B}	13.00 ± 0.06 ^{cd,A}	69.60 ± 0.62 ^{b,A}	69.46 ± 0.50 ^{b,A}	35.71 ± 0.06 ^{b}	64.40 ± 0.24 ^{a}	0.89 ± 0.00 ^{d,A}	0.55 ± 0.01 ^{d,B}
Spinach puree 1%	12.75 ± 0.01 ^{c,B}	13.04 ± 0.01 ^{c,A}	69.72 ± 0.60 ^{b,A}	69.48 ± 0.46 ^{b,A}	35.36 ± 0. 25 ^{b}	64.47 ± 0.15 ^{a}	0.84 ± 0.00 ^{e,A}	0.55 ± 0.00 ^{d,B}
Spinach puree 2%	12.96 ± 0.01 ^{b,B}	13.24 ± 0.06 ^{b,A}	68.06 ± 0.41 ^{c,A}	68.41 ± 0.45 ^{c,A}	35.67 ± 0.22 ^{b}	64.50 ± 0.26 ^{a}	1.11 ± 0.01 ^{b,A}	0.65 ± 0.01 ^{b,B}
Spinach pomace 1%	12.78 ± 0.01 ^{c,B}	12.89 ± 0.01 ^{e,A}	69.44 ± 0.48 ^{b,A}	69.29 ± 0.37 ^{b,A}	36.71 ± 0.68 ^{ab}	65.00 ± 0.16 ^{a}	0.81 ± 0.00 ^{f,A}	0.58 ± 0.00 ^{c,B}
Spinach pomace 2%	12.77 ± 0.01 ^{c,B}	13.06 ± 0.01 ^{c,A}	68.13 ± 0.62 ^{c,A}	68.57 ± 0.52 ^{c,A}	35.62 ± 0.25 ^{b}	64.61 ± 0.05 ^{a}	0.93 ± 0.01 ^{c,A}	0.48 ± 0.00 ^{e,B}
Spinach pomace 10%	14.13 ± 0.03 ^{a,B}	14.44 ± 0.01 ^{a,A}	61.39 ± 0.43 ^{d,A}	60.93 ± 0.36 ^{d,A}	37.27 ± 0.64 ^{a}	64.53 ± 0.90 ^{a}	1.94 ± 0.01 ^{a,A}	1.14 ± 0.01 ^{a,B}
(b) Red cabbage pasta and red cabbage raw material								
Raw material								
Red cabbage juice	19.23 ± 0.01	N/A	N/A	N/A	95.85 ± 0.00	N/A	10.18 ± 0.01	N/A
Red cabbage puree	16.23 ± 0.08	N/A	N/A	N/A	91.36 ± 0.02	N/A	8.26 ± 0.02	N/A
Red cabbage pomace	11.06 ± 0.01	N/A	N/A	N/A	13.14 ± 0.07	N/A	5.50 ± 0.04	N/A
Red cabbage pasta								
Control	12.49 ± 0.01 ^{b,B}	12.96 ± 0.01 ^{a,A}	70.35 ± 0.39 ^{a,A}	70.24 ± 0.26 ^{a,A}	36.16 ± 0.70 ^{c}	64.58 ± 0.23 ^{b}	0.68 ± 0.01 ^{f,A}	0.44 ± 0.01 ^{e,B}
Red cabbage juice 1%	12.46 ± 0.03 ^{b,B}	12.82 ± 0.04 ^{b,A}	68.97 ± 0.56 ^{b,A}	68.40 ± 0.51 ^{b,A}	36.41 ± 0.41 ^{bc}	64.68 ± 0.53 ^{ab}	0.78 ± 0.01 ^{c,A}	0.46 ± 0.00 ^{d,B}
Red cabbage puree 1%	12.41 ± 0.01 ^{c,B}	12.63 ± 0.03 ^{d,A}	68.88 ± 0.21 ^{b,A}	68.46 ± 0.42 ^{b,A}	37.45 ± 0.13 ^{abc}	64.79 ± 0.37 ^{b}	0.76 ± 0.00 ^{d,A}	0.50 ± 0.01 ^{c,B}
Red cabbage puree 2%	12.56 ± 0.01 ^{a,B}	12.91 ± 0.01 ^{a,A}	67.50 ± 0.58 ^{c,A}	67.32 ± 0.57 ^{c,A}	36.63 ± 0.75 ^{abc}	64.60 ± 0.72 ^{b}	0.84 ± 0.02 ^{b,A}	0.54 ± 0.02 ^{b,B}
Red cabbage pomace 1%	12.30 ± 0.01 ^{d,B}	12.59 ± 0.01 ^{d,A}	68.74 ± 0.47 ^{b,A}	68.41 ± 0.42 ^{b,A}	37.77 ± 0.33 ^{ab}	65.83 ± 0.04 ^{a}	0.73 ± 0.01 ^{e,A}	0.50 ± 0.01 ^{c,B}
Red cabbage pomace 2%	12.56 ± 0.05 ^{a,B}	12.72 ± 0.01 ^{c,A}	67.45 ± 0.57 ^{c,A}	67.30 ± 0.40 ^{c,A}	37.30 ± 0.36 ^{abc}	65.42 ± 0.46 ^{ab}	0.78 ± 0.01 ^{c,A}	0.55 ± 0.00 ^{b,B}
Red cabbage pomace 10%	12.41 ± 0.01 ^{c,B}	12.77 ± 0.03 ^{bc,A}	60.34 ± 0.58 ^{d,A}	59.96 ± 0.50 ^{d,A}	37.94 ± 0.45 ^{a}	64.30 ± 0.14 ^{b}	1.16 ± 0.01 ^{a,A}	0.95 ± 0.01 ^{a,B}

Values = mean ± standard deviation, n=3; Protein starch and ash results are based on a dry weight basis. Moisture results are based on wet weight basis. N/A = not tested. Values within a column in the same sub-table followed by the same superscripted letters are not significantly different from each other ($p > 0.05$), values followed by the same superscripted capital letter are not significantly different between cooked and uncooked samples according to the ANOVA-Duncan test.

4.3.2 Cooking performance of spinach pasta and red cabbage pasta

Optimal cooking time, cooking loss, swelling index, and water absorption index are crucial cooking quality attributes of pasta (Bustos et al., 2015). Those attributes are strongly influenced by the protein-starch matrix formed during cold extrusion (Sicignano et al., 2015). A good quality pasta has a compact protein-starch matrix, which slows diffusion of the water to the starch core and inhibits amylose leaching into cooking water, giving a longer optimal cooking time and a decreased cooking loss (Diantom et al., 2019). **Table 4.2** shows that red cabbage content increases the cooking loss significantly. The cooking loss of red cabbage pasta ranged from 4.767 to 6.163 g/100 g, compared to 4.399 g/100 g of the control sample. The cooking loss values of the spinach juice pasta and spinach puree pasta showed no significant difference at 1 g/100 g substitution level versus the control. Other spinach pasta samples show increased cooking loss (from 4.447 to 5.920 g/100 g) compared to the control. The increased cooking loss indicates a weaker gluten matrix, which may be caused by fibre disruption, competition for water between gluten protein and other compounds (such as water-soluble fibre and soluble salt) and a dilution of gluten, which is caused by the substitution of semolina with vegetable material. Moreover, introducing vegetable contents may change the water states, such as the proportion of free water and bound water. Obadi, Zhang, Shi, and Xu (2021) have shown that free water could promote the interaction of dough components and play an essential role in the formation of gluten network. The introduction of vegetable contents may reduce free water content as some water may be bound with fibre and other materials and change the hydration to some degree. Some technology, such as Differential Scanning Calorimetry (DSC) and nuclear magnetic resonance (NMR), could be used further to measure the amount of free water and bound water (Hong et al., 2021). This might be helpful in evaluating the impact of vegetable addition on pasta quality in future research.

The pasta with red cabbage pomace had a higher cooking loss than puree or juice sample at the same substitution level (red cabbage pomace 1%> red cabbage puree 1%, red cabbage pomace 1%>red cabbage juice 1%, red cabbage pomace 2%> red cabbage puree 2%). This may be because it contained less protein compared to pasta made with puree or juice, as shown in **Table 4.1**. The protein content and their properties can influence the gluten network formation and pasta structure (Bustos et al., 2015). The higher protein content in juice and puree can potentially interact with gluten, hence decreasing the disruptive effect caused by fibre and diluted gluten. Carini et al. (2012) found that carrot juice pasta has a much lower cooking loss than pasta with carrot flour. However, the substitution level of carrot juice and carrot flour in that research was not standardised. Kowalczewski et al. (2015) report the cooking loss of fresh potato juice-fortified pasta is lower than that fortified by spray-dried potato juice. All the vegetable pasta in this study has a cooking loss lower than 8 g/100 g, which is widely agreed maximum value for consumer acceptability (Bustos et al., 2015; Sissons, 2008).

Table 4.2 Cooking performance of vegetable pasta

	Optimal Cooking Time (min: s)	Cooking Loss (g/100 g)	Swelling Index (g Water/g Dry Pasta)	Water Absorption Index (g/100 g)
		Spinach Pasta		
Control	7:00	4.40 ± 0.06 ^{de}	1.86 ± 0.07 ^{a}	81.27 ± 1.42 ^{a}
Spinach juice 1%	7:00	4.37 ± 0.07 ^{e}	1.80 ± 0.02 ^{a}	80.62 ± 1.17 ^{a}
Spinach puree 1%	7:00	4.45 ± 0.09 ^{de}	1.81 ± 0.01 ^{a}	81.92 ± 1.22 ^{a}
Spinach puree 2%	7:00	4.800 ± 0.03 ^{c}	1.82 ± 0.02 ^{a}	82.09 ± 0.28 ^{a}
Spinach pomace 1%	7:00	4.50 ± 0.02 ^{d}	1.86 ± 0.01 ^{a}	80.86 ± 2.18 ^{a}
Spinach pomace 2%	7:00	5.00 ± 0.06 ^{b}	1.83 ± 0.00 ^{a}	81.93 ± 0.90 ^{a}
Spinach pomace 10%	6:30	5.92 ± 0.78 ^{a}	1.82 ± 0.07 ^{a}	74.11 ± 2.67 ^{b}
		Red Cabbage Pasta		
Red cabbage juice 1%	7:00	4.40 ± 0.06 ^{e}	1.86 ± 0.07 ^{ab}	81.27 ± 1.42 ^{ab}
Red cabbage puree 1%	7:00	4.77 ± 0.02 ^{d}	1.93 ± 0.00 ^{a}	82.16 ± 0.78 ^{a}
Red cabbage puree 2%	7:00	4.80 ± 0.07 ^{d}	1.83 ± 0.04 ^{b}	80.07 ± 1.59 ^{abc}
Red cabbage pomace 1%	7:00	4.94 ± 0.07 ^{c}	1.88 ± 0.03 ^{ab}	81.29 ± 0.34 ^{ab}
Red cabbage pomace 2%	7:00	5.08 ± 0.38 ^{b}	1.84 ± 0.03 ^{b}	77.67 ± 1.54 ^{c}
Red cabbage pomace 10%	7:00	5.07 ± 0.08 ^{b}	1.83 ± 0.06 ^{b}	79.03 ± 1.85 ^{bc}
Red cabbage juice 1%	6:15	6.16 ± 0.07 ^{a}	1.80 ± 0.01 ^{b}	73.80 ± 0.96 ^{d}

Values = mean ± standard deviation, n=3; Values within a column of the same kind of pasta followed by the same superscripted letter are not significantly different from each other ($p > 0.05$) according to the ANOVA-Duncan test.

The OCT is not changed in the vegetable pasta of all 1 g/100 g, 2 g/100 g samples, possibly because at low substitution levels, the gluten network is not significantly changed to create a measurable impact. However, at a substitution level of 10 g/100 g, spinach pomace 10%, and red cabbage pomace 10% have a shorter optimal cooking time (**Table 4.2**). The decreased optimal cooking time may be caused by decreased water absorption (from 81.27 g/100 g of control to 74.11 g/100 g of spinach pomace 10% and 73.80 g/100 g of red cabbage pomace 10%). Similar results were found by Aravind, Sissons, Egan, et al. (2012) using inulin (soluble fibre) to enrich pasta, and a lower optimal cooking time was reported. Cárdenas-Hernández et al. (2016) also found optimal cooking time was decreased when amaranth flour and amaranth leaves, and carboxymethylcellulose were added to semolina to produce pasta. In contrast, Foschia et al. (2015b) found an increased optimal cooking time when using 15 g/100 g dietary fibre (such as long-chain inulin, psyllium, Glucagel) to substitute semolina.

The swelling index and water absorption index reflect the amount of water absorbed at optimal cooking time. **Table 4.2** shows that all spinach pasta samples show the same swelling index compared to the control. Similar results were reported by Yadav et al. (2014), who found that spinach pasta has no significant difference in water absorption from the control. Red cabbage pomace samples (red cabbage pomace 1%, red cabbage pomace 2%, red cabbage pomace 10%) show a lower ($p < 0.05$) water absorption index in comparison. Red cabbage juice and puree pasta's swelling and water absorption indexes are not significantly different from the control. It is possibly because components of red cabbage pomace have less affinity to water than starch and components from red cabbage juice and puree. The results of water absorption index and swelling index of red cabbage pomace pasta are consistent with Sun-Waterhouse et al. (2013), who found elderberry juice pasta absorbs less water than other samples. In contrast, water absorption increase was observed in turnip pasta, tomato pasta, and carrot pasta (Yadav et al., 2014), as well as broad bean flour-fortified pasta (Tazrart, Zaidi, et al., 2016). This study may indicate that the swelling index and water absorption index of vegetable pasta is

dependent on the intactness or strength of the gluten network and the water-binding capacity of vegetable components.

4.3.3 Texture of spinach and red cabbage pasta

Pasta texture plays an essential role in overall quality and consumer acceptance (Desai et al., 2018; Gull et al., 2015). Elasticity is an important texture profile that is considered to be conferred by gliadins that interact non-covalently with high modular weight glutenin subunits (Sicignano et al., 2015). Elasticity (breaking distance and breaking force) of spinach pasta and red cabbage pasta is shown in **Table 4.3**. Spinach pasta has a higher breaking force ($p < 0.05$, except spinach pomace 1% and spinach pomace 2% are insignificant higher) than the control. Red cabbage addition shows no significant influence on breaking force of red cabbage juice 1%, red cabbage puree 1%, red cabbage puree 2%, and red cabbage pomace 1%. While the decreased breaking force was observed for red cabbage pomace 2% and red cabbage pomace 10%. Spinach juice 1% and spinach puree 1% have the same breaking distance as the control, while other spinach pasta samples and red cabbage pasta samples have lower breaking distance. Juice-fortified pasta shows a higher breaking distance compared to puree and pomace-fortified pasta (control > spinach juice 1% > spinach puree 1% but not statistically significant, spinach juice 1% > spinach pomace 1%, spinach puree 2%, spinach pomace 2%, spinach pomace 10% significantly, control> red cabbage juice 1% > red cabbage puree 1%, red cabbage pomace 1%, red cabbage pomace 2%, red cabbage puree 2% & red cabbage pomace 10%). At a higher substitution level of 10g/100 g. The breaking distance of spinach pomace 10% and red cabbage pomace 10% decreased dramatically. The decreased breaking distance indicates a weakening structure. Brennan and Tudorica (2007) found a higher breaking force compared to the control when adding locust bean gum and xanthan gum to pasta, respectively. The same authors reported lower breaking force when incorporating pea fibre, inulin, guar gum and bamboo fibre to durum wheat to produce pasta. Foschia et al. (2015a) found that breaking

force was decreased when durum wheat was substituted with 15g/100 g dietary fibre (inulin, psyllium and oat material). No significant difference in breaking distance was found between the optimal cooking time and 7 min (optimal cooking time of the control) of spinach pasta and red cabbage pasta samples.

Table 4.3 Elasticity and firmness of spinach and red cabbage pasta

Pasta type	Tensile test				Firmness	
	Breaking force g		Breaking distance			
	Optimal cooking time	7 min	Optimal cooking time	7 min	Optimal cooking time	7 min
Spinach Pasta (a)						
Control*	33.6±1.2^{c}		74.3±3.7^{a}		399.8±11.5^{d}	
Spinach juice 1%*	43.8±1.5^{ab}		73.1±5.1^{a}		456.9±14.6^{b}	
Spinach puree 1%*	41.2±2.2^{b}		71.3±4.9^{a}		409.3±11.1^{d}	
Spinach puree2%*	42.3±2.9^{ab}		65.7±3.0^{b}		427.5±10.5^{c}	
Spinach pomace 1%*	34.9±1.7^{c}		65.1±4.7^{b}		398.7±12.8^{d}	
Spinach pomace 2%*	35.0±1.5^{c}		64.6±1.4^{b}		414.1±8.3^{cd}	
Spinach pomace 10%*	45.4±3.9^{a,A}	45.8±1.4^{a,A}	30.8±2.5^{c,A}	30.9±1.7^{c,A}	477.56±6.57^{a,A}	428.2±19.7^{cd,B}
Red Cabbage pasta (b)						
Control*	33.6±1.2^{a}		74.3±3.7^{a}		399.8±11.5^{a}	
Red cabbage juice 1%*	32.5±1.7^{a}		66.2±3.7^{b}		325.8±11.8^{d}	
Red cabbage puree 1%*	31.4±2.2^{ab}		60.9±3.9^{c}		388.6±12.0^{ab}	
Red cabbage puree 2%*	31.1±2.2^{ab}		57.5±3.8^{c}		372.8±12.0^{bc}	
Red cabbage pomace 1%*	32.6±0.9^{a}		58.9±4.3^{c}		400.6±8.7^{a}	
Red cabbage pomace 2%*	29.5±1.9^{b}		57.4±2.4^{c}		385.9±11.4^{ab}	
Red cabbage pomace 10%*	27.04±1.80^{c,A}	25.40±2.13^{c,A}	33.4±2.1^{d,A}	34.1±2.1^{d,A}	358.5±20.0^{c,A}	329.0±11.5^{d,A}

* indicate the sample OCT is equal to 7 min. The same lower case superscripted letter mean values are not significantly different from each other for the same raw of the same vegetable pasta ($p > 0.05$). The same upper case superscripted letter mean values are not significantly different from each other ($p > 0.05$) between OCT and 7 min

Firmness is a measure of the force needed to compress pasta strands between teeth, it is an indicator of protein matrix integrity after cooking, which depends on the quality of gluten fraction (Bustos et al., 2015). **Table 4.3** shows the firmness of spinach pasta and red cabbage pasta. The spinach pasta has a greater firmness than the control (except spinach puree 1%, spinach pomace 1%, spinach pomace 2%). At the same substitution level, spinach juice 1% has a greater firmness than spinach puree 1% and spinach pomace 1%. One possible reason for this is that spinach juice 1% has fewer solid components. Those components may form discontinuities or cracks inside the pasta and result in a weakened structure. Red cabbage pasta firmness was equal to or lower than the control, while spinach pasta firmness was equal to or higher than the control. This is possible because spinach pasta has a higher protein content than red cabbage pasta (as shown in **Table 4.1**). The higher protein content may contribute to a stronger protein structure, thus mitigating the disruptive effect of dietary fibre on the gluten network. This assumption is consistent with Petitot et al. (2010), who substituted 35% of semolina with split pea or faba bean, and reported a significantly firmer pasta with a higher protein content. Jayawardena et al. (2019) used 10%-25% protein-rich beef lung powder added to durum wheat, and the resultant pasta had a significantly higher firmness and breaking force. The firmness of red cabbage juice 1% is the lowest of all tested samples, possibly because of more water swelling (see **Table 4.2** swelling index) by the starch granules which in turn created a softer texture. Foschia et al. (2015a) found that incorporating short-chain inulin leads to a dramatic decrease in pasta firmness and increased water absorption. Gull et al. (2015) reported a significantly lower firmness than the control when 2%-10% carrot pomace was added to the pasta formula. Overcooked vegetable pasta (7min cooked spinach pomace 10% and rad cabbage pomace 10%) shows lower firmness compared to OCT. Grzybowski and Donnelly (1979) reported that firmness is negatively correlated with the cooking time for traditional pasta, which means that more cooking time lowers pasta's firmness.

It may be assumed that the texture profile of vegetable pasta depends on the vegetable components. Some components, such as fibre, and sugar, may adversely affect the overall texture and cooking quality as they influence water absorption, thus causing a change in the hydration process of the starch granules and the gluten network. Fibre particles dilute the gluten and, therefore, also contribute to gluten network disruption and potentially weaken the structure. Other components, such as protein, may generally have some beneficial effects, such as strengthening the gluten network and other interactions to enhance the structure, such as increasing the firmness and breaking force. The overall texture and cooking quality are dependent on the balance of such adverse and beneficial effects from vegetable components. Spinach juice 1% in this study provides outstanding cooking and texture quality, with identical cooking loss, water absorption, and breaking distance compared to the control. It also has higher firmness and breaking force than control, thus producing *al dente* products with a firm, elastic texture. A low substitution level (1 g/100 g according to dry matter), juice form (lower solid particles), and higher protein content than durum wheat may contribute to its distinctive texture quality.

4.3.4 Colour of spinach and red cabbage pasta

Figure 4.1 shows some examples of spinach and red cabbage pasta. The colour results of vegetable pasta are shown in **Table 4.4**. The colour of spinach and red cabbage pasta is strongly influenced by vegetable addition. Red cabbage pasta has lower brightness and yellowness (less L* and b* value) and higher redness (increased a* value) compared to the control. When comparing the different forms of vegetables, the juice's dye effect is stronger than puree or pomace as red cabbage juice 1% (for both raw and cooked) has lower brightness and yellowness and more redness than red cabbage puree 1% and red cabbage pomace 1%. After cooking, the red cabbage pasta tends to be even less bright, red, and yellow, possibly due to the fact that the phytochemicals that provide the colour are water-soluble and leach into the cooking water. Chigurupati, Saiki, Gayser, and Dash (2002) found that red cabbage colour

is water-soluble and sensitive to pH change. It was found that the red cabbage colour changed from purple to deep blue when pH changed from acid to neutral. This could explain cooked red cabbage pasta tends to be bluer (lower b* value except for red cabbage puree 1%) and cooking water is slightly blue as water boils, leading to acid evaporation (as drinking water contains acidic chlorine) (Hanschen, 2020); thus, the pH of cooking water tends to be neutral. Spinach addition leads to decreased brightness, redness, and yellowness (L*, a*, b* respectively) of resultant pasta. The cooking procedure decreases the lightness, greenness, and yellowness of spinach pasta. Interestingly, the yellowness decrease of cooked spinach pasta is much lower than control (from 29.58 to 13.74 of control vs 13.05 to 11.03 of spinach juice 1%, for example), indicating that spinach reduces the yellowness decrease during the cooking. This is consistent with Nisha, Singhal, and Pandit (2004), who found that thermal treatment caused a decrease in lightness and greenness but improved yellowness of spinach puree.

Figure 4.1 Uncooked spinach pomace 2% pasta (left) and red cabbage pomace 2% pasta (right)

Table 4.4 Colour characteristics of cooked and uncooked pasta enriched with spinach and red cabbage.

	Uncooked				Cooked			
	L	a	b	Colour example	L	a	b	Colour Example
				Red Cabbage Pasta (a)				
Control	65.38 ± 0.40^{a}	-0.36 ± 0.07^{g}	29.58 ± 0.18^{a}		61.68 ± 0.30^{a}	-0.66 ± 0.02^{e}	13.74 ± 0.02^{a}	
Red cabbage juice 1%	46.46 ± 0.58^{g}	8.85 ± 0.05^{a}	-7.17 ± 0.10^{g}		41.93 ± 0.74^{f}	3.12 ± 0.12^{c}	-8.61 ± 0.16^{e}	
Red cabbage puree 1%	54.26 ± 0.33^{c}	5.91 ± 0.02^{d}	-2.22 ± 0.02^{e}		47.54 ± 0.39^{c}	-1.26 ± 0.03^{f}	-1.38 ± 0.38^{b}	
Red cabbage puree 2%	50.07 ± 0.85^{e}	6.30 ± 0.02^{c}	-5.49 ± 0.02^{f}		44.55 ± 0.35^{e}	4.47 ± 0.18^{b}	-8.68 ± 0.28^{e}	
Red cabbage pomace 1%	58.02 ± 0.09^{b}	3.25 ± 0.01^{f}	3.59 ± 0.04^{b}		49.59 ± 0.38^{b}	-1.69 ± 0.07^{g}	-2.31 ± 0.40^{c}	
Red cabbage pomace 2%	53.23 ± 0.11^{d}	4.01 ± 0.01^{e}	2.05 ± 0.02^{c}		45.29 ± 0.32^{d}	2.05 ± 0.04^{d}	-5.31 ± 0.27^{d}	
Red cabbage pomace 10%	47.12 ± 0.06^{f}	6.82 ± 0.03^{b}	-1.59 ± 0.50^{d}		36.55 ± 0.12^{g}	5.75 ± 0.06^{a}	-5.04 ± 0.04^{d}	
				Spinach Pasta (b)				
Control	65.38 ± 0.40^{a}	-0.36 ± 0.07^{a}	29.58 ± 0.18^{a}		61.68 ± 0.30^{a}	-0.66 ± 0.02^{a}	13.74 ± 0.02^{a}	
Spinach juice 1%	43.04 ± 0.21^{f}	-9.75 ± 0.09^{g}	13.05 ± 0.18^{c}		40.16 ± 0.19^{e}	-7.71 ± 0.15^{g}	11.03 ± 0.06^{b}	
Spinach puree 1%	49.56 ± 0.11^{c}	-9.59 ± 0.02^{f}	14.79 ± 0.03^{b}		43.63 ± 0.04^{c}	-6.85 ± 0.07^{f}	10.39 ± 0.26^{c}	
Spinach puree 2%	45.66 ± 0.09^{e}	-7.30 ± 0.03^{e}	10.68 ± 0.03^{e}		40.06 ± 0.41^{e}	-6.25 ± 0.13^{e}	9.27 ± 0.16^{d}	
Spinach pomace 1%	51.02 ± 0.46^{b}	-5.12 ± 0.20^{b}	12.69 ± 0.11^{d}		46.49 ± 0.31^{b}	-4.79 ± 0.19^{d}	7.93 ± 0.38^{e}	
Spinach pomace 2%	48.54 ± 0.44^{d}	-7.07 ± 0.09^{d}	10.23 ± 0.29^{f}		41.43 ± 0.26^{d}	-4.54 ± 0.27^{c}	7.36 ± 0.23^{f}	
Spinach pomace 10%	38.74 ± 0.12^{g}	-5.78 ± 0.06^{c}	7.60 ± 0.17^{g}		29.83 ± 0.08^{f}	-3.45 ± 0.09^{b}	4.95 ± 0.04^{g}	

Values = mean ± standard deviation, n=3; L a b colour is converted to R G B colour through https://www.nixsensor.com/free-color-converter/ and colour was output through EXCEL. While the colour convertor can only input integer colour number, the generated example colour is approximate. Values within a column from the same kind of pasta followed by the same superscripted letter are not significantly different from each other ($p > 0.05$), according to the ANOVA- Duncan test.

4.4 Conclusion

The results show that the juice, puree, and pomace of spinach and red cabbage behave differently when incorporated into a pasta formulation. Those differences are plausible due to heterogeneous compositions in the varied forms of vegetables. At a low substitution level (1−2 g/100 g), juice, puree, and pomace can all be used to produce pasta with acceptable cooking performance and texture quality. Juice-fortified pasta has lower cooking losses and better elasticity compared to puree and pomace-fortified pasta. Among all pasta samples in this study, the cooking performance and texture quality of spinach juice pasta were better than other vegetable pasta and comparable or even better than the control. This is probably due to its higher protein (cysteine-rich) composition and low substitution level (less gluten dilution and structure interruption). This study indicates that vegetable juice with high protein content, such as spinach juice, may be used to produce premium pasta products by the food industry.

Chapter 5

Effect of beetroot and carrot juice, puree and pomace on chemical and technological quality of fresh pasta

5.1 Introduction

Currently, consumers tend not only to satisfy their hunger but also to need additional nutrient enhancement for their well-being (Yusuf, Wojdyło, Oszmiański, & Nowicka, 2021). Therefore, the food industry and researchers make efforts to develop enriched and functional food, especially cereal-based food, to meet consumers' requirements (Kewuyemi, Kesa, & Adebo, 2022). Pasta is a staple cereal food that could be a desirable vehicle to deliver functional ingredients (Rachman, A. Brennan, et al., 2020).

Adding vegetable ingredients to pasta recipes is one strategy to increase their functionality. However, lower sensory acceptability and altered cooking properties, and an overall decreased pasta quality caused by vegetable addition have been reported (Marinelli et al., 2018; Shyam et al., 2017; Sobota et al., 2020). Minimising vegetable addition or using food additives could be helpful methods to decrease the quality impact (Peressini et al., 2019). In addition, different forms of vegetables (juice, dry power, fresh puree) may impact pasta quality differently (Oliviero & Fogliano, 2016). However, how the different forms of vegetables impacted the quality is unclear due to previous inconsistent studies. Previous studies showed how different forms of leaf vegetables (spinach and red cabbage) affect enriched pasta's physio-chemical and quality aspects (Wang, Brennan, Brennan, & Serventi, 2021). This study focuses on two root vegetables – beetroot and carrot.

Beetroot (Beta *vulgaris* L.) belongs to the family Chenopodiaceae (Chhikara et al., 2019). The edible portion of beetroot is the root, which colour is from yellow to red, depending on the variety of the beets (Chhikara et al., 2019). Beetroot is rich in health-promote nutrients, especially water-soluble nutrients (Ingle, Thorat, Kotecha, & Nimbalkar, 2017). Those water-soluble nutrients include vitamins, phenolics, nitrate, ascorbic acids, and betalains (Kazimierczak et al., 2014). Also, betalains are commercial food dyes due to the non-toxic, non-precarious, non-poisonous nature (Chhikara et al., 2019). A few studies have added beetroot compounds to pasta recipes (Mridula, Gupta, Bhadwal, Khaira, et al., 2016; Pérez

& Pérez, 2009; SIPOS et al., 2017). However, those studies only focused on beetroot juice to add into pasta formulation. Also, there were no comparisons between juice, puree and powder. Moreover, none of these studies added beetroot based on dry weight, making their results unclear and hard to compare.

Carrot (*Daucus carota* L) is one of the most popular root vegetables grown throughout the world (Sharma et al., 2012). Carrots are rich in lipid-soluble carotenoids that have significant health-promotion properties (Roohinejad, Everett, & Oey, 2014). Some studies added carrots to the recipe to produce pasta (Adegunwa, Bakare, & Akinola, 2012; Gull et al., 2015; Jalgaonkar et al., 2018). However, most of those studies were limited to carrot powder or pomace. Some researchers combined carrots other materials like millet flour. In that case, the carrot-only effect on pasta properties is questionable.

This study aims to compare the key chemical composition, cooking performance, and texture quality of beetroot and carrot pasta using their different form (juice, puree and pomace). The additional level of juice and puree (no water added, see **Table 3.3c, Table 3.3d**) was maximised (all hydration water from juice, no more additional water). It was not the same substitution level of spinach and red cabbage addition. This may give a more comprehensive view of vegetable addition influence on the physio-chemical properties of pasta.

5.2 Material and methods

5.2.1 Raw materials

As described in section 3.1

5.2.2 Vegetable preparation

As described in sections 3.2.3 and 3.2.4

5.2.3 Pasta preparation

As described in section 3.3

5.2.4 Proximate analysis

Moisture, ash and protein were described in section 3.7. Starch content was described in section 3.9.2

5.2.5 Cooking performance

As described in section 3.4

5.2.6 Texture measurement

As described in section 3.5

5.2.7 Colour measurement

As described in section 3.6

5.2.8 Statistical analysis

As described in section 3.14

5.3 Results

5.3.1 Proximate composition of beetroot and carrot pasta and raw material

The protein content of uncooked beetroot puree and beetroot juice pasta increased ($p < 0.05$) compared to the control (**Table 5.1a,** e.g., Beetroot puree 4.08%, 12.74 ± 0.04 g/100 g vs Control, 12.49

± 0.01 g/100 g). After cooking, both beetroot puree pasta and juice pasta presented lower protein ($p < 0.05$) content than their uncooked counterparts. This may be because more water-soluble protein is present in juice and puree, and cooking makes them easier to leach into the cooking water, thus causing decreased protein content. However, for beetroot pomace pasta, after cooking, the protein contents remain the same (Beetroot pomace 10%) or even higher (Beetroot pomace 3.08%, beetroot pomace 4.08%) compared to uncooked one. The tendency is similar to the control (12.96 g/100 g cooked > 12.49 g/100 g uncooked, $p < 0.05$). Beetroot juice and beetroot puree (raw material) have more protein content than semolina. It should be noted that the protein content is converted by total N in our study, which may be attributed to nitrogenous betalains in beetroot raw material (Sahni & Shere, 2016). It means that the protein content of beetroot raw material and pasta may be inaccurate. One way to reduce nitrate interference is to remove the nitrate content before Dumas total N methods to minimise its interference throughultrafiltration (Shelly et al., 2021). As for carrot pasta (**Table 5.1b**), either the cooked or uncooked carrot pasta samples showed lower ($p < 0.05$) protein content compared to the control. This is due to lower protein content in carrot juice, puree and protein than semolina. All cooked carrot pasta contains higher protein content ($p < 0.05$) than uncooked one, possibly due to more other compounds leaching into cooking water than protein.

All vegetable pasta samples contain less starch content than the control (**Table 5.1ab**, $p < 0.05$). The carrot and beetroot, which have little starch content, substituted the semolina. All pasta samples showed the same starch content when uncooked pasta compared to cooked ones except for carrot pomace 10% (cooked less than uncooked). Both beetroot pasta and carrot pasta contain more ash contents than the control ($p < 0.05$, except for cooked beetroot puree 3%). It is due to beetroot and carrot raw materials containing higher (from 5.75 g/100 g to 11.95 g/100 g) ash content than semolina (0.95 g/100 g). All cooked pasta contains less ash content than uncooked one, which indicates a cooking leach of ash content. Interestingly, beetroot pasta tends to leach more ash content during cooking

compared to carrot pasta (Beetroot pomace 10% - from 1.33g/100 g to 0.61 g/100 g vs Carrot Pomace 10% - from 1.19 g/100 g to 0.75 g/100 g). This may be because beetroot contains more water-soluble minerals that are easier to solve to the cooking water than carrot.

5.3.2 Cooking performance of beetroot pasta and carrot pasta

Optimal cooking time, cooking loss, swelling index and water absorption index are shown in **Table 5.2**. All beetroot pasta samples were characterised by lower optimal cooking time compared to the control. As for carrot pasta, only carrot pomace 10% have a lower optimal cooking time (6 min) vs control (7 min). Other carrot pasta samples remain at the same optimal cooking time as the control. Both beetroot and carrot substitution caused a higher cooking loss versus control ($p < 0.05$). At the same substitution level, juice-enriched and puree-enriched pasta samples had a lower cooking loss than pomace-enriched ones ($p < 0.05$, beetroot pomace 3.08% > beetroot juice 3.08%, carrot pomace 2.27% > carrot juice 2.27%). The highest cooking loss was found on 10% pomace substitution for both beetroot pasta (7.37 g/100 g) and carrot pasta (7.78 g/100 g). Those cooking loss values were still under 8 g/100 g, a widely agreed level of consumer acceptance (Bustos et al., 2015). Beetroot pasta and carrot addition increased ($p < 0.05$) swelling index and water absorption index, except for beetroot juice 3.08%. Carrot pasta tends to have a higher swelling index and water absorption index compared to beetroot pasta. For example, water absorption index of carrot juice 2.27% is 90.34 g/100 g, higher than 83.09 g/100 g of beetroot juice 3.08%.

Table 5.1 Proximate chemical analysis of beetroot and carrot raw material and pasta.

	Protein g/100 g dry matter		Total Starch g/100 g dry matter		Moisture g/100 g Material		Ash g/100 g dry matter	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
(a) Beetroot pasta and beetroot raw material								
Raw material								
Semolina	12.58 ± 0.11	N/A	71.42 ± 0.51	N/A	10.95 ± 0.10	N/A	0.95 ± 0.04	N/A
Beetroot juice	22.53 ± 0.04	N/A	N/A	N/A	95.35 ± 0.04	N/A	11.95 ± 0.01	N/A
Beetroot puree	16.46 ± 0.05	N/A	N/A	N/A	91.41 ± 0.07	N/A	9.46 ± 0.00	N/A
Beetroot pomace	12.22 ± 0.10	N/A	N/A	N/A	12.95 ± 0.02	N/A	7.20 ± 0.01	N/A
Beetroot Pasta								
Control	12.49 ± 0.01^{cB}	12.96 ± 0.01^{aA}	70.35 ± 0.39^{aA}	70.24 ± 0.26^{aA}	36.66 ± 1.17^{b}	65.06 ± 0.78^{b}	0.68 ± 0.01^{f}	0.44 ± 0.01^{e}
Beetroot juice 3.08%	12.46 ± 0.01^{cA}	12.33 ± 0.03^{eB}	68.32 ± 0.42^{bA}	68.73 ± 0.37^{bA}	36.41 ± 0.44^{b}	65.27 ± 0.45^{b}	1.11 ± 0.00^{b}	0.47 ± 0.01^{bc}
Beetroot puree 3.08%	12.58 ± 0.01^{bA}	12.50 ± 0.03^{dB}	68.50 ± 0.29^{bA}	68.42 ± 0.37^{bA}	35.77 ± 0.01^{b}	65.54 ± 0.02^{b}	0.94 ± 0.01^{d}	0.44 ± 0.00^{e}
Beetroot puree 4.08%	12.74 ± 0.04^{aA}	12.64 ± 0.04^{cB}	66.73 ± 0.36^{cA}	66.79 ± 0.43^{cA}	36.95 ± 0.25^{b}	65.67 ± 0.02^{b}	1.03 ± 0.01^{c}	0.48 ± 0.00^{b}
Beetroot pomace 3.08%	12.28 ± 0.07^{dB}	12.56 ± 0.04^{cdA}	68.77 ± 0.44^{bA}	68.33 ± 0.25^{bA}	36.69 ± 0.52^{b}	65.81 ± 0.14^{b}	0.88 ± 0.01^{e}	0.47 ± 0.00^{c}
Beetroot pomace 4.08%	12.31 ± 0.01^{dB}	12.83 ± 0.06^{bA}	66.93 ± 0.28^{cA}	66.94 ± 0.22^{cA}	36.57 ± 0.85^{b}	65.88 ± 0.31^{b}	0.94 ± 0.00^{d}	0.45 ± 0.00^{d}
Beetroot pomace 10%	12.60 ± 0.03^{bA}	12.56 ± 0.01^{cdA}	62.01 ± 0.64^{dA}	61.11 ± 0.52^{dA}	38.95 ± 0.46^{a}	69.25 ± 0.33^{a}	1.33 ± 0.01^{a}	0.61 ± 0.01^{a}
(b) Carrot pasta and carrot raw material								
Raw material								
Carrot juice	7.77±0.01	N/A	N/A	N/A	95.85 ± 0.00	N/A	8.04 ± 0.01	N/A
Carrot puree	6.17±0.01	N/A	N/A	N/A	91.36 ± 0.02	N/A	7.63 ± 0.01	N/A
Carrot pomace	6.44±0.01	N/A	N/A	N/A	13.14 ± 0.07	N/A	5.75 ± 0.02	N/A
Carrot Pasta								
Control	12.49 ± 0.01^{aB}	12.96 ± 0.01^{aA}	70.35 ± 0.39^{aA}	70.24 ± 0.26^{aA}	36.66 ± 1.17^{b}	65.06 ± 0.78^{c}	0.68 ± 0.01^{f}	0.44 ± 0.01^{f}
Carrot juice 2.27%	12.35 ± 0.02^{bB}	12.64 ± 0.02^{cA}	68.70 ± 0.40^{bA}	68.64 ± 0.51^{bA}	37.70 ± 0.78^{bc}	67.27 ± 0.38^{b}	0.82 ± 0.01^{c}	0.50 ± 0.01^{b}
Carrot puree 2.27%	12.29 ± 0.01^{cB}	12.63 ± 0.03^{dA}	68.75 ± 0.30^{bA}	68.60 ± 0.43^{bA}	37.00 ± 0.09^{c}	66.81 ± 0.10^{b}	0.82 ± 0.00^{d}	0.46 ± 0.00^{d}
Carrot puree 3.36%	12.29 ± 0.03^{cB}	12.47 ± 0.01^{eA}	67.47 ± 0.42^{cA}	67.43 ± 0.44^{cA}	37.06 ± 0.34^{c}	67.12 ± 0.32^{b}	0.89 ± 0.00^{b}	0.45 ± 0.01^{ef}
Carrot pomace 2.27%	12.27 ± 0.03^{cB}	12.73 ± 0.01^{bA}	68.63 ± 0.37^{bA}	68.26 ± 0.41^{bA}	39.70 ± 0.48^{a}	68.93 ± 0.39^{a}	0.78 ± 0.00^{e}	0.45 ± 0.00^{e}
Carrot pomace 3.36%	12.09 ± 0.01^{dB}	12.57 ± 0.03^{dA}	67.51 ± 0.37^{cA}	67.37 ± 0.43^{cA}	38.95 ± 0.52^{ab}	68.90 ± 0.19^{a}	0.78 ± 0.01^{e}	0.49 ± 0.01^{c}
Carrot pomace 10%	11.72 ± 0.03^{eB}	12.24 ± 0.01^{fA}	61.86 ± 0.28^{dA}	60.57 ± 0.43^{dB}	38.07 ± 0.29^{abc}	69.22 ± 0.21^{a}	1.19 ± 0.01^{a}	0.75 ± 0.01^{a}

Values = mean ± standard deviation, n=3; Protein starch and ash results are based on a dry weight basis. Moisture results are based on wet weight basis. N/A = not tested. Values within a column in the same sub-table followed by the same superscripted letters are not significantly different from each other ($p > 0.05$), values followed by the same superscripted capital letter are not significantly different between cooked and uncooked samples according to the ANOVA-Duncan test.

Table 5.2 Cooking performance of beetroot and carrot raw material and pasta.

	Optimal Cooking Time (min)	Cooking Loss (g/100 g)	Swelling Index (g Water/g Dry Pasta)	Water Absorption Index (g/100 g)
		Beetroot Pasta		
Control	7.0	4.40±0.06^{e}	1.86±0.07^{c}	81.27±1.42^{e}
Beetroot juice 3.08%	6.5	4.73±0.06^{d}	1.88±0.04^{bc}	83.09±1.72^{de}
Beetroot puree 3.08%	6.5	4.66±0.04^{de}	1.90±0.00^{bc}	86.36±0.12^{b}
Beetroot puree 4.08%	6.5	5.11±0.16^{c}	1.91±0.00^{bc}	83.64±0.66^{cd}
Beetroot pomace 3.08%	6.5	5.83±0.01^{b}	1.93±0.01^{bc}	85.17±1.73^{bcd}
Beetroot pomace 4.08%	6.5	6.03±0.03^{b}	1.93±0.03^{b}	85.90±1.42^{bc}
Beetroot pomace 10%	6.0	7.37±0.28^{a}	2.08±0.04^{a}	98.52±0.71^{a}
		Carrot Pasta		
Control	7.0	4.40±0.06^{e}	1.86±0.07^{c}	81.27±1.42^{e}
Carrot juice 2.27%	7.0	4.78±0.12^{d}	2.06±0.04^{b}	90.34±1.01^{d}
Carrot puree 2.27%	7.0	4.86±0.03^{d}	2.01±0.01^{b}	89.82±0.72^{d}
Carrot puree 3.36%	7.0	5.35±0.15^{c}	2.04±0.03^{b}	91.41±1.09^{cd}
Carrot pomace 2.27%	7.0	5.91±0.18^{b}	2.22±0.04^{a}	94.09±4.01^{bc}
Carrot pomace 3.36%	7.0	5.96±0.14^{b}	2.22±0.02^{a}	96.31±0.56^{b}
Carrot pomace 10%	6.0	7.78±0.13^{a}	2.25±0.02^{a}	101.22±0.63^{a}

Values = mean ± standard deviation, n=3; Values within a column of the same kind of pasta followed by the same superscripted letter are not significantly different from each other ($p > 0.05$) according to the ANOVA-Duncan test.

5.3.3 Texture of beetroot pasta and carrot pasta

Table 5.3 shows that the breaking force of beetroot and carrot pasta tends to be lower with a higher substitution level. Beetroot pomace 4.08%, beetroot pomace 10%, carrot puree 3.36%, carrot pomace 3.36% and carrot pomace 10% showed lower ($p < 0.05$) breaking force compared to control. Beetroot juice 3.08%, beetroot puree 3.08% and carrot juice 2.27% showed the same breaking distance versus control, while all other samples showed a lower breaking distance. At the same substitution level, beetroot pomace showed lower ($p < 0.05$) breaking distance compared to puree and juice form (beetroot pomace 3.08% < beetroot puree 3.08%, beetroot pomace 3.08% < beetroot juice 3.08%, beetroot pomace 4.08%, < beetroot puree 4.08%). As for carrot pasta, carrot puree pasta showed a similar breaking distance compared to carrot pomace pasta at the same substitution level.

All beetroot pasta samples and carrot pasta samples perform lower firmness compared to the control (except for beetroot puree 3.08% cooked at optimal cooking time). At the same substitution level, the firmness of beetroot pasta was ranked as puree > juice > pomace ($p < 0.05$). However, for carrot pasta, the firmness were at the same level for different forms (e.g. carrot juice 2.27% = carrot pomace 2.27% = carrot puree 2.27%). Only beetroot juice 3.08% present lower firmness when cooked at 7 min compared to cooked at optimal cooking time. While for other samples, no significant difference in firmness was found between the optimal cooking time and 7 min (control's optimal cooking time).

Table 5.3 Elasticity and firmness of beetroot and carrot pasta

Pasta type	Tensile test				Firmness	
	Breaking force g		Breaking distance			
	Optimal cooking time	7 min	Optimal cooking time	7 min	Optimal cooking time	7 min
(a) Beetroot pasta						
Control*	33.55±1.19^{a}		74.28±3.71^{a}		399.75±11.51^{a}	
Beetroot juice 3.08%	31.90±1.61^{abA}	33.40±3.14^{aA}	71.99±5.51^{aA}	69.30±4.02^{aA}	363.52±22.37^{bA}	334.60±7.25^{cB}
Beetroot puree 3.08%	32.16±2.12^{aA}	34.66±4.18^{aA}	67.95±7.73^{aA}	69.89±4.64^{aA}	391.44±2.97^{aA}	382.64±16.12^{bA}
Beetroot puree 4.08%	29.65±3.54^{abcA}	30.47±3.35^{abA}	59.51±7.03^{bA}	59.22±4.82^{bA}	349.18±10.55^{bA}	340.69±7.85^{cA}
Beetroot pomace 3.08%	32.73±3.77^{aA}	33.02±5.56^{aA}	52.91±4.27^{bcA}	52.89±3.79^{cA}	304.82±12.38^{cA}	297.01±6.81^{dA}
Beetroot pomace 4.08%	26.28±2.66^{bcA}	26.07±1.51^{bcA}	51.00±5.36^{cA}	50.70±2.68^{cA}	294.52±12.54^{cA}	290.47±9.39^{dA}
Beetroot pomace 10%	25.70±2.99^{cA}	25.56±3.85^{cA}	35.08±2.90^{dA}	33.94±2.65^{dA}	289.97±4.73^{cA}	287.33±3.88^{dA}
(b) Carrot pasta						
Control*	33.55±1.19^{a}		74.28±3.71^{a}		399.75±11.51^{a}	
Carrot juice 2.27%*	31.85±2.07^{ab}		72.72±4.07^{a}		343.14±12.39^{b}	
Carrot puree 2.27%*	31.92±1.98^{ab}		61.04±4.48^{b}		350.10±10.13^{b}	
Carrot puree 3.36%*	28.29±3.05^{b}		59.07±3.86^{b}		348.00±13.09^{b}	
Carrot pomace 2.27%*	31.25±2.80^{ab}		57.20±2.76^{b}		344.26±23.29^{b}	
Carrot pomace 3.36%*	30.81±2.12^{ab}		59.19±3.81^{b}		337.60±24.07^{b}	
CPO10	20.54±3.93^{cA}	22.08±1.88^{cA}	41.10±6.80^{cA}	41.64±5.05^{cA}	257.37±25.57^{cA}	243.30±8.90^{cA}

Values = mean ± standard deviation, n=9; The same lower case superscripted letter mean values are not significantly different from each other for the same raw of the same vegetable pasta ($p > 0.05$). The same upper case superscripted letter mean values are not significantly different from each other ($p > 0.05$) between OCT and 7 min.

5.3.4 Colour of beetroot pasta and carrot pasta

The colour results are shown in **Table 5.4**. Beetroot material substitution results in an increased redness (a value) but reduced brightness and yellowness (L, b value). After cooking, the beetroot pasta lost some of its redness but gained some degree of brightness and yellowness. At the same substitution level, beetroot juice caused more redness than puree and pomace (beetroot juice 3.08% > beetroot puree 3.08% > beetroot pomace 3.08%, in a value). However, after cooking, the beetroot pomace seems to lose less redness compared to juice and puree. As a result, beetroot pomace 10% has the same redness compared to beetroot juice 3.08% after cooking. Although before cooking, the red value of beetroot pomace 10% is less than beetroot juice 3.08%. Carrot pasta shows a different trend as beetroot pasta, carrot substitution caused less brightness but increased redness and yellowness. However, the brightness and reduction are not as dramatic as the beetroot ones. After cooking, the carrot pasta presents lower brightness, redness, and yellowness compared to the uncooked one.

Table 5.4 Colour of beetroot and carrot pasta

	Uncooked				Cooked			
	L	a	b	Colour example	L	a	b	Colour Example
				Beetroot pasta				
Control	65.38 ± 0.40^{a}	-0.36 ± 0.07^{f}	29.58 ± 0.18^{a}		61.68 ± 0.30^{a}	-0.66 ± 0.02^{e}	13.74 ± 0.02^{a}	
Beetroot juice 3.08%	31.58 ± 0.33^{c}	20.56 ± 0.44^{a}	5.31 ± 0.13^{c}		36.86 ± 0.43^{c}	13.55 ± 0.25^{a}	6.75 ± 0.06^{bc}	
Beetroot puree 3.08%	32.32 ± 0.33^{b}	19.64 ± 0.30^{b}	3.57 ± 0.22^{d}		37.39 ± 0.39^{b}	7.88 ± 0.28^{d}	5.44 ± 0.28^{d}	
Beetroot puree 4.08%	32.27 ± 0.54^{b}	17.37 ± 0.18^{c}	5.27 ± 0.22^{c}		34.81 ± 0.21^{d}	10.83 ± 0.66^{c}	6.90 ± 0.14^{b}	
Beetroot pomace 3.08%	32.78 ± 0.46^{b}	16.66 ± 0.61^{d}	6.00 ± 0.21^{b}		33.58 ± 0.16^{f}	12.72 ± 0.36^{b}	6.51 ± 0.20^{c}	
Beetroot pomace 4.08%	28.94 ± 0.50^{d}	15.55 ± 0.20^{e}	2.26 ± 0.19^{e}		34.16 ± 0.41^{e}	10.43 ± 0.29^{c}	4.36 ± 0.42^{e}	
Beetroot pomace 10%	25.23 ± 0.42^{e}	16.51 ± 0.26^{d}	0.85 ± 0.07^{f}		28.35 ± 0.20^{g}	13.44 ± 0.38^{a}	2.13 ± 0.12^{f}	
				Carrot Pasta				
Control	65.38 ± 0.40^{a}	-0.36 ± 0.07^{g}	29.58 ± 0.18^{f}		61.68 ± 0.30^{a}	-0.66 ± 0.02^{f}	13.74 ± 0.02^{e}	
Carrot juice 2.27%	54.34 ± 0.65^{e}	18.68 ± 0.23^{a}	38.22 ± 0.29^{a}		46.01 ± 0.49^{e}	5.62 ± 0.33^{b}	32.76 ± 0.36^{b}	
Carrot puree 2.27%	56.90 ± 0.17^{d}	9.18 ± 0.23^{e}	34.25 ± 0.12^{d}		50.30 ± 0.23^{c}	2.37 ± 0.24^{d}	30.75 ± 0.21^{c}	
Carrot puree 3.36%	57.48 ± 0.54^{c}	10.65 ± 0.21^{c}	35.64 ± 0.25^{c}		49.85 ± 0.65^{c}	4.86 ± 0.08^{c}	32.78 ± 0.24^{b}	
Carrot pomace 2.27%	59.11 ± 0.27^{b}	5.87 ± 0.15^{f}	33.68 ± 0.17^{e}		52.76 ± 0.67^{b}	0.37 ± 0.39^{e}	28.79 ± 0.61^{d}	
Carrot pomace 3.36%	57.63 ± 0.15^{c}	9.78 ± 0.44^{d}	37.54 ± 0.25^{b}		48.76 ± 0.25^{d}	4.73 ± 0.16^{c}	30.55 ± 0.29^{c}	
Carrot pomace 10%	56.63 ± 0.37^{d}	17.20 ± 0.53^{b}	39.52 ± 0.16^{a}		46.40 ± 0.21^{e}	7.34 ± 0.33^{a}	35.21 ± 0.62^{a}	

Values = mean ± standard deviation, n=10. L a b colour is converted to R G B colour through https://www.nixsensor.com/free-color-converter/ and colour was output through EXCEL. While the colour convertor can only input integer colour number, the generated example colour is proximate. Values within a column from the same kind of pasta followed by the same superscripted letter are not significantly different from each other ($p > 0.05$), according to the ANOVA- Duncan test.

5.4 Discussion

The quality parameters that affect consumers' acceptability include water absorption (swelling index & water absorption index), texture (firmness, tensile properties) and cooking loss (González et al., 2021). Good quality is characterised by low cooking loss, high firmness, breaking distance, and breaking force (Peressini et al., 2020). It is generally based on a well-formed gluten network during pasta extrusion (Simonato et al., 2019). The previous study (**Chapter 4**) reported spinach and red cabbage addition impacted those quality parameters. Data on beetroot and carrot pasta here may give a more comprehensive overview of how vegetable ingredients affect pasta quality.

Significant correlations were found between substitution level and quality parameters (**Table 5.5**). The substitution level is positively correlated with cooking loss and water absorption index (correlation factor = 0.926, $p < 0.01$; correlation factor = 0.727, $p < 0.01$, respectively). It is also negatively correlated with breaking force, breaking distance, and firmness (correlation factor = -0.878, $p < 0.01$; correlation factor = -0.860, $p < 0.01$; correlation factor = -0.795, $p < 0.01$, respectively). The more beetroot or carrot substitutes the semolina, the higher cooking loss, water absorption index but lower breaking force, breaking distance and firmness. It may indicate the more substitution, the lower quality. Similar trends were found by SIPOS et al. (2017) and Mridula, Gupta, Bhadwal, Khaira, et al. (2016), who found that beetroot juice addition reduces the overall quality of pasta products, although the quality reduction is acceptable. Gull et al. (2015) found that carrot pomace addition increased cooking loss and decreased firmness with an improved addition level. Jalgaonkar et al. (2018) and Sobota et al. (2020) reported that the carrot powder addition amount is positively correlated with cooking loss but negatively correlated with hardness, which is similar to the results of this study. At the same substitution level, the pomace-enriched pasta generally presents lower quality than puree-enriched and juice-enriched ones. For example, beetroot pomace 3.08% have a significantly higher cooking loss, lower breaking distance and firmness compared to beetroot puree 3.08% and beetroot pomace 3.08%. Carrot pomace 2.27% is characteristic of higher cooking loss, swelling index and water absorption index but lower breaking distance versus carrot juice

2.27%. It indicated that vegetable pomace material may not be good enough to produce high quality vegetable-enriched pasta compared to puree or juice form. It may be suggested that use juice or puree at a low substitution level to produce high quality vegetable enriched pasta.

Protein content is one crucial factor that influences pasta quality (Sissons, Cutillo, Marcotuli, & Gadaleta, 2021). A weak positive correlation was found between protein and firmness (**Table 5.5**, correlation factor = 0.558 at $p < 0.05$). Indicate that more protein results in firmer pasta which the consumer prefers. Similar results were reported by Sissons et al. (2021), who used different genotypes of durum wheat semolina to compare their quality. The authors found a strong positive correlation between protein content and firmness for pure durum-wheat dried pasta. As Dumas total N methods were used to evaluate the protein content in this study. It is highly possible that protein content was over-evaluated in beetroot material and beetroot pasta (Ingle et al., 2017). It may be an explanation that protein content correlation with quality factors is not that significant if the correlation test excludes the questionable beetroot pasta (**Table 5.6**). The protein content significantly correlated with all quality factors measured in this study. Strong positive correlations were found between protein contents versus firmness, breaking force, and breaking distance (correlation factor = 0.915, 0.893 and 0.832 respectively). And the protein content is negatively correlated with cooking loss, swelling index and water absorption index (correlation factor = -0.897, -0.902, and -0.942, respectively). This indicates that higher protein content results in higher breaking force and breaking distance but lower cooking loss. Pasta with those characteristics was considered a better quality product (Peressini et al., 2019). Compared to spinach pasta in the previous study (**Chapter 4**), neither carrots nor beetroot contains cysteine pigment (Preczenhak et al., 2019). It might be why both beetroot and carrot substitution cannot positively impact pasta quality on quality parameters (e.g. firmness or breaking force) as spinach does. It is plausible that the presence of some protein group in vegetable, like -the SH group in cysteine from spinach, can wane the quality reduction caused by vegetable substitution.

Beetroot could be used as a natural colouring agent to provide red colour (Chhikara et al., 2019). The colour of beetroot pasta is similar to the study by Sobota et al. (2020), who added beetroot powder and beetroot concentrate (from 2% to 8%) to pasta. The ideal colouring effect of beetroot pasta comes from beetroot juice. As the redness value of beetroot juice 3.08% is higher than beetroot pomace 10% for uncooked pasta samples. After cooking, the beetroot juice 3.08% still has a comparable redness value versus beetroot pomace 10% (13.55 of beetroot juice 3.08% vs 13.44 of beetroot pomace 10%). Carrot material addition results in lower lightness, higher redness and yellowness. Similar results were reported by Sobota et al. (2020). The authors found that colour change was highly correlated with carotenoid content. Carrot juice 2.27% shows a better colourant effect compared with carrot puree 2.27% and carrot pomace 2.27%. Both beetroot pasta and carrot pasta results showed that vegetable juice is more effective as a colourant compared with puree or pomace form, although after cooking, the colour loss of juice is more obvious. Consumers typically purchase uncooked pasta, and their appearance has an effect on their purchasing decision (Fu et al., 2013).

5.5 Conclusion

The study showed that substitution level is the essential factor that influences the quality of vegetable pasta. As the maximise substitution level of vegetable puree and juice is low (here 3.08% for beetroot juice, 4.08% for beetroot puree, 2.27% for carrot juice, 3.36% for carrot puree). The results are encouraging as all puree and juice-enriched samples shown in the study perform better quality when compared with pomace-enriched pasta. Furthermore, in accordance with the previous study (Chapter 4), the juice is beneficial regarding colourant effect as it can achieve a similar or higher colourant effect with a much lower substitution level than pomace. This study indicated that juice and puree could produce vegetable pasta with their maximum substitution level to achieve acceptable quality.

Chapter 6

Effect of vegetable juice, puree and pomace on glycaemic index of fresh pasta

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6.1 Introduction

Consumers exhibit a propensity to prioritise their health and well-being by consuming food items perceived as being healthier (Jideani et al., 2021). Therefore, there is great interest in producing health-promoting foods fortified with plant-bioactive ingredients (Simonato et al., 2019). Pasta is a staple cereal food worldwide that is widely accepted, and thus could be a good food system to incorporate with healthy ingredients (Rachman, A. Brennan, et al., 2020). The glycemic index (GI) of pasta is lower than other staple foods such as bread and rice (Zou et al., 2015). Papoutsis et al. (2021) found that some phytochemicals, such as polyphenols, saponins, and proteins from vegetables, could deliver α-amylase and α-glucosidase inhibition effects. Phenolic compounds from vegetables have been shown to affect starch degradation and reduce the potential glycaemic impact of starchy foods (Mu et al., 2021). A high level of dietary fibre has also been reported to attenuate starch hydrolysis and glycaemic response (Dhingra, Michael, Rajput, & Patil, 2012). Hence, adding vegetable ingredients to pasta may be one option to produce low-GI pasta.

Several researchers have focused on the glycaemic response of vegetable pasta. Lu et al. (2018) substituted 5%-15% semolina with mushroom powder (white button mushroom, shiitake mushroom, porcini mushroom). The authors found that the extent of starch degradation decreased significantly compared to the control using *in vitro* starch digestion. Chusak et al. (2020) substituted semolina with 5-15% ripe gac fruit (*Momordica cochinchinensis*) powder and 10%-15% unripe gac fruit powder. Again, a remarkably reduced starch digestibility was reported. The authors suggested the reduction is due to phenolic compounds and carotenoids. Using these by-products in foods has consumer acceptability issues, but consumers generally perceive them to be advantageous from a nutritional basis (Reißner et al., 2021). However, limited research has been carried out to compare the nutritional value of pasta, which is developed by adding different forms of vegetables to pasta formulas.

Therefore, pasta enriched with other forms of vegetables, such as puree, juice, and pomace, was investigated in this study. The aim was to determine how different forms of vegetables influence the

glycaemic response and what factors may cause such influence. Two types of leaf vegetables (spinach and red cabbage) and two types of root vegetables (beetroot and carrot) were used. Spinach was reported to be an α-amylase inhibitor, and its inhibition effect can compete with acarbose, a known antidiabetic agent (Vyas, 2017). Red cabbage and beetroot are rich in water-soluble phytochemicals. Those phytochemicals were believed to present α-glucosidase activity and α-amylase inhibition effect (Papoutsis et al., 2021). Carrot is rich in carotenoids, a lipid-rich nutrient. Chau, Chen, and Lee (2004) reported that insoluble fibre extraction from carrot exhibited glucose absorption and α-amylase inhibition effect. Different forms of spinach and red cabbage were added to pasta at the same substitution level in this study. In contrast, for beetroot and carrot pasta samples, their juice and puree forms of pasta were substituted at a maximum level.

6.2 Material and methods

6.2.1 Raw materials

As described in section 3.1

6.2.2 Vegetable preparation

As described in section 3.2

6.2.3 Pasta preparation

As described in section 3.3

6.2.4 Dietary fibre and starch

As described in section 3.9

6.2.5 *In vitro* starch digestion

As described in section 3.10

6.2.6 α-amylase inhibition

As described in 3.11

6.3 Results and discussion

6.3.1 General effects of vegetable addition effects on the mimic glycaemic index

The amount of reducing sugars released over 120 min digestion is shown in **Figure 6.1abcd**. Only control, juice pasta and 10% pomace pasta results were shown in **Figure 6.1abcd.** It was because the unclear (lots of points stacked together) figure would appear if all the results were included. Reducing sugars reached a peak value at either 20 min or 60 min for all pasta samples. This observation was similar to the previous study conducted by Lu et al. (2018). At 120 min, all vegetable pasta with 10% pomace substitution showed a lower released reducing sugar compared to the control. However, as for juice-substituted vegetable pasta, only spinach juice 1% and beetroot juice 3.08% showed a significantly lower reducing sugar at 120 min versus control. **Figure 6.2** shows the standardised incremental area under the curve (AUC) of vegetable pasta compared to the control. All vegetable pasta samples with 10% pomace substitution (including spinach pomace 10%, red cabbage pomace 10%, beetroot pomace 10%, and carrot pomace 10%) showed a significantly lower ($p < 0.05$) AUC compared to the control. There are some researchers who substituted the semolina with other materials and observed decreased AUC (Jayawardena et al., 2019; Simonato et al., 2019). Except for those 10% pomace samples, only spinach juice 1%, spinach puree 1%, and beetroot puree 4.08% showed a significantly lower ($p < 0.05$) AUC compared to the control. This may indicate that selecting proper vegetables and their form is crucial to develop low-GI vegetable pasta products.

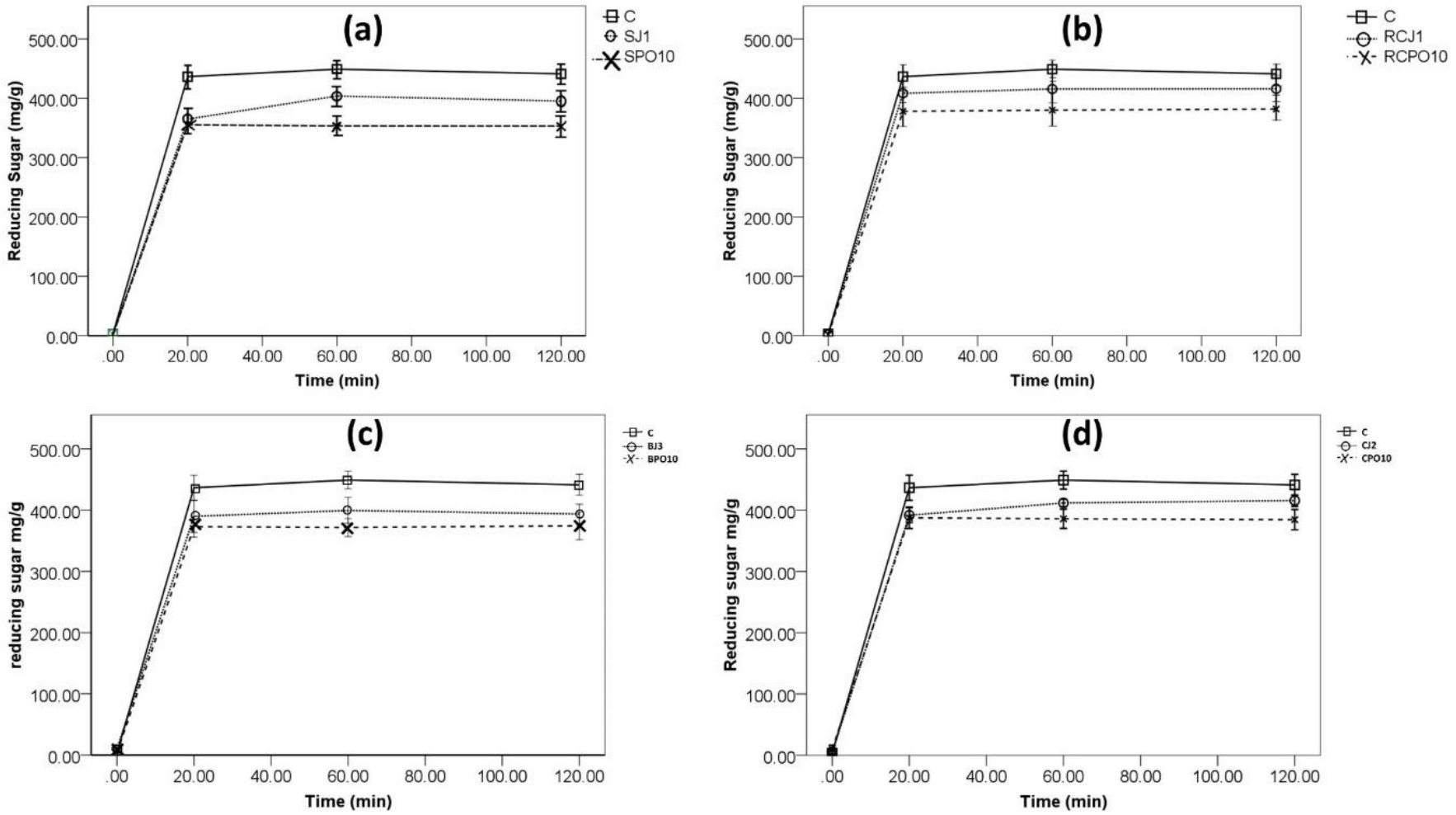

Figure 6.1 reducing sugar release vs time (a) reducing sugar release vs. time of control (C), spinach juice 1% pasta (SJ1), and spinach pomace 10% pasta (SPO10); **(b)** reducing sugar release vs. time of control (C), red cabbage juice 1% pasta (RCJ1), and red cabbage pomace 10% pasta (RCPO10); **(c)** reducing sugar release vs. time of control (C), beetroot juice 3.08% pasta (BJ3), beetroot pomace 10% pasta (BPO10); **(d)** reducing sugar release vs. time of control (C), carrot juice 2.27% pasta (CJ2), carrot pomace 10% pasta (CPO10)

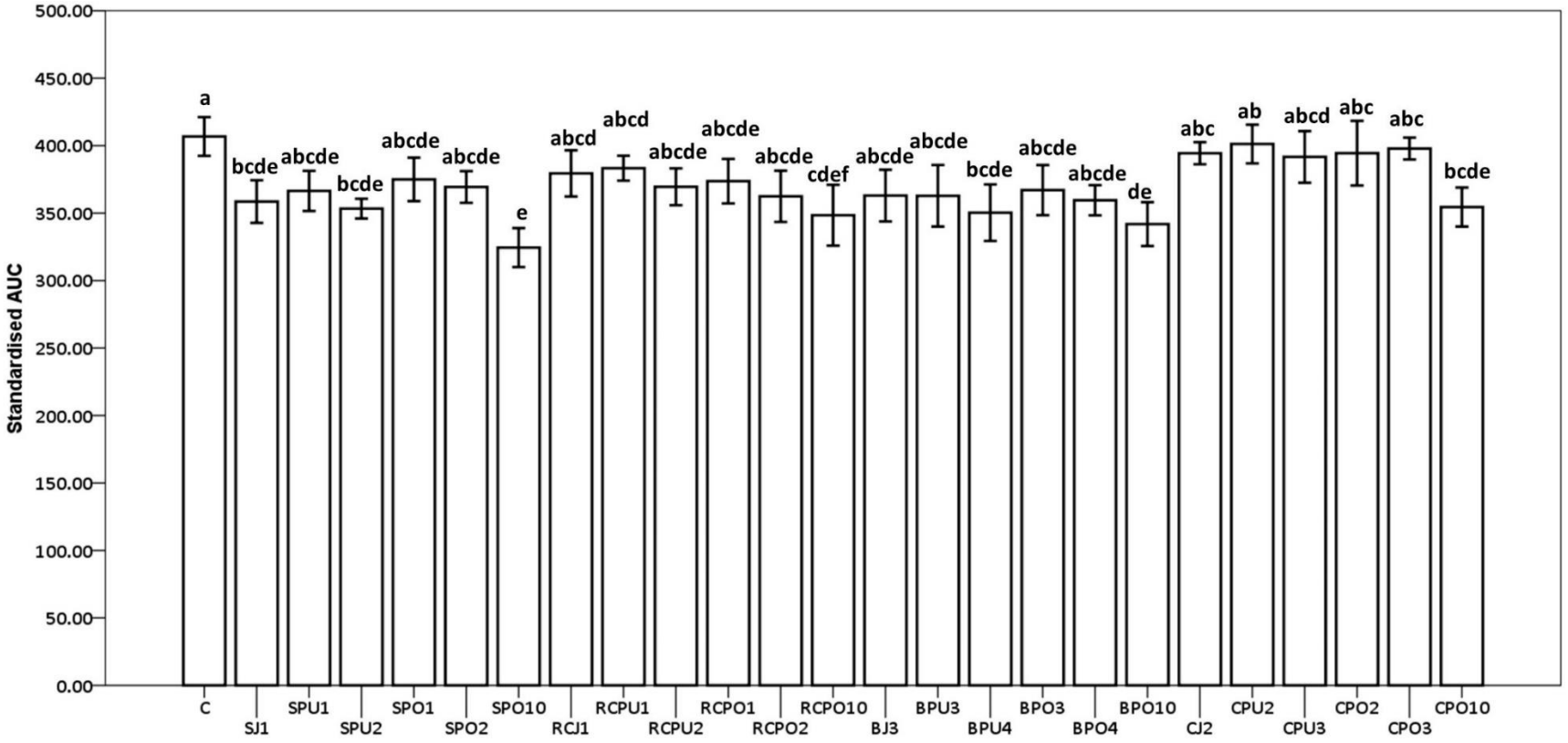

Figure 6.2. **Value for the area under the curve (AUC) of pasta samples** C, control; SJ1, spinach juice 1%; SPU1, spinach puree 1%; SPU2, spinach puree 2%, SPO1, spinach pomace 1%; SPO2, spinach pomace 2%; SPO10, spinach pomace 10%; RCJ1, red cabbage juice 1%; RCPU1, red cabbage puree 1%; RCPU2, red cabbage puree 2%; RCPO1, red cabbage pomace 1%; RCPO2, red cabbage pomace 2%; RCPO10, red cabbage pomace 10%; BJ3, beetroot juice 3.08%; BPU3, beetroot puree 3.08%, BPU4, beetroot puree 4.08%; BPO3, beetroot pomace 3.08%; BPO4, beetroot pomace 4.08%; BPO10, beetroot pomace 10%; CJ2, carrot juice 2.27%; CPU2 carrot puree 2.27%; CPU3, carrot puree 3.36%; CPO2, carrot pomace 2.27%; CPO3, carrot pomace 3.36%; CPO10, carrot pomace 10%; C: control sample. Values = mean ± standard deviation, n=3. Bars followed by the same letter are not significantly different from each other ($p > 0.05$) according to the ANOVA-Turkey test.

6.3.2 *In Vitro* Starch Digestion Versus α-amylase inhibition effect

Spinach juice 1% and spinach puree 2% pasta had lower GI than the control, showing a decreased AUC (**Figure 6.2**), possibly due to α-amylase inhibition of spinach, as shown in **Table 6.1**. The extracts from spinach show a higher α-amylase inhibition ability than other vegetable materials. The 50% inhibition concentration (IC_{50}) was 0.047, 0.053, 0.240 mg/mL, for spinach juice, spinach puree, and spinach pomace, respectively. Therefore, the pasta samples such as spinach juice 1% and spinach pomace 10% pasta also presented measurable α-amylase inhibition effect. Also, spinach juice showed a higher inhibition effect than puree and pomace (lower IC_{50} value). It may explain that at the same 1% substitution level, Spinach juice 1% performed a lower AUC while spinach puree 1% and, spinach pomace 1% did not. Barkat, Singh, Jayaprakasha, and Patil (2018) have reported a high α-amylase inhibition ability (comparable to acarbose, a known α-amylase inhibitor) of spinach materials. The authors showed that the α-amylase inhibition effect of spinach was influenced by harvest day (weakest at 20 days, strongest at 60 days).

Red cabbage, beetroot and carrot also showed some α-amylase inhibition ability, but their inhibition effect was much lower than spinach. As the results show (**Table 6.1**), red cabbage pasta samples (red cabbage juice 1% and red cabbage pomace 10%) showed unmeasurable α-amylase inhibition ability. It may be valuable to mention that raw red cabbage pasta did present α-amylase inhibition ability (red cabbage juice 1% IC_{50} = 59.32 mg/mL), but after cooking, this activity decreased to unmeasurable (IC_{50} > 100 mg/mL), as shown in **Table 6.1**. It may be because water-soluble phytochemicals leach into the cooking water, and their activity decreases. Thermal-induced degradation of phytochemicals may be another reason for the reduction of α-amylase inhibition activity. McDougall et al. (2005) found that extracts of phenolic compounds from red cabbage express no α-amylase inhibition at a low dose (100 μg), and the inhibition effects rank the weakest at a higher dose (500 μg) compared with strawberry, blackcurrant, blueberry, raspberry, and green tea.

Table 6.1 α-amylase Inhibition of Raw Materials and Spinach Pasta Sample

Sample	inhibition IC_{50} value	
	Raw Material	
Spinach juice	0.047 mg/mL	
Spinach puree	0.054 mg/mL	
Spinach pomace	0.240 mg/mL	
Red cabbage juice	4.59 mg/mL	
Red cabbage puree	3.02 mg/mL	
Red cabbage pomace	12.54 mg/mL	
Beetroot juice	2.96 mg/mL	
Beetroot puree	4.71 mg/mL	
Beetroot pomace	27.18 mg/mL	
Carrot juice	>100 mg/mL	
Carrot Puree	>100 mg/mL	
Carrot Pomace	>100 mg/mL	
	Pasta Sample	
Pasta Sample	Cooked	Uncooked
Spinach Juice 1%	6.57 mg/mL	3.59 mg/mL
Spinach Pomace 10%	3.12 mg/mL	2.98 mg/mL
Red cabbage juice 1%	>100 mg/mL	59.32 mg/mL
Red cabbage pomace 1%	>100 mg/mL	45.67 mg/mL
Beetroot juice 3.08%	>100 mg/mL	10.97 mg/mL
Beetroot pomace 10%	>100 mg/mL	>100 mg/mL
Carrot juice 2.27%	>100 mg/mL	>100 mg/mL
Carrot pomace 10%	>100 mg/mL	>100 mg/mL

The raw beetroot juice showed a higher α-amylase inhibition effect than red cabbage (IC_{50} = 2.96 mg/ml beetroot juice vs IC_{50} = 4.59 mg/ml of red cabbage juice). Beetroot puree has a lower α-amylase inhibition effect than beetroot juice (IC_{50} = 4.71 mg/ml for beetroot puree vs IC_{50} = 2.96 mg/ml for beetroot juice), but it is close to red cabbage juice. It may be one reason that beetroot puree 4.08% showed a lower AUC than the control, even though no measurable α-amylase inhibition effect was found in cooked beetroot pasta. Oboh, Obayiuwana, Aihie, Iyayi, and Udoh (2020) have reported that beetroot juice could be used to manage hyperglycemia and diabetes as beetroot juice expressed α-amylase inhibition effect with IC_{50} at 1.78 mg/ml. Like red cabbage, the cooked beetroot showed a dramatically lower inhibition effect (higher IC_{50}) compared to the uncooked one. As a result, no significantly lower AUC was found for beetroot juice 3.08% pasta. Indicating that for the vegetable, where α-amylase inhibition is derived from the water-soluble phytochemicals, it may not effectively lower glycaemic response as expected.

Carrot material ranked the lowest α-amylase inhibition effect among all tested vegetables (IC_{50} > 100 mg/ml). Only carrot pomace 10% pasta showed a lower AUC compared to the control. Yusuf et al. (2021) found that carrot varieties exhibit dramatically different α-amylase inhibition abilities. The authors reported IC_{50} value of α-amylase inhibition of different carrot varieties ranged from 107 mg/mL to 808 mg/mL. The authors also found that the weakest inhibition effect comes from the most popular type that is widely available in the market. It is suggested that the α-amylase inhibition activity is derived from the bioactive compounds of plants. Those compounds, which included glycosides, polysaccharides, steroids and terpenoids, could inhibit α-amylase through competitive, uncompetitive, and non-competitive ways (Mentreddy, 2007). Furthermore, the α-amylase inhibition effect of vegetable extracts may come from phenolic compounds. Barrett, Farhadi, and Smith (2018) have shown that some phenols can interact with enzymes such as α-amylase, which results in blocking their catalytic sites, thus reducing their activity. Takahama and Hirota (2018) showed that flavonoids in foods could interact with starch to form a starch-flavonoid complex through covalent bonds and hydrophobic interactions. Those complexes help to suppress amylose-iodine formation

and further suppress amylose digestion through porcine pancreas α-amylase catalyzed starch digestion and digestive enzyme activity measurement.

Table 6.2 Starch content of vegetable pasta

Leaf Vegetable			
(a)		**(b)**	
Pasta Type	Starch content g/100 g dw	Pasta Type	Starch content g/100 g dw
Control	70.24 ± 0.26[a]	Control	70.24 ± 0.26[a]
Spinach juice 1%	69.46 ± 0.50[b]	Red cabbage juice 1%	68.40 ± 0.51[b]
Spinach puree 1%	69.48 ± 0.46[b]	Red cabbage puree 1%	68.46 ± 0.42[b]
Spinach puree 2%	68.41 ± 0.45[c]	Red cabbage puree 2%	67.32 ± 0.57[c]
Spinach pomace 1%	69.29 ± 0.37[b]	Red cabbage pomace 1%	68.41 ± 0.42[b]
Spinach pomace 2%	68.57 ± 0.52[c]	Red cabbage pomace 2%	67.30 ± 0.40[c]
Spinach pomace 10%	60.93 ± 0.36[d]	Red cabbage pomace 10%	59.96 ± 0.50[d]
Root Vegetable			
(c)		**(d)**	
Pasta Type	Starch content g/100 g dw	Pasta Type	Starch content g/100 g dw
Control	70.24 ± 0.26[a]	Control	70.24 ± 0.26[a]
Beetroot juice 3.08%	68.73 ± 0.37[b]	Carrot juice 2.27%	68.64 ± 0.51[b]
Beetroot puree 3.08%	68.42 ± 0.37[b]	Carrot puree 2.27%	68.60 ± 0.43[b]
Beetroot puree 4.08%	66.79 ± 0.43[c]	Carrot puree 3.36%	67.43 ± 0.44[c]
Beetroot pomace 3.08%	68.33 ±0.25[b]	Carrot pomace 2.27%	68.26 ± 0.41[b]
Beetroot pomace 4.08%	66.94 ± 0.22[c]	Carrot pomace 3.36%	67.37 ± 0.43[c]
Beetroot pomace 10%	61.11 ± 0.52[d]	Carrot pomace 10%	60.57 ± 0.43[d]

Values = mean ± standard deviation, n=3; dw = dry weight; The same superscripted letter mean values are not significantly different from each other for the same raw of the same vegetable pasta ($p > 0.05$).

6.3.3 *In Vitro* Starch Digestion Versus Total Starch Content

Except for the 10% substitution pasta samples and some spinach pasta samples (with measurable α-amylase inhibition effect), only beetroot puree 4.08% pasta showed a lower AUC compared to control among all other samples. It may indicate that a certain substitution level may be required to achieve a lower AUC. Cleary and Brennan (2006) replaced 2.5%, 5%, 7.5%, and 10% semolina with barley β-glucan fibre. The authors found that pasta with 5%, 7.5%, and 10% replacement exhibited lower reducing sugar than the control whereas the sample with 2.5% substitution showed the same reducing sugar release. Lu et al. (2018) incorporated 5%, 10%, and 15% shiitake mushroom powder into pasta. The authors found that 10% and 15% incorporation with lower AUC, but 5% incorporation showed the same AUC compared to the control. Chusak et al. (2020) substituted semolina with gac fruit (*Momordica cochinchinensis*) powder and found a significantly decreased AUC when 10–15% semolina was replaced with fruit powder, while the same AUC compared to control was found with 5% semolina replacement. Many studies, which substitute semolina (or wheat) with other materials, show a reduced glycaemic response (Biney & Beta, 2014; Jayawardena et al., 2019; Peressini et al., 2020; Tazrart, Zaidi, et al., 2016). The lower AUC may be partially due to a reduction in overall starch content (**Table 6.2**) caused by semolina substitution.

To find out the relationship between Starch content and glycaemic response. The pasta samples were divided into three groups depending on their α-amylase inhibition effect, and the correlation test was applied to them respectively (**Table 6.3**). The tests show a positive correlation between starch content and AUC (correlation factor = 0.806, $p \leq 0.05$) for spinach pasta. A positive correlation was also found between starch content and AUC for red cabbage & beetroot pasta (correlation factor = 0.739, $p \leq 0.01$). When the α-amylase inhibition effect is low, a strong positive correlation was found between starch content and AUC was observed for carrot pasta (correlation factor = 0.983, $p \leq$

0.01). It may be suggested that when excluding the α-amylase inhibition effect, the starch content is an essential factor that affects the glycaemic response. All these results indicate that higher AUC was related to that higher starch content if their α-amylase inhibition activity was similar.

Table 6.3 Correlation test

(a) Correlation test AUC vs Starch & Fibre spinach pasta (High α-amylase inhibition effect)				
	Starch	SDF	IDF	TDF
AUC	0.803*	-0.766*	-0.753	-0.758*
P-value	0.03	0.045	0.051	0.048
(b) Correlation test AUC vs Starch & Fibre Beetroot & red cabbage pasta (medium α-amylase inhibition effect)				
	Starch	SDF	IDF	TDF
AUC	0.739**	-0.628*	-0.659**	-0.678**
P-value	0.004	0.022	0.014	0.011
(c) Correlation test AUC vs Starch & Fibre Carrot pasta (low α-amylase inhibition effect)				
	Starch	SDF	IDF	TDF
AUC	0.983**	-0.959**	-0.939**	-0.945**
P-value	0.001	0.001	0.002	0.001

* $p < 0.05$, ** $p < 0.001$; SDF, soluble dietary fibre; IDF insoluble dietary fibre; TDF, total dietary fibre.

6.3.4 *In Vitro* Starch Digestion Versus Dietary Fibre

Figure 6.3 shows the dietary fibre profile of spinach, red cabbage, beetroot and carrot pasta. Vegetable substitution increases the soluble dietary fibre (SDF), insoluble dietary fibre (IDF), and total dietary fibre (TDF) content of all vegetable pasta samples compared to the control (although some increases were not statistically significant). A substitution level of 10% pomace of spinach, red cabbage, beetroot and carrot in this study increased the TDF, IDF and SDF the most dramatically. The dietary fibre of vegetable pasta is dependent on fibre from raw materials and possible cooking loss. Sobota and Zarzycki (2013) found that cooking impacts TDF, IDF and SDF of pasta product, and that impact depends on pasta type and cooking time. **Table 6.3** shows the correlation factor between glycaemic response and dietary fibre. For spinach pasta, negative correlations were found between SDF vs AUC (correlation factor = −0.766, $p \leq 0.05$), TDF vs AUC (correlation factor = −0.758, $p \leq 0.05$). For beetroot and red cabbage pasta, negative correlations were found between SDF vs AUC (correlation factor = −0.959, $p \leq 0.01$), IDF vs AUC (correlation factor = −0.939, $p \leq 0.01$), and TDF vs AUC (correlation factor = −0.945, $p \leq 0.05$). Furthermore, for carrot pasta, strong positive correlations

were found between SDF vs AUC (correlation factor = −0.628, $p \leq 0.05$), IDF vs AUC (correlation factor = −0.659, $p \leq 0.01$), and TDF vs AUC (correlation factor = −0.678, $p \leq 0.05$). Those outcomes showed that more soluble, insoluble, and total fibre accompanied with vegetable materials results in lower AUC. Chau et al. (2004) have illustrated that fibre can reduce post-prandial serum glucose via hindering the diffusion of glucose, retarding α-amylase action. Meenu and Xu (2019) found that dietary fibre altered the water-binding activity of pasta samples and restricted starch swelling during cooking influencing starch digestion. Peressini et al. (2020) tested AUC of soluble-fibre enriched pasta. Authors found a decreased AUC with Barley Balance®, psyllium but increased AUC for inulin and inulin HPX when 15% semolina was substituted by soluble fibre. Researchers suggested that changes in pasta structure induced by fibre enrichment contribute to the glycaemic response of pasta products. Although dietary fibre is believed to deliver health benefits such as preventing constipation, reducing bowel transit time, and selectively promoting of beneficial microbiota (Kalala et al., 2018). The dietary fibre can negatively affect the pasta quality due to the disruption of the gluten matrix (Marinelli et al., 2018) and lead to competition with starch to bind with protein (Vignola et al., 2018) and water (Foschia et al., 2013).

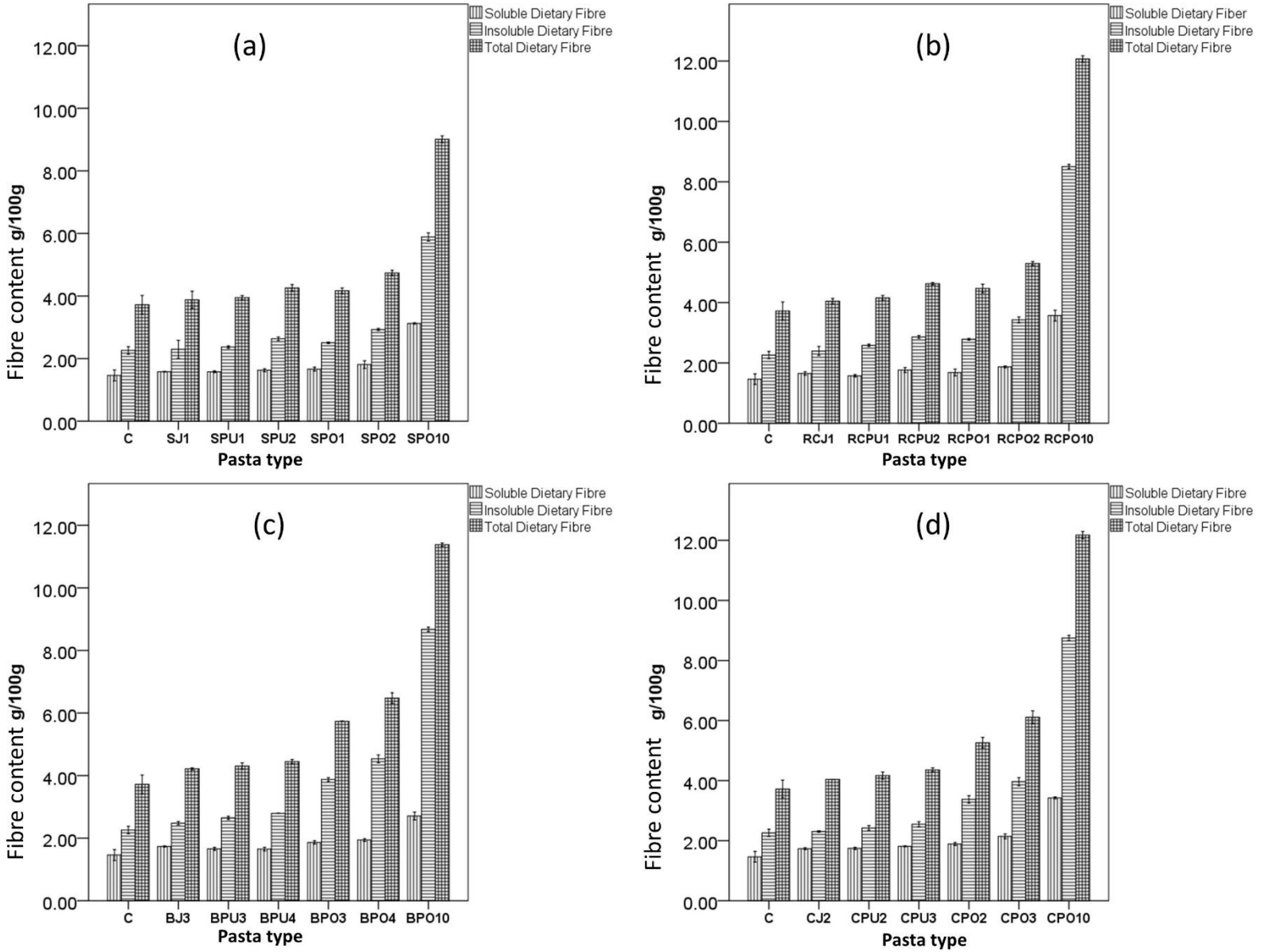

Figure 6.3 dietary fibre profile of spinach (a), red cabbage (b), beetroot (c) and carrot (d) pasta

C, control; SJ1, spinach juice 1%; SPU1, spinach puree 1%; SPU2, spinach puree 2%, SPO1, spinach pomace 1%; SPO2, spinach pomace 2%; SPO10, spinach pomace 10%; RCJ1, red cabbage juice 1%; RCPU1, red cabbage puree 1%; RCPU2, red cabbage puree 2%; RCPO1, red cabbage pomace 1%; RCPO2, red cabbage pomace 2%; RCPO10, red cabbage pomace 10%; BJ3, beetroot juice 3.08%; BPU3, beetroot puree 3.08%, BPU4, beetroot puree 4.08%; BPO3, beetroot pomace 3.08%; BPO4, beetroot pomace 4.08%; BPO10, beetroot pomace 10%; CJ2, carrot juice 2.27%; CPU2 carrot puree 2.27%; CPU3, carrot puree 3.36%; CPO2, carrot pomace 2.27%; CPO3, carrot pomace 3.36%; CPO10, carrot pomace 10%; C: control sample. Results are expressed as Mean ± standard deviation.

6.4 Conclusion

The addition of vegetable ingredients was meaningful in enhancing the nutritional value of pasta. *In vitro* starch digestion results suggested that the glycaemic response was modulated by a combined effect of the following several factors: starch and dietary fibre content, influenced by the amount of vegetables substituting the semolina. Another factor is the α-amylase inhibition effect of vegetable materials, which is the native properties of vegetable varieties. Moreover, vegetables rich in water-soluble nutrients may not be suitable for developing low GI pasta, as the α-amylase inhibition effect is reduced dramatically after cooking, like beetroot pasta and red cabbage pasta. Different forms of vegetables perform differently regarding those factors. The food industry could use the outcome of the study to choice proper vegetable and their suitable form to create low GI pasta. Results showed that spinach juice could reduce the GI of resultant pasta even at only 1% substitution.

Chapter 7

Nutritional composition of fresh vegetable juice, puree and pomace pasta

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7.1 Introduction

Consumers tend to increase their health-consciousness. Therefore, their demand for health-promoting food keeps rising (González et al., 2021). This has shifted the food industry's interest in developing and producing healthy food high in fibre, vitamins, minerals, and other health promotional ingredients. Vegetables contain an extremely wide range of phytochemicals or bioactive functional compounds that are associated with health benefits (Thakur & Sharma, 2018). However, studies showed that the consumption of vegetable ingredients is low in developed countries like Australia (Lea, Worsley, & Crawford, 2005). The ingrained habit of consumers prevents them from serving enough vegetables (Padayachee, Day, Howell, & Gidley, 2017). In this case, combining vegetable ingredients with staple food could be an option to improve vegetable intake. Pasta is a primaeval staple food which takes second place after bread in world cereal product consumption (Tazrart, Lamacchia, Zaidi, & Haros, 2016). The main advantages of pasta products are convenience, long shelf life, low cost, and abundant variety of shapes (Papanikolaou, 2020). Therefore, it may be an excellent food vehicle for delivering health-promotion ingredients such as vegetables (Nilusha, Jayasinghe, Perera, & Perera, 2019).

Numerous studies investigated the effect of vegetable ingredients on pasta quality. Marinelli et al. (2018) substituted 15% semolina with grape marc (residues of wine-making) flour. The authors found an increased total phenolic content and bioaccessible phytochemicals compared to the control. Gaita et al. (2018) used grape pomace, typically winery waste, to produce pasta. A noticeable increase in phenolic content and antioxidant capacity were reported. Sęczyk et al. (2016) added 5% carob (*Ceratonia siliqua* L.) flour to pasta and reported 2-fold, 18-fold and 3-fold improvement in phenolics content, antiradical activity and reducing power compared to control. Other authors utilised vegetable juice to increase pasta's nutrition (Kowalczewski et al., 2015; Sun-Waterhouse et al., 2013). Sun-Waterhouse et al. (2013) added elderberry juice concentrate to pasta. An increased total antioxidant activity, total phenolic

content, anthocyanins, and total fibre content were observed. The above studies only use one form of fruit and vegetables. None of them compared pomace powder, juice, and whole fruit when they were added to pasta. Vegetable juice, puree, powder and pomace powder contain different nutrients due to they may come from various parts of vegetables (Francis & Phelps, 2003). In addition, juice, puree and pomace processing methods can also lead to various nutrients (Majerska, Michalska, & Figiel, 2019). Therefore, their impact on the nutritional value of vegetable pasta is different. Vegetable pasta produced by various forms of vegetables, including puree, juice, and pomace, was investigated. Two leaf vegetables (spinach and red cabbage) and two root vegetables (beetroot and carrot) were selected to produce vegetable pasta. Total phenolic content, antioxidant capacities, β-carotene, mineral and dietary fibre content were measured.

Spinach (*Spinacia oleracea*) is rich in phenolic compounds and flavonoids, mainly including para-coumaric, ferulic acid, and quercetin (Roberts & Moreau, 2016). Also, spinach is a good source of lipid-soluble carotenoids (lutein, β-carotene and violaxanthin) and green chlorophyll (Vyas, 2017). What is more, spinach was reported to be high in mineral compounds like Zinc and iron (Giri, Armstrong, & Rajashekar, 2016). Thus, spinach pasta may be used to supplement those minerals that are lacking in cereals. Red cabbage (*Brassica oleracea var. capitata f. rubra*) is rich in bioactive compounds like phenols, isothiocyanates, vitamins A, B, C and anthocyanins (Chauhan, Tiwari, & Singh, 2016). Water-soluble anthocyanins are reported as dominant antioxidant pigments in red cabbage (Tabart, Pincemail, Kevers, Defraigne, & Dommes, 2018). Those anthocyanins were reported to correlate with promoting anti-inflammatory properties and reducing cancer risk (Drozdowska, Leszczyńska, Koronowicz, Piasna-Słupecka, & Dziadek, 2020). Adding red cabbage material to pasta could provide purple pasta with strong antioxidant properties.

Beetroot (*B. vulgaris* L.) contains varieties of health benefits phytochemicals. Those phytochemicals include phenols, betacyanins, betalains, nitrates, carotenoids, flavonoids, vitamins, and minerals (Chhikara et al., 2019). The betacyanins and betalains of beetroot are widely used water-soluble nature food colourants (Bazaria & Kumar, 2018). In addition, beetroot is a gold mine of antioxidants with anti-inflammatory and anti-carcinogenic effects (Oboh et al., 2020). Previous researchers tried to add beetroot juice to pasta, and enhanced nutritional properties were reported (Mridula, Gupta, Bhadwal, Khaira, et al., 2016; SIPOS et al., 2017). However, there is no comparison of beetroot juice pasta with other forms of beetroot, like beetroot pomace. Those comparisons will be shown in this study. Carrot contains polyphenols, polyacetylenes, and lipid-soluble carotenoids (Sharma et al., 2012). Those pigments have been reported to have varieties of health-promotion effects (Šeregelj et al., 2020). Therefore, carrots can be considered functional ingredients and have been used to enrich pasta (Adegunwa et al., 2012; Gull et al., 2015; Jalgaonkar et al., 2018). It should be noted that adding juice and puree to pasta has limitations due to the limited techno-functional properties of the pasta, such as cooking quality and consumer appearance. Juice contains low solid content (typically 5–15%), which can dilute the solid components of pasta and form a weaker pasta matrix (Wu, Shen, Sinha, & Hui, 2011). Thus achieving a high substitution level based on the dry matter may be impossible when using juice or puree due to excessive hydration. The preliminary study (**Table 7.1**) found that vegetable powder (spinach and red cabbage pasta samples) showed no significant difference compared to vegetable puree when added to pasta in key technical tests such as elasticity, firmness, and cooking loss. However, the antioxidant ability of powder-enriched pasta was lower than puree-enriched one, hence the puree was used instead.

Table 7.1 Key technical quality and antioxidant capacity of vegetable powder pasta and vegetable puree pasta (spinach pasta & red cabbage pasta)

Pasta Type	Key Technical Quality				Antioxidant capacity	
	Cooking Loss (g/100 g)	Firmness g	Breaking Distance mm	Breaking Force g	Total Phenolic content (mg GAE/100 g)	FRAPS (µmol Fe^{2+} / g)
			Spinach Pasta			
Control	4.40 ± 0.06^{c}	399.75 ± 11.51^{c}	74.28 ± 3.71^{a}	33.55 ± 1.19^{d}	441.21 ± 4.11^{e}	1.26 ± 0.10^{d}
Spinach puree 1%	4.45 ± 0.09^{c}	409.32 ± 11.09^{bc}	71.30 ± 4.88^{a}	41.18 ± 2.23^{b}	490.34 ± 8.18^{c}	2.38 ± 0.32^{bc}
Spinach powder 1%	4.48 ± 0.04^{c}	401.54 ± 9.76^{c}	68.95 ± 4.26^{ab}	37.13 ± 1.95^{c}	473.67 ± 5.95^{d}	2.28 ± 0.30^{c}
Spinach puree 2%	4.80 ± 0.03^{b}	427.46 ± 10.49^{a}	65.69 ± 3.00^{b}	42.34 ± 2.87^{ab}	521.60 ± 2.73^{a}	3.27 ± 0.43^{a}
Spinach powder 2%	4.91 ± 0.05^{a}	419.67 ± 11.25^{ab}	65.21 ± 2.69^{b}	38.12 ± 3.67^{c}	510.66 ± 7.96^{b}	2.84 ± 0.26^{ab}
			Red Cabbage pasta			
Control	4.40 ± 0.06^{c}	399.75 ± 11.51^{a}	74.28 ± 3.71^{a}	33.55 ± 1.19^{a}	441.21 ± 4.11^{e}	1.26 ± 0.10^{e}
Red cabbage puree 1%	4.80 ± 0.07^{b}	388.59 ± 11.96^{ab}	60.90 ± 3.93^{b}	31.41 ± 2.24^{ab}	713.13 ± 9.74^{b}	5.48 ± 0.10^{c}
Red cabbage powder 1%	4.88 ± 0.05^{ab}	396.34 ± 9.95^{a}	59.65 ± 4.21^{b}	31.66 ± 2.62^{ab}	629.24 ± 8.31^{d}	4.45 ± 0.21^{d}
Red cabbage puree 2%	4.94 ± 0.07^{a}	372.80 ± 11.96^{c}	57.45 ± 3.78^{b}	31.12 ± 2.23^{ab}	778.85 ± 7.11^{a}	8.38 ± 0.06^{a}
Red cabbage powder 2%	4.98 ± 0.06^{a}	382.66 ± 8.94^{bc}	57.51 ± 4.04^{b}	30.97 ± 1.79^{b}	679.64 ± 10.15^{c}	6.95 ± 0.12^{b}

Values = mean ± standard deviation. For cooking loss, total pehnolic content, FRAPS results n=3; For Firmness, Breaking distance and breaking force, n=9; Values within a column followed by the same letter are not significantly different from each other ($p > 0.05$) at the same pasta group, according to the ANOVA- Duncan test

7.2 Material and Methods

7.2.1 Total Phenolic content (TPC)

See 3.12.1 and 3.12.4

7.2.2 Antioxidant capacities

See 3.12

7.2.3 β-carotene Analysis

See 3.13

7.2.4 Mineral Contents

See 3.8

7.3 Results and discussion

7.3.1 Total Phenolic content (TPC)

Total Phenolic Contents (TPC) results of raw, cooked, and digested pasta is shown in **Table 7.2**. A higher ($p < 0.05$) total phenolic contents were found in all vegetable-fortified pasta samples compared to the control for the raw pasta. At the same substitution level, juice-fortified pasta shows higher total phenolic content compared to puree-fortified pasta and pomace-fortified pasta (spinach juice 1% > spinach puree 1% > spinach pomace 1%, $p < 0.05$; Red cabbage juice 1% > red cabbage puree 1% > red cabbage pomace 1%, $p < 0.05$; Beetroot juice 3.08% > Beetroot Puree 3.08% > Beetroot pomace 3.08%,

$p < 0.05$). Only carrot pasta is exceptional; carrot juice 2.27% shows no significant difference from carrot puree 2.27% and carrot pomace 2.27%. That observation could come from different processing methods of raw vegetable materials. The juice and puree used in this study were frozen after being prepared, just deforested before adding to pasta. The procedure makes the puree and juice fresh and keeps their nutrients. However, for pomace samples, the processing involving in traditional air-oven drying. Karam et al. (2016) found a significant change in the total phenolic contents before and after air-oven drying in multiple vegetable samples. For spinach, red cabbage, and carrot pasta, 10% pomace substitution ranked the highest total phenolic contents among other samples. However, beetroot pomace 10% showed a lower total phenolic contents value compared to beetroot juice 3.08%. This indicated that beetroot juice is superior to beetroot pomace in improving total phenolic contents. The results may be supported by the outcome reported by Vasconcellos et al. (2016). The authors found that the total phenolic contents of fresh beetroot juice were 3-times more than freeze-dried beetroot chips, 4 times higher than spray-dried beetroot powder. Cooked pasta had a lower ($p < 0.05$) Total phenolic contents than raw pasta for all the pasta samples, indicating a loss during cooking. Fares et al. (2010) reported that boiling water could degrade sensitive phenols while releasing some bond phenolics. The quantity relationship between the total phenolic contents of cooked and uncooked pasta remains similar. For example, the rank of the total phenolic contents of carrot pasta keeps the same before and after cooking (**Table 7.2**).

After digestion, the total phenolic contents of all pasta samples increased dramatically compared to cooked pasta (e.g. spinach juice 1% increased from 317 to 1866 mg GAE/100 g). This trend was similar to the one reported by Koehnlein et al. (2016), who tested the total phenolic contents of most consumed foods of Brazilian diet before and after digestion. The authors found that the total phenolic contents of egg pasta increased 6 times after digestion. In addition, Similar trends were found by Padalino et al. (2019), who reported that *in vitro* digestion increased the total phenolic contents value of semolina

pasta from 79 mg GAE/100 g to 940 mg GAE/100 g. In cereals, phenolics can be conjugated with sugars, cell wall polysaccharides, or amines (Koehnlein et al., 2016). *In vitro* digestion can release those conjugated phenolics. This is because starch and proteins, with which those phenols bond, are hydrolysed (Gawlik-Dziki, Dziki, Baraniak, & Lin, 2009). In this study, the total phenolic contents of control pasta increased from 232 mg GAE/100 g before digestion to 1725 mg GAE/100 g after digestion. The difference between them indicates semolina itself can release lots of bonded phenols after digestion. The vegetable-fortified pasta retained a higher ($p < 0.05$) total phenolic contents compared to the control after *in vitro* digestion. This illustrates more bioavailable phenolics from vegetable pasta than from control pasta. Those results were similar to those reported by Padalino et al. (2019). The authors added *Salicornia europaea* extract to pasta and found improved total phenolic contents compared to durum wheat pasta after *in vitro* digestion. After *in vitro* digestion, the difference in total phenolic contents between samples decreased. For example, cooked carrot pomace 10% has much higher total phenolic contents vs cooked carrot puree 3.36% (495.18 mg GAE/100 g vs 316.17 mg GAE/100 g). However, after *in vitro* digestion, there is no significant difference between them (1979 mg GAE/100 g of carrot pomace 10% vs 1913 mg GAE/100 g of carrot puree 3.36%, $p \geq 0.05$). This makes vegetable pasta with a low substitution level more practical than that with a high one. It is because vegetable pasta with low substitution levels only loses moderate quantities of total phenolic contents, while their technical quality (as reported in **Chapter 3 & 4**) is much higher than the high-substitution level samples.

Table 7.2 Total phenolic content of vegetable pasta

(a) Leaf vegetables	TPC (mg GAE/100 g)			(b) Root vegetables	TPC (mg GAE/100 g)		
Pasta Type	Raw	Cooked	Digested	Pasta Type	Raw	Cooked	Digested
Spinach pasta				Beetroot pasta			
Control	441.21 ± 4.11^{e}	231.51 ± 2.73^{d}	1725.35 ± 33.30^{d}	Control	441.21 ± 4.11^{e}	231.51 ± 2.73^{g}	1725.35 ± 33.30^{c}
Spinach juice 1%	571.42 ± 18.36^{b}	317.18 ± 4.26^{b}	1866.28 ± 13.52^{b}	Beetroot juice 3.08%	889.09 ± 9.58^{a}	718.82 ± 6.66^{b}	1894.72 ± 33.14^{ab}
Spinach puree 1%	490.34 ± 8.18^{d}	273.78 ± 4.34^{c}	1788.08 ± 17.95^{c}	Beetroot puree 3.08%	779.46 ± 14.10^{b}	643.59 ± 12.32^{c}	1917.73 ± 28.19^{ab}
Spinach puree 2%	521.60 ± 2.73^{c}	326.77 ± 9.35^{b}	1869.32 ± 34.96^{b}	Beetroot puree 4.08%	890.18 ± 11.50^{a}	758.66 ± 6.33^{a}	1941.92 ± 32.15^{a}
Spinach pomace 1%	455.55 ± 7.52^{e}	265.91 ± 1.62^{c}	1780.86 ± 21.09^{c}	Beetroot pomace 3.08%	524.80 ± 15.09^{e}	358.99 ± 12.32^{f}	1832.12 ± 14.43^{b}
Spinach pomace 2%	498.53 ± 13.66^{cd}	314.95 ± 5.84^{b}	1853.78 ± 49.06^{b}	Beetroot pomace 4.08%	552.26 ± 11.45^{d}	379.52 ± 15.31^{e}	1839.28 ± 75.26^{b}
Spinach pomace 10%	849.77 ± 29.08^{a}	620.66 ± 14.12^{a}	2022.89 ± 23.30^{a}	Beetroot pomace 10%	736.37 ± 4.47^{c}	570.63 ± 10.17^{d}	1940.44 ± 84.62^{a}
Red cabbage pasta				Carrot Pasta			
Control	441.21 ± 4.11^{g}	231.51 ± 2.73^{f}	1725.35 ± 33.30^{d}	Control	441.21 ± 4.11^{d}	231.51 ± 2.73^{d}	1725.35 ± 33.30^{c}
Red cabbage juice 1%	803.87 ± 3.68^{b}	531.61 ± 4.07^{b}	1934.79 ± 26.16^{b}	Carrot juice 2.27%	513.43 ± 6.90^{c}	288.61 ± 12.25^{c}	1886.83 ± 48.19^{b}
Red cabbage puree 1%	713.13 ± 9.74^{d}	367.02 ± 2.04^{d}	1896.90 ± 25.61^{bc}	Carrot puree 2.27%	511.43 ± 5.44^{c}	287.24 ± 1.85^{c}	1892.25 ± 27.99^{b}
Red cabbage puree 2%	778.85 ± 7.11^{c}	496.86 ± 10.65^{c}	1922.11 ± 43.40^{b}	Carrot puree 3.36%	532.10 ± 3.04^{b}	316.17 ± 4.97^{b}	1913.51 ± 33.60^{ab}
Red cabbage pomace 1%	548.76 ± 3.12^{f}	284.85 ± 5.22^{e}	1832.25 ± 28.93^{c}	Carrot pomace 2.27%	509.28 ± 14.95^{c}	287.16 ± 1.50^{c}	1847.04 ± 53.83^{b}
Red cabbage pomace 2%	599.35 ± 4.59^{e}	369.30 ± 4.60^{d}	1894.10 ± 66.62^{bc}	Carrot pomace 3.36%	542.90 ± 1.82^{b}	311.98 ± 3.10^{b}	1918.52 ± 37.84^{ab}
Red cabbage pomace 10%	1139.69 ± 7.20^{a}	790.65 ± 8.05^{a}	2069.87 ± 26.91^{a}	Carrot Pomace 10%	773.80 ± 4.01^{a}	495.18 ± 6.53^{a}	1979.62 ± 21.67^{a}

Values = mean ± standard deviation, n=3; Values within a column from the same kind of pasta followed by the same superscripted letter are not significantly different from each other ($p > 0.05$), according to the ANOVA- Duncan test.

7.3.2 Antioxidant capacities

The antioxidant capacities of foods depend on compounds such as phenolic content, vitamin C, vitamin E and carotenoids (Wootton-Beard, Moran, & Ryan, 2011). Antioxidant activity from vegetables is generally believed to reduce the risk of chronic diseases and the risk of cardio- and cerebrovascular and neurological diseases (Jideani et al., 2021). Antioxidant capacities, including Ferric Reducing Antioxidant Power (FRAP) and ABTS radical inhibition capacities of raw, cooked, and digested pasta, are shown in **Table 7.3** and **Table 7.4,** respectively. A strong positive correlation was found (correlation factor = 0.901, $p \leq 0.01$) between FRAPS values and ABTS values. This may reflect the high reliability of those antioxidant results. Rumpf, Burger, and Schulze (2023) reported a positive correlation between ABTS and FRAPS when lignin's antioxidant capacities were tested. A higher FRAP and ABTS value was observed in all vegetable pasta samples compared to the control. This is applied to uncooked, cooked and digested pasta. Those results illustrated that the incorporation of spinach, red cabbage, beetroot and carrot material could improve the radical scavenging activities of pasta samples, indicating that pasta is a good medium to incorporate with vegetable material to improve human nutrition.

For the raw pasta, the antioxidant capacities of vegetable pasta ranked as red cabbage 10% > spinach pomace 10% > beetroot pomace 10% > carrot pomace 10% ($p < 0.05$ for both FRAP and ABTS results). It is unexpected that the antioxidant capacities of beetroot pasta are lower than red cabbage pasta and spinach pasta. Studies have shown that the antioxidant capacity of fresh beetroot is high and comparable to fresh red cabbage (Patel, Chaubey, Das, & Pandey, 2019; Shashirekha et al., 2015; Žitňanová et al., 2006). The reason for the under-expected antioxidant capacities of beetroot pasta may be due to the freeze-drying procedure before antioxidant capacity measurement. Dalmau, Eim, Rosselló, Cárcel, and Simal (2019) showed that the antioxidant activity of fresh beetroot cubes was reduced by 52% after freeze-drying, according to FRAP assay measurement. The authors suggested that cellular

structure change during freeze-drying provoked the degradation of molecules, thus reducing the antioxidant activity. It has been reported that most vegetables lost their antioxidant capacity more or less after freeze-drying (Kamiloglu et al., 2016). However, some vegetables, such as freeze-dried tomatoes, have an improved antioxidant ability (Jorge et al., 2014). This means that comparing antioxidant capacities between different vegetable pasta may not be as accurate as expected. At the same substitution level, juice-fortified pasta samples showed the highest antioxidant value compared to puree and pomace-fortified pasta samples. This is applied to red cabbage, spinach and beetroot-fortified pasta in their raw, cooked and digested forms. The results regarding carrot pasta are slightly different. Carrot juice 2.27% only showed a higher ($p < 0.05$) antioxidant capacity than carrot puree 2.27% and pomace 2.27% in cooked and digested samples. It may indicate that vegetable juice could be a solid choice to add to pasta formula to improve pasta's antioxidant capacity compared to puree and pomace.

After cooking, most vegetable pasta shows a lower antioxidant capacity (except for spinach puree 1%, spinach pomace 1%, spinach pomace 2%, red cabbage juice 1%, red cabbage puree 1% and red cabbage puree 2%) than uncooked ones. Similar results were reported by Simonato et al. (2019), who added olive pomace to pasta and found reduced antioxidants in cooked pasta compared with its uncooked counterpart. The less antioxidant capacity of cooked pasta may be due to the phytochemical leach into the cooking water (Lu et al., 2018). Another reason may be thermal-induced phytochemical oxidation and degradation (Gaita et al., 2018). Spinach, beetroot, and carrot pasta showed a higher antioxidant capacity when comparing the digested sample with the cooked one. Several studies have reported that *in vitro* digestion could improve the antioxidant activity of enriched pasta (Bustos et al., 2020; Ziółkiewicz et al., 2023). The authors suggested that antioxidant phytochemicals may undergo depolymerisation during *in vitro* digestion. Thus, those phenols express their additional antioxidant abilities and an improved antioxidant capacity was observed. However, after digestion, the antioxidant capacity of red cabbage pasta decreased. This may be due to the different antioxidant stability of red

cabbage materials compared with other vegetables. The antioxidant capacities of red cabbage materials are mainly from its water-soluble anthocyanin (Podsędek, Redzynia, Klewicka, & Koziołkiewicz, 2014). McDougall, Fyffe, Dobson, and Stewart (2007) reported that anthocyanin content from red cabbage was stable in gastric digestion but suffered a heavy loss (around 73%) during pancreatic digestion. Such changes may explain the decreased antioxidant capacity of red cabbage pasta after *in vitro* digestion.

Table 7.3 Antioxidant capacity-FRAP assay

(a)Leaf vegetables	FRAP (µmol Fe^{2+}/g dw)			(b)Root vegetables	FRAP (µmol Fe^{2+}/g dw)		
Pasta Type	Raw	Cooked	Digested	Pasta Type	Raw	Cooked	Digested
Spinach pasta				Beetroot pasta			
Control	1.260 ± 0.103$^{f, B}$	0.830 ± 0.030$^{f, C}$	1.665 ± 0.039$^{f, A}$	Control	1.260 ± 0.103$^{e, B}$	0.830 ± 0.030$^{g, C}$	1.666 ± 0.039$^{g, A}$
Spinach juice 1%	3.721 ± 0.120$^{b, B}$	3.320 ± 0.077$^{b, C}$	4.481 ± 0.144$^{b, A}$	Beetroot juice 3.08%	3.036 ± 0.031$^{b, A}$	1.910 ± 0.056$^{b, C}$	2.956 ± 0.041$^{b, B}$
Spinach puree 1%	2.379 ± 0.323$^{e, B}$	1.963 ± 0.094$^{e, C}$	2.932 ± 0.032$^{e, A}$	Beetroot puree 3.08%	2.650 ± 0.091$^{c, A}$	1.584 ± 0.072$^{d, B}$	2.554 ± 0.047$^{d, A}$
Spinach puree 2%	3.270 ± 0.043$^{c, B}$	3.168 ± 0.025$^{c, C}$	4.248 ± 0.045$^{c, A}$	Beetroot puree 4.08%	3.105 ± 0.118$^{b, A}$	1.714 ± 0.032$^{c, C}$	2.737 ± 0.047$^{c, B}$
Spinach pomace 1%	2.224 ± 0.302$^{e, B}$	2.004 ± 0.048$^{e, C}$	2.944 ± 0.022$^{e, A}$	Beetroot pomace 3.08%	2.090 ± 0.083$^{d, A}$	1.252 ± 0.029$^{f, C}$	2.146 ± 0.071$^{f, B}$
Spinach pomace 2%	2.791 ± 0.049$^{d, B}$	2.691 ± 0.072$^{d, B}$	3.745 ± 0.049$^{d, A}$	Beetroot pomace 4.08%	2.714 ± 0.100$^{c, A}$	1.471 ± 0.026$^{e, C}$	2.421 ± 0.039$^{e, B}$
Spinach pomace 10%	9.342 ± 0.284$^{a, A}$	8.607 ± 0.060$^{a, B}$	9.804 ± 0.234$^{a, A}$	Beetroot pomace 10%	5.425 ± 0.051$^{a, A}$	3.467 ± 0.053$^{a, C}$	4.853 ± 0.061$^{a, B}$
Red cabbage pasta				Carrot Pasta			
Control	1.260 ± 0.103$^{f, B}$	0.830 ± 0.030$^{g, C}$	1.666 ± 0.039$^{f, A}$	Control	1.260 ± 0.103$^{c, B}$	0.830 ± 0.030$^{f, C}$	1.666 ± 0.039$^{f, A}$
Red cabbage juice 1%	8.521 ± 0.121$^{b, A}$	8.467 ± 0.121$^{b, A}$	4.488 ± 0.107$^{b, B}$	Carrot juice 2.27%	2.256 ± 0.036$^{b, B}$	1.754 ± 0.007$^{b, C}$	3.306 ± 0.051$^{b, A}$
Red cabbage puree 1%	5.481 ± 0.103$^{c, A}$	5.286 ± 0.075$^{d, A}$	3.320 ± 0.030$^{c, B}$	Carrot puree 2.27%	2.280 ± 0.081$^{b, B}$	1.421 ± 0.018$^{e, C}$	2.714 ± 0.052$^{e, A}$
Red cabbage puree 2%	8.383 ± 0.055$^{b, A}$	8.163 ± 0.072$^{c, B}$	4.372 ± 0.051$^{b, C}$	Carrot puree 3.36%	2.390 ± 0.066$^{b, B}$	1.588 ± 0.025$^{c, C}$	3.034 ± 0.109$^{c, A}$
Red cabbage pomace 1%	4.034 ± 0.042$^{e, A}$	3.908 ± 0.080$^{f, A}$	2.794 ± 0.043$^{e, B}$	Carrot pomace 2.27%	2.259 ± 0.041$^{b, B}$	1.408 ± 0.042$^{e, C}$	2.684 ± 0.037$^{e, A}$
Red cabbage pomace 2%	4.869 ± 0.082$^{d, A}$	4.788 ± 0.103$^{e, A}$	3.146 ± 0.089$^{d, B}$	Carrot pomace 3.36%	2.365 ± 0.090$^{b, B}$	1.486 ± 0.014$^{d, C}$	2.827 ± 0.065$^{d, A}$
Red cabbage pomace 10%	13.645 ± 0.084$^{a, A}$	13.315 ± 0.077$^{a, B}$	6.263 ± 0.130$^{a, C}$	Carrot Pomace 10%	3.629 ± 0.046$^{a, B}$	2.750 ± 0.049$^{a, C}$	5.090 ± 0.057$^{a, A}$

Values = mean ± standard deviation, n=3; dw = dry weight; The same lowercase superscripted letters within a row of the same kind of vegetable pasta are not significantly different from each other ($p > 0.05$), according to the ANOVA-Turkey's test. dw = dry weight; The same uppercase superscripted letters within a column of the same kind of vegetable pasta are not significantly different from each other ($p > 0.05$).

Table 7.4 ABTS Antioxidant capacity

(a)Leaf vegetables	TE (µmol/g dw)			(b)Root vegetables	TE (µmol/g dw)		
Pasta Type	Raw	Cooked	Digested	Pasta Type	Raw	Cooked	Digested
	Spinach pasta				Beetroot pasta		
Control	6.50 ± 0.18$^{e, B}$	4.65 ± 0.30$^{e, C}$	7.58 ± 0.26$^{e, A}$	Control	6.50 ± 0.18$^{d, B}$	4.65 ± 0.30$^{d, C}$	7.58 ± 0.26$^{d, A}$
Spinach juice 1%	10.53 ± 0.12$^{b, B}$	10.07 ± 0.17$^{b, C}$	11.25 ± 0.11$^{b, A}$	Beetroot juice 3.08%	10.24 ± 0.16$^{b, A}$	7.49 ± 0.30$^{b, C}$	9.48 ± 0.12$^{b, B}$
Spinach puree 1%	9.35 ± 0.26$^{cd, B}$	9.06 ± 0.04$^{d, B}$	9.94 ± 0.13$^{d, A}$	Beetroot puree 3.08%	9.56 ± 0..5$^{b, A}$	6.57 ± 0.11$^{c, C}$	8.45 ± 0.09$^{c, B}$
Spinach puree 2%	10.38 ± 0.12$^{b, B}$	9.92 ± 0.14$^{bc, C}$	10.73 ± 0.08$^{c, A}$	Beetroot puree 4.08%	10.26 ±0.16$^{b, A}$	7.47 ±0.18$^{b, C}$	8.77 ± 0.10$^{c, B}$
Spinach pomace 1%	9.05 ± 0.09$^{d, B}$	9.03 ± 0.12$^{d, B}$	10.03 ± 0.08$^{d, A}$	Beetroot pomace 3.08%	8.36 ± 0.11$^{c, A}$	6.09 ± 0.11$^{c, C}$	7.74 ± 0.14$^{d, B}$
Spinach pomace 2%	9.56 ± 0.13$^{c, B}$	9.38 ± 0.04$^{cd, B}$	10.47 ± 0.13$^{c, A}$	Beetroot pomace 4.08%	8.54 ± 0.12$^{c, A}$	6.27 ± 0.12$^{c, C}$	7.89 ± 0.18$^{d, B}$
Spinach pomace 10%	13.87 ± 0.13$^{a, B}$	12.48 ± 0.32$^{a, C}$	14.93 ± 0.21$^{a, A}$	Beetroot pomace 10%	11.17 ± 0.57$^{a, A}$	9.24 ± 0.25$^{a, C}$	10.12 ± 0.13$^{a, B}$
	Red cabbage pasta				Carrot Pasta		
Control	6.50 ± 0.18$^{e, B}$	4.65 ± 0.30$^{e, C}$	7.58 ± 0.26$^{e, A}$	Control	6.50 ± 0.18$^{d, B}$	4.65 ± 0.30$^{d, C}$	7.58 ± 0.26$^{d, A}$
Red cabbage juice 1%	12.16 ± 0.16$^{b, A}$	12.10 ± 0.15$^{b, A}$	11.05 ± 0.11$^{b, B}$	Carrot juice 2.27%	8.23 ± 0.26$^{b, B}$	6.57 ± 0.10$^{b, C}$	10.38 ± 0.31$^{b, A}$
Red cabbage puree 1%	11.47 ± 0.11$^{c, A}$	11.26 ± 0.09$^{c, A}$	10.20 ± 0.12$^{c, B}$	Carrot puree 2.27%	7.85 ± 0.08$^{bc, B}$	5.90 ± 0.22$^{c, C}$	9.07 ± 0.09$^{c, A}$
Red cabbage puree 2%	12.01 ± 0.11$^{b, A}$	11.85 ± 0.15$^{b, A}$	10.96 ± 0.11$^{b, B}$	Carrot puree 3.36%	8.08 ± 0.14$^{bc, B}$	6.15 ± 0.09$^{bc, C}$	9.50 ± 0.10$^{c, A}$
Red cabbage pomace 1%	10.81 ± 0.13$^{d, A}$	10.37 ± 0.17$^{d, B}$	9.49 ±0.10$^{d, C}$	Carrot pomace 2.27%	7.68 ± 0.13$^{c, B}$	5.76 ± 0.10$^{c, C}$	8.92 ± 0.23$^{c, A}$
Red cabbage pomace 2%	11.06 ± 0.06$^{d, A}$	10.63 ± 0.11$^{d, B}$	9.83 ± 0.14$^{cd, C}$	Carrot pomace 3.36%	7.89 ± 0.12$^{bc, B}$	5.84 ± 0.09$^{c, C}$	9.54 ± 0.41$^{c, A}$
Red cabbage pomace 10%	16.06 ± 0.13$^{a, A}$	15.89 ± 0.19$^{a, A}$	13.51 ± 0.25$^{a, B}$	Carrot Pomace 10%	9.67 ± 0.11$^{a, B}$	8.36 ± 0.08$^{a, C}$	11.40 ± 0.24$^{a, A}$

Values = mean ± standard deviation, n=3; dw = dry weight; The same lowercase superscripted letters within a row of the same kind of vegetable pasta are not significantly different from each other ($p > 0.05$), according to the ANOVA-Turkey's test. The same uppercase superscripted letters within a column of the same kind of vegetable pasta are not significantly different from each other ($p > 0.05$).

7.3.3 β-carotene of carrot and spinach pasta

Carotenoids are lipid-soluble phytochemicals that have been reported to have some health benefits (Rao & Rao, 2007). The health benefits include preventing chronic diseases (Bohn, 2019), reducing the risk of cardiovascular disease (Di Pietro, Di Tomo, & Pandolfi, 2016), preventing obesity (Coronel, Pinos, & Amengual, 2019), and some other benefits (Nabi et al., 2020). Those benefits are primarily generated by the antioxidant potential of carotenoids (Eggersdorfer & Wyss, 2018). Some carotenoids could provide health benefits through additional mechanisms (Eggersdorfer & Wyss, 2018). For example, β-carotene has health benefits through its ability to be converted to vitamin A (Miller, Coronel, & Amengual, 2020). β-carotene is one of vegetables' six most abundant carotenoids (Coronel et al., 2019). β-carotene was found abundantly in carrot and spinach material (Marx, Stuparic, Schieber, & Carle, 2003; Yuan et al., 2019). **Table 7.5** shows the β-carotene content of spinach and carrot raw material and pasta (The chromatographic separation of β-carotene is shown in **Figure 7.1**). The spinach juice and spinach puree contained the same amount ($p < 0.05$) of β-carotene, while spinach pomace contained less β-carotene than spinach juice and puree ($p < 0.05$). Similar situation was found in carrot material although β-carotene content of carrot materials is much higher than spinach materials. The pomace raw material contains lower β-carotene may be due to the long time air-drying (7 h of 60 °C) to produce them. Hiranvarachat, Suvarnakuta, and Devahastin (2008) found around 15% of β-carotene loss from blenched carrot samples after 7 h of hot air drying. The author contributed that loss to isomerization degradation, which transfers β-carotene to its cis form, and thermal degradation. Marx et al. (2003) found that blenching prevents nonenzymatic browning reaction and causes a higher value of β-carotene of pasteurised carrot juice. This may be another reason that spinach pomace has lower β-carotene as spinach pomace preparation was not involved in blench processing but was related to hot air drying.

As a result, juice and puree-enriched pasta contain a higher β-carotene than pomace-enriched one when the substitution level is the same (**see Table 7.5** spinach juice 1% = spinach puree 1% > spinach pomace 1%; Carrot juice 2.27% = carrot puree 2.27% > carrot pomace 2.27%;). This applies to uncooked, cooked and digested pasta samples. All spinach and carrot pasta samples contained higher β-carotene content than the control, indicating that pasta is a compelling medium to deliver β-carotene. Compared to raw pasta, cooked pasta contains a comparable amount of β-carotene, which suggests that cooking does not cause a loss of β-carotene in spinach and carrot pasta. It is not like the total phenolic content loss observed during pasta cooking. It may be suggested that β-carotene is not water-soluble, so spinach and carrot pasta cooked in boiled water does not cause β-carotene loss to the cooking water. However, after *in vitro* digestion, the β-carotene was lower ($p < 0.05$) than in cooked pasta samples, which showed the loss of β-carotene during digestion. Courraud, Berger, Cristol, and Avallone (2013) reported a more than 50% β-carotene loss for raw and cooked spinach during digestion. The authors also showed less than 10% β-carotene loss during the digestion of carrot juice. It was hypothesized that the loss depends on the food matrix and raw material processing. For the pasta samples, digestion caused the decrease of β-carotene. The range of decrease was from 20.4% (carrot pomace 10%) to 28.0% (carrot juice 2.27%). After digestion, all spinach and carrot pasta samples contain more β-carotene than the control, which illustrates spinach pasta can deliver significantly more bioavailable β-carotene than traditional durum pasta.

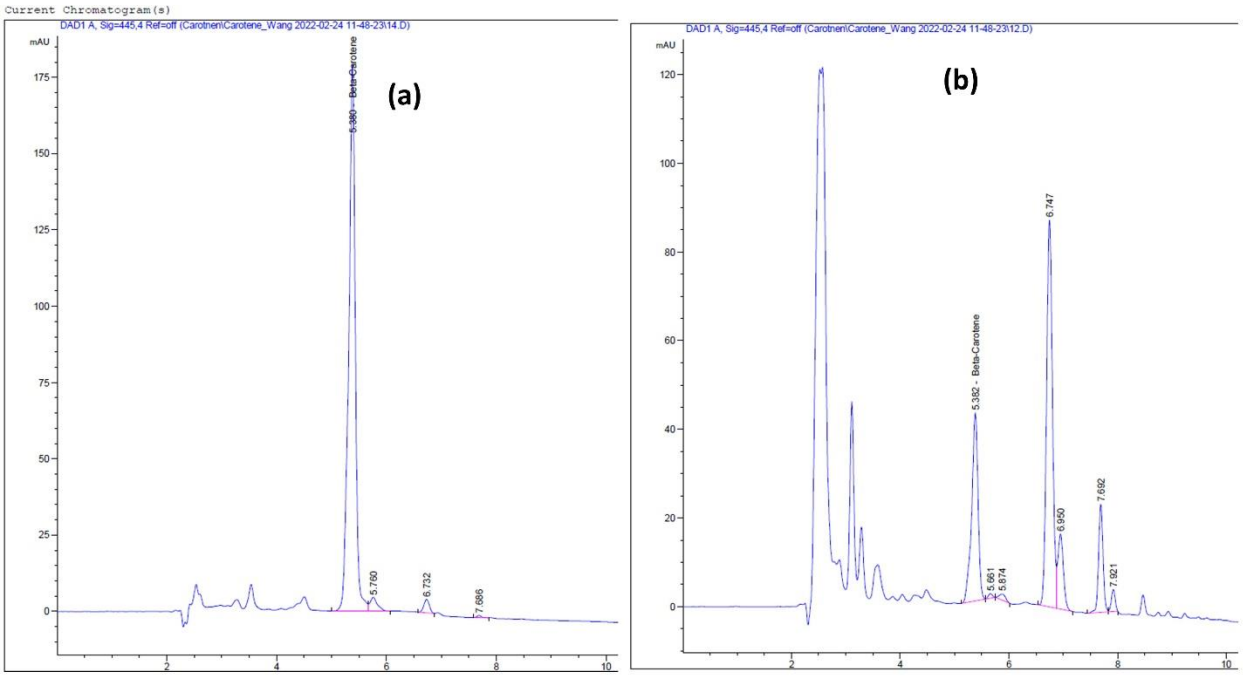

Figure 7.1 Chromatography of β-carotene of (a) digested carrot pomace 10%, (b) digested spinach pomace 10%

Table 7.5 β-carotene content of spinach and carrot pasta and raw material

Raw material of spinach and carrot µg/g			
Spinach Juice	27.618 ± 0.793^{c}		
Spinach Puree	28.240 ± 0.783^{c}		
Spinach pomace	17.862 ± 0.571^{d}		
Carrot Juice	270.508 ± 1.578^{a}		
Carrot Puree	273.188 ± 1.086^{a}		
Carrot Pomace	92.483 ± 0.746^{b}		
	Spinach pasta		
Pasta Type	Uncooked µg/g	Cooked µg/g	Digested µg/g
C	0.034 ± 0.002$^{f, A}$	0.033 ± 0.001$^{e, A}$	0.026 ± 0.001$^{f, B}$
Spinach Juice 1%	0.319 ± 0.012$^{d, A}$	0.312 ± 0.013$^{c, A}$	0.235 ± 0.011$^{d, B}$
Spinach Puree 1%	0.305 ± 0.009$^{d, A}$	0.302 ± 0.008$^{c, A}$	0.228 ± 0.011$^{d, B}$
Spinach Puree 2%	0.577 ± 0.008$^{b, A}$	0.572 ± 0.014$^{b, A}$	0.424 ± 0.006$^{b, B}$
Spinach Pomace 1%	0.192 ± 0.011$^{e, A}$	0.183 ± 0.010$^{d, A}$	0.136 ± 0.005$^{e, B}$
Spinach Pomace 2%	0.361 ± 0.005$^{c, A}$	0.357 ± 0.008$^{c, A}$	0.272 ± 0.008$^{c, B}$
Spinach Pomace 10%	1.985 ± 0.014$^{a, A}$	2.002 ± 0.150$^{a, A}$	1.497 ± 0.026$^{a, B}$
	Carrot Pasta		
Pasta Type	Uncooked µg/g	Cooked µg/g	Digested µg/g
Control	0.034 ± 0.002$^{e, A}$	0.033 ± 0.001$^{e, A}$	0.026 ± 0.001$^{e, B}$
Carrot juice 2.27%	6.155 ± 0.342$^{b, A}$	6.022 ± 0.180$^{b, A}$	4.336 ± 0.098$^{b, B}$
Carrot puree 2.27%	6.156 ± 0.060$^{b, A}$	6.135 ± 0.161$^{b, A}$	4.350 ± 0.070$^{b, B}$
Carrot puree 3.36%	9.339 ± 0.074$^{a, A}$	9.356 ± 0.233$^{a, A}$	7.413 ± 0.098$^{a, B}$
Carrot pomace 2.27%	1.898 ± 0.049$^{d, A}$	1.879 ± 0.073$^{d, A}$	1.434 ±0.077$^{d, B}$
Carrot pomace 3.36%	2.905 ± 0.058$^{c, A}$	2.976 ± 0.553$^{c, A}$	2.183 ± 0.079$^{c, B}$
Carrot Pomace 10%	9.155 ± 0.234$^{a, A}$	9.327 ± 0.089$^{a, A}$	7.429 ± 0.200$^{a, B}$

Values = mean ± standard deviation, n=2; The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other ($p > 0.05$), according to the ANOVA-Turkey's test. The same uppercase superscripted letters within a column are not significantly different from each other ($p > 0.05$).

7.3.4 Mineral content

Minerals are among those essential nutrients that are needed by the human body to achieve its functions (Gharibzahedi & Jafari, 2017). Minerals could be divided into macro-minerals and micro-minerals (Martínez-Ballesta et al., 2010). Macro-minerals of vegetable pasta are shown in **Table 7.6abcd**.

Calcium is an essential element for human bones and teeth. It is also involved in the regulation of nerve function and the immune system (Li et al., 2018). Results show that all vegetable-enriched pasta contains more calcium compared to the control ($p < 0.05$). Among these vegetables, spinach is most effective in improving the calcium amount of enriched pasta (Spinach pomace 10% contains 2685 mg/kg calcium, much higher than all other samples see **Table 7.6a**). However, after cooking, the calcium in cooked pasta is equal to or higher than the uncooked one. It may be because the calcium in those vegetables was present as insoluble compounds. Therefore, the calcium in vegetable pasta was not leaching into the cooking water. After cooking, other contents leach into the cooking water and the proportion of calcium in the dry weight increase. This may indicate vegetable enrichment can improve bioaccessible calcium in resultant pasta. However, Martínez-Ballesta et al. (2010) questioned the bioavailability of calcium in vegetables due to their Insoluble nature. One example comes from Manivannan, Viswanathan, and Sundaram (2022), who showed that calcium in spinach is bound with oxalates, and the bioavailability is very low (less than 10%).

Potassium (K) and sodium (Na) are needed for the maintenance of electrolyte and fluid balance in the human body (McLean & Wang, 2021). They also regulate blood pressure, muscle contraction, and nerve transition (Gharibzahedi & Jafari, 2017). Vegetable addition increased the potassium content of uncooked vegetable pasta ($p < 0.05$). As for sodium content, spinach pasta, beetroot pasta, and carrot pasta show a higher sodium content than the control, while red cabbage pasta showed the same sodium content as the control (**Table 7.6bd**). However, after cooking, both potassium and sodium suffer heavy losses. This may be due to the water-soluble nature of these two minerals. As a result, the sodium content of all red cabbage pasta and most spinach pasta (except for spinach pomace 2% and spinach pomace 10%) was reduced to undetectable, even though the majority of vegetable pasta samples retained potassium after cooking (except for the potassium of carrot juice 2.27%). It was suggested that plant potassium is high in solubility and can quickly disperse in the digestive tract, resulting in a high (>

90%) bioavailability (Stone, Martyn, & Weaver, 2016). This makes vegetable pasta useful for delivering soluble minerals like potassium.

Phosphorus (P) is present in every cell (Gharibzahedi & Jafari, 2017). It plays roles in cell growth, energy regulation, and kidney performance regulation (Bird & Eskin, 2021). All the uncooked vegetable pasta contains more phosphorus than the control (**Table 7.6ac**, $p < 0.05$). However, after cooking, beetroot pasta has equal (beetroot juice 3.08%, $p \geq 0.05$) or less phosphorus (all other beetroot pasta, $p < 0.05$) compared to the control, while other vegetable pasta still has a higher phosphorus content versus the control. It indicates that beetroot material may not be suitable for improving the phosphorus content of vegetable pasta, while spinach, red cabbage and carrot could be. Magnesium (Mg) is needed for protein formation, muscle contraction, and nerve transmission (Al Alawi, Majoni, & Falhammar, 2018). Spinach and red cabbage material improved the magnesium content of the resultant pasta compared to the control ($p < 0.05$) for the uncooked and cooked pasta. This improvement is much less obvious for beetroot and carrot materials. Among beetroot and carrot pasta samples, only carrot pomace 10% showed a higher magnesium content than the control. Cooking causes a loss of phosphorous and magnesium for all the vegetable pasta samples, although that loss is much less than potassium and sodium. **Table 7.6bd** also showed a calculated summary of those selected macro-minerals. All vegetable pasta contains higher mineral contents compared to the control for both uncooked and cooked samples. At the same substitution level, juice-enriched pasta tends to have more bioaccessible (data from cooked pasta) minerals than puree and pomace enriched ones for spinach and red cabbage pasta. As for beetroot and carrot pasta, the situation is in the contrast, the pomace enriched pasta contains the highest bioaccessible minerals while juice enriched pasta contains the lowest when the substitution level is the same. Those results indicates that different vegetables (and their different forms) have inconsistent effect on the mineral content of vegetable pasta.

Iron (Fe) and Zinc (Zn) are two selected micro-minerals that have been measured. Iron is essential for the biosynthesis of haemoglobin, a crucial component of human red blood cells (Gharibzahedi & Jafari, 2017). Inadequate iron intake may lead to nutritional anaemia in humans, a condition that can have negative health consequences (Camaschella, 2015). Those consequences included mental malfunction and a lower growth rate in children (Pasricha, Tye-Din, Muckenthaler, & Swinkels, 2021). The iron content of raw materials and vegetable pasta is shown in **Table 7.7ab** (Only showed spinach pasta due to other vegetable pasta samples having undetectable micro-minerals). For spinach raw materials, the iron amount ranked as spinach juice (380.7 mg/kg) > spinach puree (175.0 mg/kg) > spinach pomace (97.4 mg/kg). Red cabbage and beetroot showed a much lower iron content versus spinach (from 25.8 mg/kg of red cabbage pomace to 42.1 mg/kg of beetroot puree). As a result, only spinach pasta (spinach juice 1%, spinach puree 2%, spinach pomace 10%) showed measurable iron content. After cooking, those three spinach pasta samples contain the same amount of iron as the uncooked ones. This indicates that spinach pasta could deliver bioaccessible iron. However, a lot of researchers have reported that the bioavailability of spinach iron is low (Panda & Shinde, 2016; Roberts & Moreau, 2016; Vyas, 2017). Therefore, further research on the bioavailability of iron from vegetable pasta may be needed. Zinc is a vital component of the structural architecture of numerous enzymes (Chasapis, Ntoupa, Spiliopoulou, & Stefanidou, 2020). Lack of zinc in the diet may result in slow growth, delayed skeletal and sexual maturity, and decreased immune function (Gharibzahedi & Jafari, 2017). Only spinach pomace 10% showed detectable zinc, which may infer that a high substitution level of spinach was required to deliver zinc in vegetable pasta. Luo and Xie (2012) reported that the bioavailability of iron and zinc in vegetables is much lower than that in meat and other animal-origin materials. The authors suggested that phytate and polyphenols in vegetables could form an insoluble complex and lower the bioavailability of these minerals. Boiling did not cause losses of iron and zinc from spinach

pasta, indicating that iron and zinc present in spinach pasta are insoluble. Thus, their bioavailability was questionable.

Table 7.6 (a) Macro minerals (calcium, magnesium, phosphorus) of spinach and red cabbage pasta

Pasta Type	Calcium mg/kg		Magnesium mg/kg		Phosphorus mg/kg	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Control	248.3 ± 7.4^{i}	243.3 ± 2.7^{j}	320.4 ± 26^{i}	246.6 ± 6.1^{i}	738.9 ± 9.6^{h}	696.1 ± 8.4^{i}
Spinach juice 1%	465.1 ± 6.1^{e}	476.9 ± 13.8^{e}	475.6 ± 5.1^{c}	390.0 ± 6.6^{c}	842.2 ± 6.0^{d}	725.8 ± 5.9^{fg}
Spinach puree 1%	485.4 ± 6.6^{e}	492.2 ± 8.5^{e}	375.0 ± 4.3^{fg}	280.0 ± 5.5^{gh}	842.1 ± 4.4^{d}	740.9 ± 5.4^{f}
Spinach puree 2%	568.4 ± 7.1^{c}	583.8 ± 14.^{3c}	424.9 ± 4.6^{d}	322.6 ± 3.2^{e}	970.6 ± 6.8^{b}	789.5 ± 5.8^{cd}
Spinach pomace 1%	522.5 ± 6.5^{d}	545.0 ± 10.2^{d}	364.8 ± 4.1^{g}	298.1 ± 7.5^{fg}	788.0 ± 6.6^{f}	703.8 ± 1.1^{hi}
Spinach pomace 2%	838.8 ± 7.9^{b}	875.6 ± 11.9^{b}	408.9 ± 6.5^{e}	343.4 ± 4.2^{d}	821.3 ± 1.5^{e}	714.8 ± 4.7^{gh}
Spinach pomace 10%	2685.2 ± 14.2^{a}	2784.9 ± 20.1^{a}	801.5 ± 8.9^{b}	715.7 ± 5.0^{b}	1076.4 ± 4.4^{a}	804.1 ± 6.8^{bc}
Red cabbage juice 1%	280.7 ± 5.5^{gh}	280.7 ± 10.5^{gh}	327.8 ± 3.3^{i}	241.0 ± 9.5^{i}	929.1 ± 4.0^{c}	812.2 ± 3.8^{b}
Red cabbage puree 1%	284.0 ± 5.0^{gh}	293.0 ± 11.1^{fgh}	345.1 ± 6.9^{h}	270.1 ± 7.0^{h}	843.7 ± 6.5^{d}	773.8 ± 3.0^{de}
Red cabbage puree 2%	317.0 ± 8.0^{f}	321.0 ± 2.6^{f}	364.5 ± 3.9^{g}	303.1 ± 1.7^{ef}	931.1 ± 3.9^{c}	835.0 ± 4.4^{a}
Red cabbage pomace 1%	270.7 ± 9.1^{hi}	274.0 ± 13.7^{hi}	387.5 ± 7.5^{f}	292.4 ± 10.1^{fg}	764.2 ± 3.7^{g}	702.2 ± 2.8^{hi}
Red cabbage pomace 2%	297.2 ± 8.1^{fg}	307.4 ± 2.0^{fg}	471.8 ± 5.5^{c}	347.1 ± 5.6^{d}	791.9 ± 8.3^{f}	707.4 ± 4.6^{hi}
Red cabbage pomace 10%	515.4 ± 7.5^{d}	554.3 ± 6.1^{cd}	975.4 ± 1.5^{a}	758.5 ± 11.0^{a}	976.6 ± 3.2^{b}	761.5 ± 7.7^{e}

Values = mean ± standard deviation, n=3; The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other in one table ($p > 0.05$), according to the ANOVA-Turkey's test.

Table 7.6 (b) Macro minerals (potassium, sodium, summary) of spinach and red cabbage pasta

Pasta Type	Potassium mg/kg		Sodium mg/kg		Summary	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Control	358.9 ± 7.4^{k}	Trace	Trace	Trace	1666.6 ± 21.5^{l}	1279.3 ± 19.6^{k}
Spinach juice 1%	3215.0 ± 17.8^{c}	642.0 ± 9.5^{d}	38.8 ± 1.9^{c}	Trace	5037.0 ± 24.4^{c}	2234.6 ± 20.8^{e}
Spinach puree 1%	1499.3 ± 5.9^{f}	598.3 ± 8.7^{e}	38.8 ± 0.7^{c}	Trace	3240.7 ± 21.3^{h}	2111.5 ± 10.0^{f}
Spinach puree 2%	2393.0 ± 12.2^{d}	871.0 ± 9.5^{c}	78.2 ± 2.1^{b}	Trace	4434.7 ± 51.6^{d}	2566.8 ± 31.3^{c}
Spinach pomace 1%	1311.7 ± 12.6^{g}	281.0 ± 10.1^{h}	18.1 ± 1.2^{e}	Trace	3005.0 ± 19.4^{i}	1827.9 ± 26.7^{g}
Spinach pomace 2%	2076.4 ± 10.1^{e}	588.3 ± 3.5^{f}	34.20 ± 2.0^{d}	10.0 ± 0.4^{b}	4179.2 ± 18.6^{e}	2502.1 ± 14.8^{d}
Spinach pomace 10%	7061.2 ± 11.5^{a}	2507.7 ± 6.1^{a}	163.3 ± 2.6^{a}	54.5 ± 2.2^{a}	11787.4 ± 10.6^{a}	6866.9 ± 25.5^{a}
Red cabbage juice 1%	2395.3 ± 17.2^{d}	209.3 ± 3.6^{h}	Trace	Trace	3932.8 ± 23.6^{f}	1543.1 ± 18.8^{i}
Red cabbage puree 1%	1186.2 ± 5.9^{h}	125.4 ± 3.9^{k}	Trace	Trace	2659.0 ± 13.1^{j}	1462.3 ± 11.7^{j}
Red cabbage puree 2%	2073.9 ± 10.0^{e}	216.9 ± 6.0^{i}	Trace	Trace	3686.4 ± 24.0^{h}	1676.0 ± 14.8^{h}
Red cabbage pomace 1%	747.0 ± 5.0^{j}	182.7 ± 4.5^{j}	Trace	Trace	2169.3 ± 24.8^{k}	1451.3 ± 24.3^{j}
Red cabbage pomace 2%	1137.4 ± 6.7^{i}	355.7 ± 4.4^{g}	Trace	Trace	2698.1 ± 11.1^{j}	1717.2 ± 15.6^{h}
Red cabbage pomace 10%	3763.1 ± 6.8^{b}	1566.6 ± 6.2^{b}	Trace	Trace	6230.4 ± 11.9^{b}	3641.0 ± 17.7^{b}

Values = mean ± standard deviation, n=3; The summary is the sum of calcium, magnesium, phosphorus, potassium and sodium**.** The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other in one table ($p > 0.05$), according to the ANOVA-Turkey's test.

Table 7.6 (c) Macro minerals (calcium, magnesium, phosphorus) of beetroot and carrot pasta

Pasta Type	Calcium mg/kg		Magnesium mg/kg		Phosphorus mg/kg	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Control	248.3 ± 7.4^{j}	243.3 ± 2.7^{h}	320.4 ± 26^{bcd}	246.6 ± 6.1^{bcde}	738.9 ± 9.6^{f}	696.1 ± 8.4^{de}
Beetroot juice 3.08%	306.0 ± 6.4^{gh}	316.6 ± 12.0^{e}	321.6 ± 9.3^{bcd}	239.3 ± 7.6^{de}	808.7 ± 7.0^{de}	676.0 ± 13.4^{ef}
Beetroot puree 3.08%	344.7 ± 7.1^{ef}	350.7 ± 13.5^{d}	324.3 ± 10.6^{bc}	235.9 ± 6.3^{de}	789.3 ± 1.9^{e}	645.7 ± 5.1^{g}
Beetroot puree 4.08%	365.7 ± 5.1^{de}	382.2 ± 9.1^{c}	338.8 ± 5.5^{ab}	257.6 ± 9.3^{abcd}	807.8 ± 6.4^{de}	657.4 ± 5.7^{fg}
Beetroot pomace 3.08%	386.2 ± 13.6^{cd}	393.7 ± 3.4^{cd}	310.1 ± 6.7^{cde}	227.8 ± 7.7^{e}	836.7 ± 7.4^{c}	666.7 ± 6.0^{fg}
Beetroot pomace 4.08%	428.6 ± 4.3^{b}	439.8 ± 2.8^{b}	348.4 ± 5.2^{a}	267.3 ± 4.0^{abc}	848.1 ± 6.5^{c}	659.6 ± 8.1^{fg}
Beetroot pomace 10%	723.9 ± 14.7^{a}	763.9 ± 5.3^{a}	346.8 ± 5.3^{a}	272.6 ± 7.5^{ab}	1005.7 ± 5.0^{a}	667.1 ± 6.5^{fg}
Carrot juice 2.27%	274.5 ± 7.2^{i}	274.3 ± 13.6^{g}	302.8 ± 7.8^{efg}	259.6 ± 11.4^{abcd}	827.0 ± 8.6^{cd}	743.4 ± 5.8^{c}
Carrot puree 2.27%	295.6 ± 5.1^{hi}	295.1 ± 3.8^{fg}	295.8 ± 3.5^{fg}	236.9 ± 9.3^{de}	803.5 ± 8.6^{e}	715.9 ± 9.3^{d}
Carrot puree 3.36%	321.5 ± 6.4^{fg}	345.3 ± 5.2^{d}	308.9 ± 6.3^{cde}	246.4 ± 6.2^{cde}	833.5 ± 10.0^{c}	771.7 ± 3.0^{b}
Carrot pomace 2.27%	380.9 ± 9.2^{cd}	401.5 ± 3.9^{cd}	285.5 ± 4.7^{gh}	225.5 ± 11.5^{e}	774.8 ± 4.8^{e}	739.1 ± 3.0^{c}
Carrot pomace 3.36%	395.9 ± 3.7^{c}	409.0 ± 2.5^{c}	269.9 ± 8.1^{h}	245.3 ± 4.5^{cde}	806.9 ± 6.1^{de}	761.1 ± 10.1^{bc}
Carrot pomace 10%	727.7 ± 8.6^{a}	783.3 ± 6.0^{a}	324.1 ± 5.2^{bc}	276.9 ± 15.6^{a}	928.0 ± 8.1^{b}	843.1 ± 9.5^{a}

Values = mean ± standard deviation, n=3; The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other in one table ($p > 0.05$), according to the ANOVA-Turkey's test.

Table 7.6 (d) Macro minerals (potassium, sodium, summary) of beetroot and carrot pasta

Pasta Type	Potassium mg/kg		Sodium mg/kg		Summary	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Control	358.9 ± 7.4^{k}	Trace	Trace	Trace	1666.6 ± 21.5^{j}	1279.3 ± 19.6^{j}
Beetroot juice 3.08%	1961.7 ± 5.8^{f}	229.5 ± 4.5^{h}	178.5 ± 1.3^{h}	88.66 ± 0.7^{f}	3576.5 ± 27.3^{f}	1590.0 ± 8.0^{h}
Beetroot puree 3.08%	1982.7 ± 9.4^{f}	332.7 ± 11.1^{f}	185.0 ± 2.9^{gh}	92.5 ± 2.0^{f}	3626.0 ± 6.8^{f}	1690.8 ± 11.8^{g}
Beetroot puree 4.08%	2472.5 ± 16.1^{c}	442.4 ± 11.9^{e}	237.8 ± 2.5^{ef}	132.9 ± 5.5^{cd}	4222.8 ± 23.7^{d}	1899.3 ±24.6^{e}
Beetroot pomace 3.08%	2128.5 ± 24.8^{e}	476.8 ± 6.3^{d}	190.7 ± 4.3^{gh}	97.8 ± 2.3^{f}	3852.2 ± 41.4^{e}	1962.9 ± 14.3^{d}
Beetroot pomace 4.08%	2609.8 ± 8.8^{b}	605.9 ± 12.6^{c}	244.0 ± 6.7^{e}	124.0 ± 3.6^{de}	4478.9 ± 12.9^{c}	2183.3 ± 16.2^{c}
Beetroot pomace 10%	5013.7 ± 12.7^{a}	1312.2 ± 12.6^{a}	547.9 ± 4.6^{b}	277.6 ± 11.0^{b}	7638.0 ± 8.1^{a}	3463.4 ± 27.6^{a}
Carrot juice 2.27%	1056.4 ± 7.6^{h}	Trace	265.3 ± 8.3^{d}	141.5 ± 5.1^{c}	2726.0 ± 8.8^{h}	1498.8 ± 18.9^{i}
Carrot puree 2.27%	853.3 ± 28.5^{i}	166.0 ± 5.7^{i}	196.9 ± 4.5^{g}	96.5 ± 2.6^{f}	2445.0 ± 30.0^{i}	1593.7 ± 1.5^{h}
Carrot puree 3.36%	1207.0 ± 8.1^{g}	247.5 ± 3.9^{gh}	283.8 ± 12.9^{c}	136.2 ± 5.5^{cd}	2954.5 ± 30.1^{g}	1797.2 ± 16.0^{f}
Carrot pomace 2.27%	791.0 ± 9.0^{j}	256.1 ± 4.8^{g}	160.3 ± 6.7^{i}	87.7 ± 1.9^{f}	2392.5 ± 16.0^{i}	1766.6 ± 17.1^{f}
Carrot pomace 3.36%	1020.8 ± 4.0^{h}	334.9 ± 10.8^{f}	225.3 ± 6.0^{f}	112.9 ± 3.9^{e}	2718.8 ± 20.3^{h}	1963.2 ± 3.1^{d}
Carrot pomace 10%	2303.6 ± 7.2^{d}	837.1 ± 6.1^{b}	628.3 ± 3.2^{a}	302.8 ± 8.5^{a}	4911.8 ± 7.5^{b}	3106.4 ± 3.0^{b}

Values = mean ± standard deviation, n=3; The summary is the sum of calcium, magnesium, phosphorus, potassium and sodium**.** The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other in one table ($p > 0.05$), according to the ANOVA-Turkey's test.

Table 7.7 Micro mineral (iron & zinc) of vegetable raw materials and spinach pasta

(a) Micro Mineral raw material		
Raw material	Fe mg/kg	Zn mg/kg
Semolina	Trace	Trace
Spinach juice	380.7 ± 4.0^{a}	105.7 ± 3.5^{a}
Spinach puree	175.0 ± 5.6^{b}	98.3 ± 2.1^{b}
Spinach pomace	97.4 ± 1.6^{c}	74.4 ± 1.6^{c}
Red cabbage juice	34.8 ± 1.0^{ef}	Trace
Red cabbage puree	32.4 ± 1.0^{fg}	Trace
Red cabbage pomace	25.8 ± 0.8^{g}	Trace
Beetroot Juice	40.6 ± 0.6^{de}	Trace
Beetroot Puree	42.1 ± 2.7^{d}	Trace
Beetroot Pomace	37.1 ± 1.1^{def}	Trace
Carrot Juice	Trace	Trace
Carrot Puree	Trace	Trace
Carrot Pomace	Trace	Trace

(b) Micromineral spinach pasta samples				
	Fe mg/kg		Zn mg/kg	
	uncooked	cooked	uncooked	cooked
Control	Trace	Trace	Trace	Trace
Spinach juice 1%	3.8 ±1.1^{bA}	3.7 ± 0.9^{bA}	Trace	Trace
Spinach puree 1%	Trace	Trace	Trace	Trace
Spinach puree 2%	3.4 ± 0.9^{bA}	3.6 ± 1.5^{bA}	Trace	Trace
Spinach Pomace 1%	Trace	Trace	Trace	Trace
Spinach Pomace 2%	Trace	Trace	Trace	Trace
Spinach Pomace 10%	9.5 ± 1.2^{aA}	9.59 ± 0.7^{aB}	4.53 ± 0.6^{A}	5.33 ± 0.7^{A}

Values = mean ± standard deviation, n=3; The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other ($p > 0.05$), according to the ANOVA-Turkey's test. The same uppercase superscripted letters within a column are not significantly different from each other ($p > 0.05$).

7.4 Conclusion

The consumption of traditional pasta, semolina-based, is common as a staple food. Nonetheless, pasta's high starch but low antioxidant capacity exhibits a nutritional imbalance. This study showed that incorporating diverse vegetables in their different forms may compensate for the imbalanced nutrition of traditional pasta. The results are encouraging as the nutrients from vegetables could be delivered by vegetable pasta. The water-soluble nutrients, like total phenolic contents and soluble minerals of vegetables, were proven to be superior to traditional pasta. Even though some nutrients, such as soluble minerals and antioxidants, were lost during cooking. The lipid-soluble nutrient β-carotene could also be delivered even though only 1% semolina was substituted. Furthermore, *in vitro* digestion tests showed that vegetable pasta's total phenolic content, β-carotene, and antioxidant capacity are retained in vegetable pasta. Some minerals, such as iron, zinc, and calcium, in vegetable pasta exhibit limited solubility. The low solubility of these minerals may further result in diminished bioavailability. Therefore, vegetable pasta may not be practical for delivering those nutrients. It would be helpful to undertake further research on how to improve the bioavailability of insoluble minerals in vegetable pasta.

Chapter 8

General discussion and conclusion

8.1 Summary

This study utilised spinach, red cabbage, beetroot and carrot in their juice, puree and pomace form and incorporated them in semolina to produce vegetable pasta. Experiments were conducted to evaluate the vegetable pasta samples' physicochemical properties, technical quality, and nutritional characteristics. All the vegetable pasta samples showed better nutritional properties than the traditional pasta. Specifically, the vegetable pasta samples exhibited higher levels of total phenolic content, dietary fibre, and mineral content, as well as a stronger antioxidant capacity. A lower predictive glycaemic response was commonly observed in pasta with a high level of vegetable substitution. The exception was spinach pasta, which showed a lower glycaemic response with only 1% juice substitution. However, high substitution levels of vegetable material caused a significantly lower technical quality of vegetable pasta. At the same lower substitution level, juice and puree-enriched pasta samples generally have better technical quality than pomace-enriched ones. The study exhibits that incorporating vegetable juice or puree to produce pasta results in a superior nutritional profile with a slight decrease in technical quality.

8.2 Discussion

The inclusion of vegetable ingredients in pasta could be a potential strategy for enhancing its functionality. However, many studies have reported a decline in the technical quality of pasta resulting from the addition of vegetables, including reduced sensory acceptability and changes in cooking properties (Hosein, Charles, Ramoo, Prout, & Roberts, 2018; Jalgaonkar et al., 2018; Simonato et al., 2019). Chapters 4 and 5 showed that vegetable pasta with high substitution levels (10% in this study) generally had significantly lower quality compared to the one with a lower substitution level. This observation is consistent with some other studies (Bouacida et al., 2017; Cárdenas-Hernández et al., 2016; Mridula, Gupta, Bhadwal, Khaira, et al., 2016; Simonato et al., 2019).

It is widely accepted that protein content plays a key role in pasta structure (Peressini et al., 2020). In pasta, protein gluten entraps the starch granules and acts as the backbone of the pasta structure (Ooms & Delcour, 2019). Thus, a high technical quality of pasta was maintained. In contrast, vegetable materials contain more dietary fibre than semolina, as reported in chapter 6. Dietary fibre has also been frequently reported to impact the quality of enriched pasta due to its ability to disrupt the gluten network and compete for the water with starch granules (Foschia et al., 2015a; Padalino et al., 2017; Peressini et al., 2020). High substitution of semolina with vegetable material introduces vegetable fibres and dilutes the gluten content, resulting in the lower technical quality of vegetable pasta. In addition, juice, puree, and pomace behave differently when incorporated into the pasta formulation. Those differences could be partially due to the phytochemical composition of various forms of vegetables. For example, spinach juice contains more protein but less fibre than spinach pomace, which may explain why spinach juice 1% has lower cooking losses and better elasticity and firmness than spinach pomace 1%. Moreover, spinach pasta had higher firmness, elasticity and lower cooking loss, or generally better technical quality than other vegetable pasta. This is potentially due to its high-protein and relatively low-fibre nature.

Chapter 6 reported the starch-digestibility of pasta made from durum wheat semolina, which was partially substituted by puree, juice or pomace from spinach, red cabbage, beetroot and carrot. Part of the pasta samples exhibited lower predictive glycaemic response compared to the control. Those included 10% substitution pasta samples (spinach pomace 10%, red cabbage pomace 10%, beetroot pomace 10% and carrot pomace 10%), some other spinach pasta samples (spinach juice 1%, spinach puree 2%) and one other beetroot sample (beetroot puree 4.08%). A significant reason for spinach juice 1% and puree 2% exhibiting lower glycaemic response may be α-amylase. This study showed that spinach ranked the highest α-amylase inhibition effect compared with other vegetables (red cabbage, beetroot and carrot). Barkat et al. (2018) have reported a high α-amylase inhibition ability (comparable to acarbose, a known α-amylase inhibitor) of spinach materials. Among vegetable materials, juice has a higher α-amylase inhibition effect compared to puree and pomace. This may be because of juice's higher phenolic compounds than puree and pomace (as reported in chapter 7). The

α-amylase inhibition activity is likely due to the presence of phenolic compounds (Barkat et al., 2018). Certain phenols are capable of interacting with α-amylase enzymes, resulting in the blockage of their catalytic sites and a consequent reduction in their activity (Takahama & Hirota, 2018).

All 10% pomace pasta samples showed a lower glycaemic response, indicating a certain substitution level may be required to achieve a lower glycaemic response. Chusak et al. (2020) substituted semolina with gac fruit (*Momordica cochinchinensis*) powder and found a significantly decreased glycaemic response when 10–15% semolina was replaced with fruit powder, while the same glycaemic response compared to control was found with 5% semolina replacement. The correlation test was positive for starch content and glycaemic response. Other studies also reported similar correlations (Mu et al., 2021; Peressini et al., 2020), indicating that the starch content is an essential factor that affects the glycaemic response. Dietary fibre could also be an important factor influencing vegetable pasta's glycaemic response. The work of Chau et al. (2004) showed that fibre could reduce post-prandial serum glucose by hindering the diffusion of glucose, retarding α-amylase action. Brennan and Tudorica (2008) reported that dietary fibre could alter the water-binding activity of pasta samples, inhibiting starch swelling during cooking and influencing starch digestion. Results showed that dietary fibre is negatively correlated with a glycaemic response. Dietary fibre and starch results illustrate that vegetable addition cause less starch but more dietary fibre in the resultant pasta, which further leads to lower glycaemic response.

Chapter 7 reported the nutritional value of vegetable pasta which is produced by various forms of vegetables, including puree, juice, and pomace. Higher total phenolic content (TPC) and antioxidant capacity were found in all vegetable pasta for uncooked, cooked and digested samples, indicating that vegetable incorporation can improve pasta's total phenolic contents and antioxidant ability. Cooked pasta tends to have a lower phenolic content and antioxidant capacity than uncooked pasta in all samples. Similar results were reported by some other researchers (Carini et al., 2012; Sęczyk et al., 2016; Shreenithee & Prabhasankar, 2013). It is suggested that phenolic compounds and water-soluble antioxidants could be lost to cooking water during cooking (Lu et al., 2018) or undergo

thermal degradation (Fares et al., 2010). After digestion, the phenolic contents of all vegetable pasta samples increased dramatically compared to cooked pasta. Other studies presented similar observations (Koehnlein et al., 2016; Padalino et al., 2019). The authors explained that *in vitro* digestion could release conjugated phenolics and improve the total phenolic content. This study showed that *in vitro* digestion decreased the difference in phenolic contents between samples. For example, cooked carrot pomace 10% has much higher total phenolic contents than cooked carrot puree 3.36% (495.18 mg GAE/100 g vs 316.17 mg GAE/100 g). However, after *in vitro* digestion, there is no significant difference between them (1979 mg GAE/100 g of carrot pomace 10% vs 1913 mg GAE/100 g of carrot puree 3.36%, $p \geq 0.05$). The finding may suggest that vegetable pasta formulations with low substitution levels are more practical compared to those with high substitution levels. This can be attributed to the fact that vegetable pasta formulations with low substitution levels incur minimal sacrifice in phenolic content while exhibiting superior technical quality attributes (as reported in Chapters 3 and 4) when compared to high-substitution level samples.

As for antioxidant capacity, inconsistent results were shown after *in vitro* digestion. Improved antioxidant capacity was found in spinach, beetroot and carrot pasta, while decreased antioxidant capacity was found in red cabbage pasta. The improved antioxidant capacity could be due to the depolymerisation of antioxidant phytochemicals (Bustos et al., 2020; Ziółkiewicz et al., 2023). The decreased antioxidant capacity of red cabbage pasta may be due to low anthocyanin antioxidant activity at high pH conditions of pancreatic digestion (McDougall et al., 2007). The β-carotene test showed that juice and puree-enriched pasta contain more bioavailable β-carotene content than pomace-enriched ones when at the same substitution level. It is potentially because of a long time oven air drying to produce pomace, which leads to degradation and isomerisation of β-carotene (Marx et al., 2003). In addition, juice and puree-enriched pasta trends to contain more phenolic contents and antioxidant capacity compared to pomace-enriched pasta. Those results suggested that puree and juice may be more suitable to produce vegetable pasta than pomace in regard to improving the nutritional value of vegetable pasta.

Chapter 7 also reported the mineral content of vegetable pasta. The mineral content of vegetable pasta was significantly higher than the control. At the same substitution level, juice-enriched pasta tends to have more bioaccessible minerals than puree and pomace-enriched ones for spinach and red cabbage pasta. In contrast, pomace-enriched pasta contains the highest bioaccessible minerals for beetroot and carrot pasta, while juice-enriched pasta contains the lowest when the substitution level is the same. These results showed that the impact of different vegetables (and their various forms) on the mineral content of vegetable pasta is not uniform. The actual bioavailability of insoluble minerals (such as calcium, iron and zinc) was questioned by some authors (Chasapis et al., 2020; Luo & Xie, 2012). However, other authors suggested that soluble minerals such as potassium and sodium from vegetable materials are highly bioavailable (Stone et al., 2016). Although some soluble minerals are lost during cooking, cooked vegetable pasta still contains more soluble minerals than the control.

8.3 Recommendations for future work

This study showed that both vegetable juice, puree and pomace could improve the nutritional value of vegetable pasta. At the same low substitution level (from 1 to 4.1%), juice and puree-enriched pasta generally showed better nutritional value and technical quality than pomace-enriched ones. However, the physical limitations make a high-level substitution of juice and puree challenging. Future studies could apply concentration technology (such as evaporation and reserve osmosis) to vegetable juice and puree. Then a higher solid content of juice and puree could make it practicable to improve the substitution level. Thus, further nutritional improvement of vegetable pasta could be expected. However, with the increased vegetable addition, the impact of bound water from hydration water could be significant. Evaluation of free water and bound water of vegetable pasta or changing the formula to keep the free water constant (instead of total water) could be suggested. The technical quality and sensory test of that concentrated vegetable-enriched pasta needs to be studied to ensure acceptable quality. Furthermore, *in vivo* tests for the bioavailability of nutrients

could be used to give a more comprehensive understanding of the nutritional value of vegetable pasta.

Appendix A Demonstration of equations

According to many studies, typically, pasta hydration level is between 27 ml-33 ml water per 100g solid material. 30 ml water per 100g solid is a widely accepted level. In that case, the study set hydration at 30 ml per 100g solid material for all the samples. The maximum substitution (or "upper limit") was calculated based on this.

The juice or puree itself contained a high amount of water. So the maximum substitution is that all the hydration water comes from juice or puree, it means if added more juice, or puree, water will exceed the aim hydration level (when the hydration level was set at 30 ml water per 100 g solid).

So the equation based on following:

Pasta formula 100g solids + 30 ml (g) water.

Then come to the equations:

In Equation 2: $(100 - \boldsymbol{a})$= water content in juice (g/100 g), so $\frac{x(100-\boldsymbol{a})}{100}$ is water provided by juice or puree as x is the weight (gram) of juice/puree. As described above, the upper limit comes from all hydration water comes from puree or juice so $\boldsymbol{h} = \frac{x(100-\boldsymbol{a})}{100}$ this is **Equation 2** written in another form.

Equation 1 is based on hydration defined as 100 g solid content combined with $\boldsymbol{h}$ mL(g) water, it means, based on the definition of hydration, 100 g solid in total. $\boldsymbol{a}x/100$ is the solid content from juice or puree y is the weight of semolina. Solid from semolina + solid from juice or puree contribute to total solid. So $y + \boldsymbol{a}x/100 = 100$

Equation 3 is substitution level $\boldsymbol{s} = 100 - y$ it should be noticed that this s based on all hydration water comes from juice and puree.

Equations 1-3 aim to confirm that substitution level $\boldsymbol{s}$ is achievable.

For example, assuming that carrot juice contains 94 g/100 g water, it contains 100-94 = 6 g/100 g ($\boldsymbol{a}$) solid. To achieve the upper limit of substitution. All hydration water comes from carrot juice, put **Equation 2** into use $\boldsymbol{h} = \frac{x(100-\boldsymbol{a})}{100}$ will be $\mathbf{30} = \frac{x(100-\mathbf{6})}{100} \rightarrow x = 30 \div 0.94 = 31.91$ then input data to **Equation 1** $y = 100 - \frac{30 \div 0.94 \times 6}{100} = 98.09$ put y into **Equation 3**, the substitution level (upper limit) $\boldsymbol{s = 100 - 98.09 = 1.91}$ **, it means to assume that carrot juice contains 94g/100 g water, the upper limit of substitution is 1.91 g/100 g when hydration level set as 30 g/100 g**

Because all hydration water already comes from juice, this 1.91 g/100 g will be the upper limit if set the hydration level at 30 mL/100g.

It also means that if not concentrated carrot juice, and if the substitution level is set above 1.91 g/100 g, for example, 3 g/100 g, it will be unachievable. **Equation 4** is used to calculate if you want to achieve a certain substitution level s. The solid content of juice or puree should be higher than $\boldsymbol{a}$. Otherwise, it will be unachievable.

Appendix B Reagent detail

0.04M dinitro-salicylic acid (DNS) solution

VOLUME OF DNS SOLUTION	200 ML	100 ML
NAOH	**6.4 g in 80 mL H_2O**	3.2 g in 40 mL H_2O
DNS	**2 g in 80 mL 2M NaOH**	1 g in 40 mL 2M NaOH
POTASSIUM SODIUM TARTRATE TETRAHYDRATE	**60 g and make up to 100 mL with H_2O**	30 g and make up to 50 mL with H_2O

Appendix C Detailed dietary fibre data

(a) spinach pasta dietary fibre						
	SDF g/100 g dm		IDF g/100 g dm		TDF g/100 g dm	
Sample	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Control	$1.48 \pm 0.04^{d,A}$	$1.46 \pm 0.18^{c,A}$	$2.13 \pm 0.24^{d,A}$	$2.26 \pm 0.12^{d,A}$	$3.60 \pm 0.20^{d,A}$	$3.72 \pm 0.30^{d,A}$
Spinach juice 1%	$1.58 \pm 0.18^{cd,A}$	$1.58 \pm 0.01^{c,A}$	$2.27 \pm 0.27^{cd,A}$	$2.30 \pm 0.29^{d,A}$	$3.85 \pm 0.29^{cd,A}$	$3.88 \pm 0.28^{cd,A}$
Spinach puree 1%	$1.57 \pm 0.01^{cd,A}$	$1.58 \pm 0.03^{c,A}$	$2.35 \pm 0.07^{cd,A}$	$2.37 \pm 0.04^{cd,A}$	$3.92 \pm 0.08^{cd,A}$	$3.95 \pm 0.07^{cd,A}$
Spinach puree 2%	$1.66 \pm 0.05^{c,A}$	$1.63 \pm 0.05^{bc,A}$	$2.57 \pm 0.10^{bc,A}$	$2.63 \pm 0.06^{bc,A}$	$4.23 \pm 0.05^{c,A}$	$4.26 \pm 0.11^{c,A}$
Spinach pomace 1%	$1.65 \pm 0.07^{c,A}$	$1.66 \pm 0.07^{bc,A}$	$2.49 \pm 0.13^{bc,A}$	$2.51 \pm 0.03^{cd,A}$	$4.13 \pm 0.19^{c,A}$	$4.17 \pm 0.09^{c,A}$
Spinach pomace 2%	$1.83 \pm 0.06^{b,A}$	$1.89 \pm 0.12^{b,A}$	$2.86 \pm 0.06^{b,A}$	$2.93 \pm 0.04^{b,A}$	$4.69 \pm 0.12^{b,A}$	$4.78 \pm 0.09^{b,A}$
Spinach pomace 10%	$3.13 \pm 0.05^{a,A}$	$3.13 \pm 0.02^{a,A}$	$5.68 \pm 0.12^{a,B}$	$5.89 \pm 0.13^{a,A}$	$8.82 \pm 0.07^{a,B}$	$9.01 \pm 0.11^{a,A}$

Values = mean ± standard deviation, n=3; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; TDF, total dietary fibre; The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other ($p > 0.05$), according to the ANOVA-Turkey's test. The same uppercase superscripted letters within a column are not significantly different from each other between cooked and uncooked samples ($p > 0.05$).

(b) red cabbage pasta dietary fibre						
	SDF g/100 g dm		IDF g/100 g dm		TDF g/100 g dm	
Sample	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Control	$1.48 \pm 0.04^{d,A}$	$1.46 \pm 0.18^{d,A}$	$2.13 \pm 0.24^{e,A}$	$2.26 \pm 0.12^{f,A}$	$3.60 \pm 0.20^{e,A}$	$3.72 \pm 0.30^{f,A}$
Red cabbage juice 1%	$1.61 \pm 0.03^{bcd,A}$	$1.65 \pm 0.18^{bcd,A}$	$2.37 \pm 0.11^{de,A}$	$2.40 \pm 0.15^{ef,A}$	$3.99 \pm 0.09^{d,A}$	$4.04 \pm 0.09^{ef,A}$
Red cabbage puree 1%	$1.59 \pm 0.02^{cd,A}$	$1.57 \pm 0.04^{cd,A}$	$2.52 \pm 0.15^{cd,A}$	$2.58 \pm 0.04^{de,A}$	$4.11 \pm 0.13^{d,A}$	$4.16 \pm 0.08^{de,A}$
Red cabbage puree 2%	$1.70 \pm 0.05^{bcd,A}$	$1.77 \pm 0.08^{bc,A}$	$2.75 \pm 0.06^{c,A}$	$2.86 \pm 0.05^{c,A}$	$4.45 \pm 0.01^{c,A}$	$4.63 \pm 0.04^{c,A}$
Red cabbage pomace 1%	$1.74 \pm 0.05^{bc,A}$	$1.68 \pm 0.11^{bcd,A}$	$2.69 \pm 0.11^{cd,A}$	$2.78 \pm 0.03^{cd,A}$	$4.43 \pm 0.15^{c,A}$	$4.47 \pm 0.14^{cd,A}$
Red cabbage pomace 2%	$1.84 \pm 0.08^{b,A}$	$1.87 \pm 0.03^{b,A}$	$3.32 \pm 0.13^{b,A}$	$3.43 \pm 0.10^{b,A}$	$5.16 \pm 0.05^{b,A}$	$5.29 \pm 0.07^{b,A}$
Red cabbage pomace 10%	$3.34 \pm 0.23^{a,A}$	$3.59 \pm 0.18^{a,A}$	$8.01 \pm 0.13^{a,B}$	$8.50 \pm 0.07^{a,A}$	$11.45 \pm 0.10^{a,B}$	$12.07 \pm 0.11^{a,A}$

Values = mean ± standard deviation, n=3; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; TDF, total dietary fibre; The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other ($p > 0.05$), according to the ANOVA-Turkey's test. The same uppercase superscripted letters within a column are not significantly different from each other between cooked and uncooked samples ($p > 0.05$).

(c) beetroot pasta dietary fibre						
	SDF g/100 g dm		IDF g/100 g dm		TDF g/100 g dm	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Control	$1.48 \pm 0.04^{e,A}$	$1.46 \pm 0.18^{d,A}$	$2.13 \pm 0.24^{e,A}$	$2.26 \pm 0.12^{f,A}$	$3.60 \pm 0.20^{e,A}$	$3.72 \pm 0.30^{e,A}$
Beetroot juice 3.08%	$1.73 \pm 0.06^{cd,A}$	$1.74 \pm 0.02^{bc,A}$	$2.47 \pm 0.02^{d,A}$	$2.48 \pm 0.05^{e,A}$	$4.19 \pm 0.07^{d,A}$	$4.21 \pm 0.04^{d,A}$
Beetroot puree 3.08%	$1.65 \pm 0.05^{d,A}$	$1.66 \pm 0.05^{cd,A}$	$2.61 \pm 0.08^{d,A}$	$2.65 \pm 0.05^{de,A}$	$4.27 \pm 0.14^{d,A}$	$4.31 \pm 0.10^{d,A}$
Beetroot Puree 4.08%	$1.67 \pm 0.02^{d,A}$	$1.65 \pm 0.06^{cd,A}$	$2.77 \pm 0.02^{d,A}$	$2.78 \pm 0.01^{d,A}$	$4.44 \pm 0.04^{d,A}$	$4.45 \pm 0.07^{d,A}$
Beetroot Pomace 3.08%	$1.86 \pm 0.06^{bc,A}$	$1.86 \pm 0.05^{bc,A}$	$3.81 \pm 0.04^{c,A}$	$3.87 \pm 0.06^{c,A}$	$5.67 \pm 0.10^{c,A}$	$5.74 \pm 0.01^{c,A}$
Beetroot Pomace 4.08%	$1.96 \pm 0.06^{b,A}$	$1.94 \pm 0.05^{b,A}$	$4.43 \pm 0.17^{b,A}$	$4.54 \pm 0.13^{b,A}$	$6.39 \pm 0.23^{b,A}$	$6.48 \pm 0.17^{b,A}$
Beetroot Pomace 10%	$2.69 \pm 0.09^{a,A}$	$2.71 \pm 0.13^{a,A}$	$7.94 \pm 0.16^{a,B}$	$8.67 \pm 0.07^{a,A}$	$10.62 \pm 0.07^{a,B}$	$11.38 \pm 0.05^{a,A}$

Values = mean ± standard deviation, n=3; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; TDF, total dietary fibre; The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other ($p > 0.05$), according to the ANOVA-Turkey's test. The same uppercase superscripted letters within a column are not significantly different from each other between cooked and uncooked samples ($p > 0.05$).

(d) carrot pasta dietary fibre						
	SDF g/100 g dm		IDF g/100 g dm		TDF g/100 g dm	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Control	$1.48 \pm 0.04^{d,A}$	$1.46 \pm 0.18^{d,A}$	$2.13 \pm 0.24^{e,A}$	$2.26 \pm 0.12^{e,A}$	$3.60 \pm 0.20^{e,A}$	$3.72 \pm 0.30^{e,A}$
Carrot juice 2.27%	$1.75 \pm 0.08^{c,A}$	$1.74 \pm 0.03^{c,A}$	$2.28 \pm 0.10^{d,A}$	$2.30 \pm 0.03^{de,A}$	$4.02 \pm 0.19^{d,A}$	$4.04 \pm 0.01^{d,A}$
Carrot puree 2.27%	$1.74 \pm 0.05^{c,A}$	$1.74 \pm 0.04^{c,A}$	$2.36 \pm 0.08^{d,A}$	$2.42 \pm 0.08^{de,A}$	$4.10 \pm 0.13^{d,A}$	$4.17 \pm 0.12^{d,A}$
Carrot puree 3.36%	$1.84 \pm 0.05^{c,A}$	$1.81 \pm 0.02^{c,A}$	$2.36 \pm 0.13^{d,A}$	$2.55 \pm 0.08^{d,A}$	$4.20 \pm 0.08^{d,A}$	$4.36 \pm 0.07^{d,A}$
Carrot pomace 2.27%	$1.84 \pm 0.08^{c,A}$	$1.89 \pm 0.05^{c,A}$	$3.28 \pm 0.12^{c,A}$	$3.38 \pm 0.12^{c,A}$	$5.13 \pm 0.02^{c,A}$	$5.26 \pm 0.17^{c,A}$
Carrot pomace 3.36%	$2.06 \pm 0.09^{b,A}$	$2.15 \pm 0.08^{b,A}$	$3.81 \pm 0.15^{b,A}$	$3.97 \pm 0.14^{b,A}$	$5.86 \pm 0.24^{b,A}$	$6.11 \pm 0.22^{b,A}$
Carrot pomace 10%	$3.45 \pm 0.08^{a,A}$	$3.43 \pm 0.03^{a,A}$	$8.01 \pm 0.17^{a,B}$	$8.75 \pm 0.09^{a,A}$	$11.46 \pm 0.09^{a,B}$	$12.18 \pm 0.12^{a,A}$

Values = mean ± standard deviation, n=3; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; TDF, total dietary fibre; The same lowercase superscripted letters within a row of the same kind of pasta or raw materials are not significantly different from each other ($p > 0.05$), according to the ANOVA-Turkey's test. The same uppercase superscripted letters within a column are not significantly different from each other between cooked and uncooked samples ($p > 0.05$).

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