The utilisation of grape pomace as a value added ingredient: The role of grape phenolic compounds on functional foods

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by

Khanh Nhu Tran

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Abstract

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Grape pomace, the residue obtained after making wine, contains large quantities of polyphenols, dietary fibre, and has a high antioxidant capacity. The contents of the valuable compositions in grape pomace are believed to be much higher than the ones extracted from the grape to wine. The main polyphenols detected in grape pomace include hydroxycinnamic acids, hydroxybenzoic acids, flavan-3-ols, flavonols, and stilbenes. Cereal and cereal-based products are popular foods all over the world. However, they have a high carbohydrate content and a low polyphenol and antioxidant content. In addition, too high an intake of these highly digestible starch foods is believed to be involved in global issues of obesity and high blood sugar levels. Consumers' demand for healthier and natural origin products has been increasing in recent decades, along with the awareness of the importance of "fresh" products. Hence, the use of plant parts to fortify cereal-based products has attracted much research, resulting in proven benefits to human health. Grape pomace has been added to many types of product, including starchy food and dairy products. Overall, cereal-based products have been confirmed to be an apparopriate food matrix to apply grape pomace based on its nutritional and physical characteristics.

In this study, three white grape pomaces (Sauvignon Blanc, Pinot Gris, and Gewürztraminer) and two red grape pomace (Merlot and Pinot Noir) obtained from a New Zealand winery were the main objects. Analysis of compositions of these grape pomaces showed a high content of total phenolic compounds, in which the red grape pomaces had higher concentrations than the white ones. Between varieties, Pinot Noir had the highest content of phenolic compounds, followed by Merlot, Pinot Gris, and Gewürztraminer, while Sauvignon Blanc possessed the lowest value. The results also showed that the concentration ranges of total phenolic content found in New Zealand grape pomaces were similar to the values found in grape pomaces obtained elsewhere such as Brazil, France or Italy. Further analysis by HPLC found that catechin and epicatechin were the most abundant phenolics in all pomaces. A significant concentration of malvidin-3-o-glucoside was found in Merlot and Pinot Noir pomaces, but was not detected in white pomaces. The antioxidant capacity was reflected in the total phenolic content of each pomace with the red pomaces showed a better antioxidant capacity than the white pomaces.The red pomaces had a significantly higher content of both soluble and insoluble dietary fibre content. In contrast, white pomaces had a higher sugar content than the red pomaces.

Replacement of wheat flour with different levels (5 %, 10 %, and 15 %) of grape pomace powder was performed to investigate the change in physicochemical and nutritional properties of cookies. Inclusion of pomace powder caused a significant reduction ($p < 0.05$) of both the thickness and diameter of the cookies. The cookies hardness decreased significantly ($p < 0.05$) with increased supplement levels, but there was no difference ($p > 0.05$) in the reduction between grape varieties. The colour of cookies also changed significantly ($p < 0.05$), they became darker with higher levels of addition (L^* value decreased), the increase of a^* value and decrease of b^* value indicated that the final products got redder and bluer.

The total phenolic content of cookies increased with increasing of pomace addition. The results of scavenging capacity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric ion reducing antioxidant power (FRAP) were in agreement with the phenolic content of cookies. The amount of sugar released during the *in vitro* digestion of cookies was attenuated due to the fortification with grape pomaces. Samples with the addition of 15% Pinot Noir and Merlot showed the greatest reduction in reducing sugar release. All other samples reduced the content of sugar in comparison with the control sample.

Fortification of rice starch using grape pomace powder was conducted by using a rapid visco-analyser (RVA) to investigate their impacts in another type of food matrix, namely a paste. Pasting properties of paste significantly changed ($p < 0.05$) with the addition of pomace powder. The peak viscosity, breakdown, final viscosity, and setback of the pastes decreased significantly ($p < 0.05$), and the pasting temperature increased. The pasting profile of starch is defined by the apparent ratio of amylose to amylopectin; hence the inclusion of grape pomace powder changed this ratio and the final pasting profile. In an *in vitro* digestion, the general trend of reducing sugar released was similar to the control paste and pastes with pomace powder, but the actual amounts of reducing sugars released were lower than the control. A higher reduction was observed in samples with Pinot Noir powder. Such reductions were attributed to the content of polyphenols and dietary fibre.

The influences of polyphenols from grape pomaces were tested in the mean of extracts instead of traditional powder. The changes in pasting profile were similar to the changes observed with grape pomace powder, means that peak viscosity, breakdown, final viscosity and setback decreased with the increase of added level of polyphenol extracts. The temperature increased but the range of variation was much lower than the ones with pomace powder inclusion. The extracts showed the similar impacts to reduce sugar released during *in*

vitro digestion process in comparison with pomace powder enrichment. In addition, the total phenolic content and antioxidant capacity before and after digestion were in similar trend with pomace powder samples did. The α-amylase inhibitory was also tested on these samples, with the highest inhibition rate was attributed to Pinot Noir (64.3%)

Keywords: grape pomace, Sauvignon Blanc, Pinot Gris, Gewürztraminer, Merlot, Pinot Noir, grape polyphenols, antioxidant capacity, dietary fibre, cereal-based product, cookies, paste, glycaemic index, α-amylase inhibition

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Abbreviations list

GP	grape pomace
GPs	grape pomaces
GPP	grape pomace powder
GPE	grape pomace extract
GPPs	grape pomace powders
GPEs	grape pomace extracts
WGP	white grape pomaces
RGP	red grape pomaces
RGPP	red grape pomace powder
WGPP	white grape pomace powder
SA	Sauvignon Blanc
PG	Pinot Gris
GE	Gewürztraminer
ME	Merlot
PN	Pinot Noir
SA5	Sample with 5% replacement of Sauvignon Blanc (powder or extract)
PG5	Sample with 5% replacement of Pinot Gris (powder or extract)
GE5	Sample with 5% replacement of Gewürztraminer (powder or extract)
ME5	Sample with 5% replacement of Merlot (powder or extract)
PN5	Sample with 5% replacement of Pinot Noir (powder or extract)
SA10	Sample with 10% replacement of Sauvignon Blanc (powder or extract)
PG10	Sample with 10% replacement of Pinot Gris (powder or extract)
GE10	Sample with 10% replacement of Gewürztraminer (powder or extract)
ME10	Sample with 10% replacement of Merlot (powder or extract)
PN10	Sample with 10% replacement of Pinot Noir (powder or extract)
SA15	Sample with 15% replacement of Sauvignon Blanc (powder or extract)
PG15	Sample with 15% replacement of Pinot Gris (powder or extract)
GE15	Sample with 15% replacement of Gewürztraminer (powder or extract)
ME15	Sample with 15% replacement of Merlot (powder or extract)
PN15	Sample with 15% replacement of Pinot Noir (powder or extract)
TPC	total phenolic content
AC	antioxidant capacity
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
FRAP	Ferric ion reducing antioxidant power
GAE	gallic acid equivalent
DF	dietary fibre
IDF	insoluble dietary fibre

SDF	soluble dietary fibre
TDF	total dietary fibre
DM	dried matter
mhl	million hectolitres
OIV	International Organisation of Vine and Wine
UAE	ultrasound assisted extraction

Chapter 1

Introduction

1.1 Background

Grapes (*Vitis vinifera* L.) are one of the world's largest fruit crop which in 2018 were cultivated in 7.4 million hectares globally, producing 77.8 million tonnes of grapes (International Organisation of Vine and Wine, 2019). The production includes 7% of dried grapes, 36% of table grape and the rest (57%) was grown for wine grape. The total volume of world wine production was around 292 million hectolitres (mhL), generating a trade of approximately 30 billion euros in 2018 (International Organisation of Vine and Wine, 2019). Data also stated that global wine consumption rose from 240 mhL in 2014 to 245 mhL in 2018 and that wine consumption is still growing. The popularity of wine may be attributed to the "French paradox" theory, which suggests that a moderate intake of alcohol, especially wine, may help to prevent cardiovascular diseases (Renaud & de Lorgeril, 1992). This theory has been confirmed by a number of researchers (Castaldo *et al*., 2019; Haseeb, Alexander, & Baranchuk, 2017; Tariba, 2011).

In the process of winemaking, the global winemaking industry discards millions of tonnes of grape pomace annually, including grape skins (60%), seeds (15%), and minor quantities of stems and pulps of the grape (Domínguez, Sanchez-Hernandez, & Lores, 2017; Nawaz, Shi, Mittal, & Kakuda, 2006). It has been estimated that the total annual production of grape pomace worldwide may account for 30% in weight of input materials (Teixeira *et al*., 2014), which in turn, creates problems of waste treatment. Figure 1.1 illustrates the quantity of grape pomace remaining after the winemaking process. Modern techniques have been applied to solve this matter. A long-standing and common way is the use of grape pomace as soil fertilisers, but the high levels of phenolics may lead to the prevention of seed germination (Kammerer, Claus,

Carle, & Schieber, 2004). Hence, grape pomace is only used in limited amounts only for this purpose.

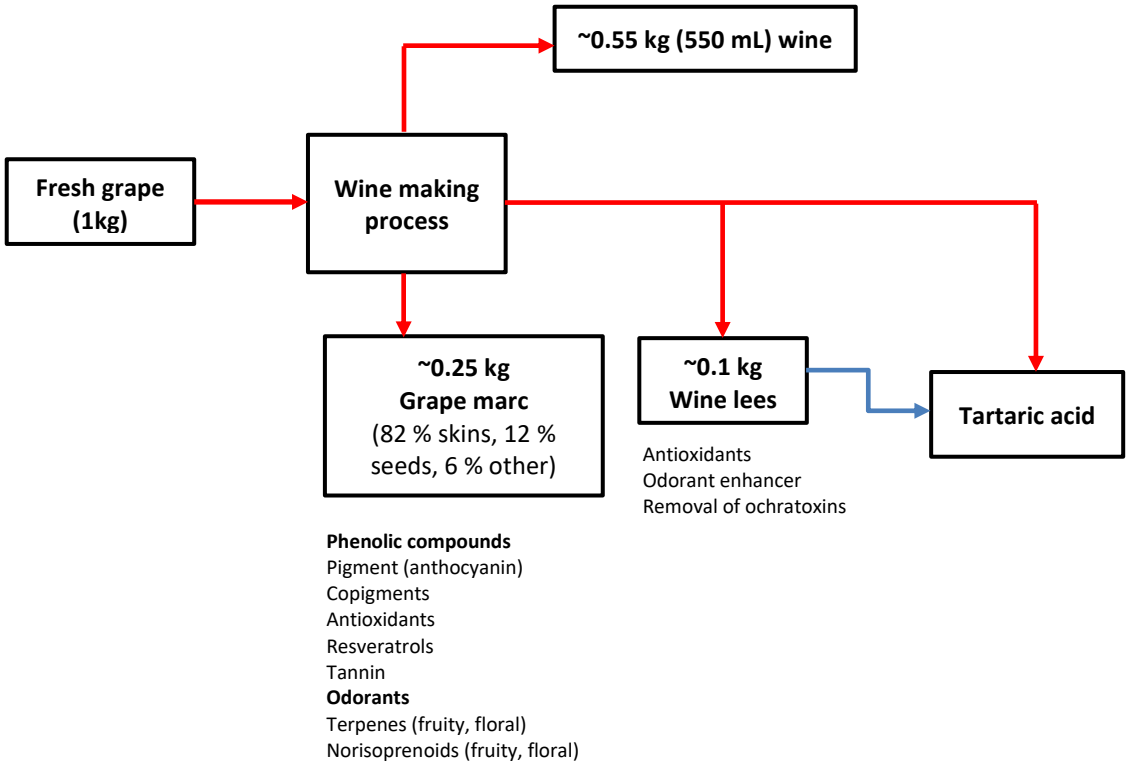


Figure 1.1. Yield of primary winemaking residues and substances obtained

Adapted from Pedroza, Salinas, Alonso, & Zalacain, (2017)

Grape pomace has also been added to animal feeds, but the high content of lignin in grape pomace has been shown to cause a reduction in animal digestion ability as the lignins inhibit cellulolytic and proteolytic enzymes while increasing rumen bacteria (Fontana, Antoniolli, & Bottini, 2013). In addition, while some researchers have considered grape pomace to be a good source of antioxidants, others have claimed that it has no nutritional value for domestic animals (Spigno, Marinoni, & Garrido, 2017). Despite some drawbacks, the benefits of grape pomace are high and many teams have focused on waste recovery from grape pomace production. The Raisinor France introduced ED95, bioethanol, that is derived from grape marc, which is compatible with the diesel engine cycle. In Australia, grape pomace has been used to produce

compost and tartaric acids, commercially. The WholeWine company in the USA has produced sixteen different flours based on varieties of grape pomace. However, extraction of bioactive compounds from grape pomace is one the most favourable options as it maintains the bioactivity of target molecules, preserves the high quantity and quality of bioactive compounds, and gives high market value for final products (Ribeiro *et al*., 2015b; Spigno *et al*., 2017; Teixeira *et al*., 2014; Teixeira, Eiras-Dias, Castellarin, & Gerós, 2013a).

It has been estimated that only 35 – 40 % of grape phenolic compounds are transferred to the wine, which means that 60 – 65% are left in the pomace after the winemaking process (Baoshan, Ricardo-da-Silva, & Spranger, 2001). The general phytochemical profile of grape pomace has previously been characterised confirming the presence of various valuable constituents that possess health-promoting effects and other positive impacts on food systems. Typical classes of phenolic compounds found in grape pomace include flavanols, flavonols, anthocyanins, stilbenes, and hydroxybenzoic acids (Fontana *et al*., 2013). The levels of bioactive compounds in grape pomace have been shown to be higher than in the by-products of many other agricultural products such as cider apple pomace, strawberry residues, or pear residues (Deng, Penner, & Zhao, 2011). Despite the abundance of valuable compounds, the recovery and utilisation of grape pomace remain problematic due to a number of obstacles such as the limitation of extraction methods, or the natural transformation of compounds during processing. For example, the high temperatures required in the Soxhlet extraction technique degrades the quality of phenolic compounds (Hogervorst, Miljić, & Puškaš, 2017). Nonetheless, many promising schemes have been introduced to effectively exploit this natural source of compounds (Fontana *et al*., 2013; Galanakis, 2012; Teixeira *et al*., 2014).

Along with raising awareness and customer demand for natural products related to food and human health, grape pomace has shown potential to be a natural additive to replace synthetic

chemicals which are currently used in the food industry, pharmaceuticals and cosmetics. In the food industry, grape pomace products have been proven to prevent lipid oxidation and thus prolong the storage life of various products such as beef patties (García-Lomillo *et al*., 2017), chicken (Sampaio, Saldanha, Soares, & Torres, 2012; Sáyago-Ayerdi, Brenes, & Goñi, 2009), and fish (Maestre, Micol, Funes, & Medina, 2010; Pazos, Gallardo, Torres, & Medina, 2005). Enrichment of food ingredients is another possibility, with a number of papers reporting that a small amount of grape pomace powder added to food during processing may lead to the improvement of food components and shelf life extension (Hoye & Ross, 2011; Martin-Carron, García-Alonso, Goñi, & Saura-Calixto, 1997). In terms of pharmaceutical uses, recent studies have illustrated the potential activity of grape pomace extracts in the prevention of cancer (Pino-García *et al*., 2017), gastrointestinal and colonic digestion (Pino-García, González-SanJosé, Rivero-Pérez, García-Lomillo, & Muniz, 2016). The effect of grape pomace extracts in antimicrobial strategies have also been studied, with evidence of the extracts of grape pomace being able to prevent, or inhibit, harmful organisms such as *E. coli* (Ahn, Gru, & Mustapha, 2015) or *Pseudomonas* (Lorenzo, Sineiro, Amado, & Franco, 2014).

This thesis has a dual focus. Firstly, it investigated the phytochemicals of New Zealand grape pomace using five grape varieties collected from the winery at Lincoln University, New Zealand. Secondly, it examined the possibility of incorporation of grape pomace into two food matrices: cookies and boiling starch, in regards to their bioavailability and bioaccessibility. In this study, pomace was used from three white grape varieties and two red grape varieties. All of these grape varieties belong to the *V. vinifera* L., the common grapevine, which is the species native to the Mediterranean region, Central Europe and southwestern Asia. Normal grapevines are liana with the length up to 32 m long with flaky bark, alternate and palmately lobed leaves. The fruit is a berry, known as grape, with the diameter of up to 6 mm for wild species, and 30 mm

for cultivated ones. Grapes may be green, red or purple in colour. Depending upon use, grapes are categorised into table grapes (eaten fresh), wine grape (processed into wine, vinegar or juice) and dried grape (raisins). In the winemaking process, white wine is made by removing grape skins before fermentation, while for red wine the skins are discarded after fermentation. This makes the differences in taste and colour for the two wines. In this study, white grape pomace included Sauvignon Blanc (SB), Pinot Gris (PG) and Gewürztraminer (GE); red grape pomace was derived from Merlot (ME) and Pinot Noir (PN).

1.2 Research gap

A large number of researchers have investigated the phenolic profile of grapes and grape pomace all over the world. Research has been conducted in the largest winemaking areas so that the phytochemical profiles of endemic grape pomace have been reported in Italy *(Fiori et al*., 2014), France (Ky, Crozier, Cros, & Teissedre, 2014), Brazil (Ribeiro *et al*., 2015b) and Argentina (Lingua, Fabani, Wunderlin, & Baroni, 2016a). Although the quantitative and qualitative distribution of polyphenols in grape pomace varies significantly between grape varieties, regions and weather conditions, most of the research has illustrated that grape pomace is an abundant source of phenolic compounds. However, little attention has been paid to grape pomace produced from local vineyards in New Zealand. One study conducted by a research group in University of Otago and Lincoln University concluded that grape pomace generated from cool climate area such as New Zealand has the similar bioactive range to grape pomace collected from warm climate regions (for example Spain or Turkey) (Cheng et al., 2012a). Hence, it is worth investigating the possibility of enrichment food products with local grape pomace in terms of physical effects and nutritional properties.

1.3 Aim of research

The aim of this study was to determine the phytochemical profiles of some New Zealand grape pomace and to investigate the feasibility of using New Zealand grape pomace as the source of phenolic compounds, antioxidants and dietary fibre to fortify cereal-based food products.

1.4 Research objectives

1. To work with the pomace of five different New Zealand grape varieties, and prepare extracts and powder from the pomace.

2. To study the phytochemical profile of New Zealand grape pomace, including phenolic compounds, anthocyanins.

3. To evaluate the addition of different levels of grape pomace powder to cereal-based products and to investigate physical parameters of such products.

4. To investigate the nutritional quality of grape pomace fortified cereal-based products with reference to the starch, protein and phenolic compounds digestibility, and antioxidant activity.

1.5 Hypothesis

1. Grape pomace will improve cereal-based food products physical properties, phenolic content, and hence, the antioxidant activity.

2. Addition of grape pomace powder will decrease the starch digestibility of food matrices by inhibiting α-amylase activity.

3. Addition of grape pomace powder will increase the phenolic content and antioxidant capacity of food matrices.

Chapter 2

Literature review

This chapter reviews the current information, and summarises, knowledge about grape pomace and the utilisation of grape pomace over the world. Based on the summaries, the knowledge about compositions of grape pomace will be provided, and the differences between grape varieties and cultivated regions will be discussed. Extraction methods play a crucial role in grape pomace composition in terms of both the quantity and quality of phenolic compounds. Hence, advancement in extraction technology using different extraction techniques for the extraction of grape pomace is of interest in this chapter. This chapter also presents critical evaluations regarding the applications of grape pomace to enhance the quality of foodstuffs in terms of physicochemical, technological, nutritional and sensory aspects.

2.1 Grape pomace compositions

The phytochemical composition of grape pomace depends on a number of factors including varieties, cultivation practices, geographic regions, climate, extraction techniques or winery processing. There are two main types of wine: red and white wine, each requiring different processes which are illustrated in Figure 2.1. The main difference lies in the time of fermentation, in which only clear grape juice is fermented without the presence of grape solids to make white wine, while red wine is produced by fermenting all the grape parts together: skins, seeds, pulps and juice, the pomace being removed afterwards. This difference makes red wines taste stronger and more like grape skins, while white wines taste milder and more like grape juice. In addition, red wines are only made from dark-skinned grapes, while white wines can be made from any grapes. These processes also lead to the differences in the composition of grape pomaces, which will be discussed in the following chapters.

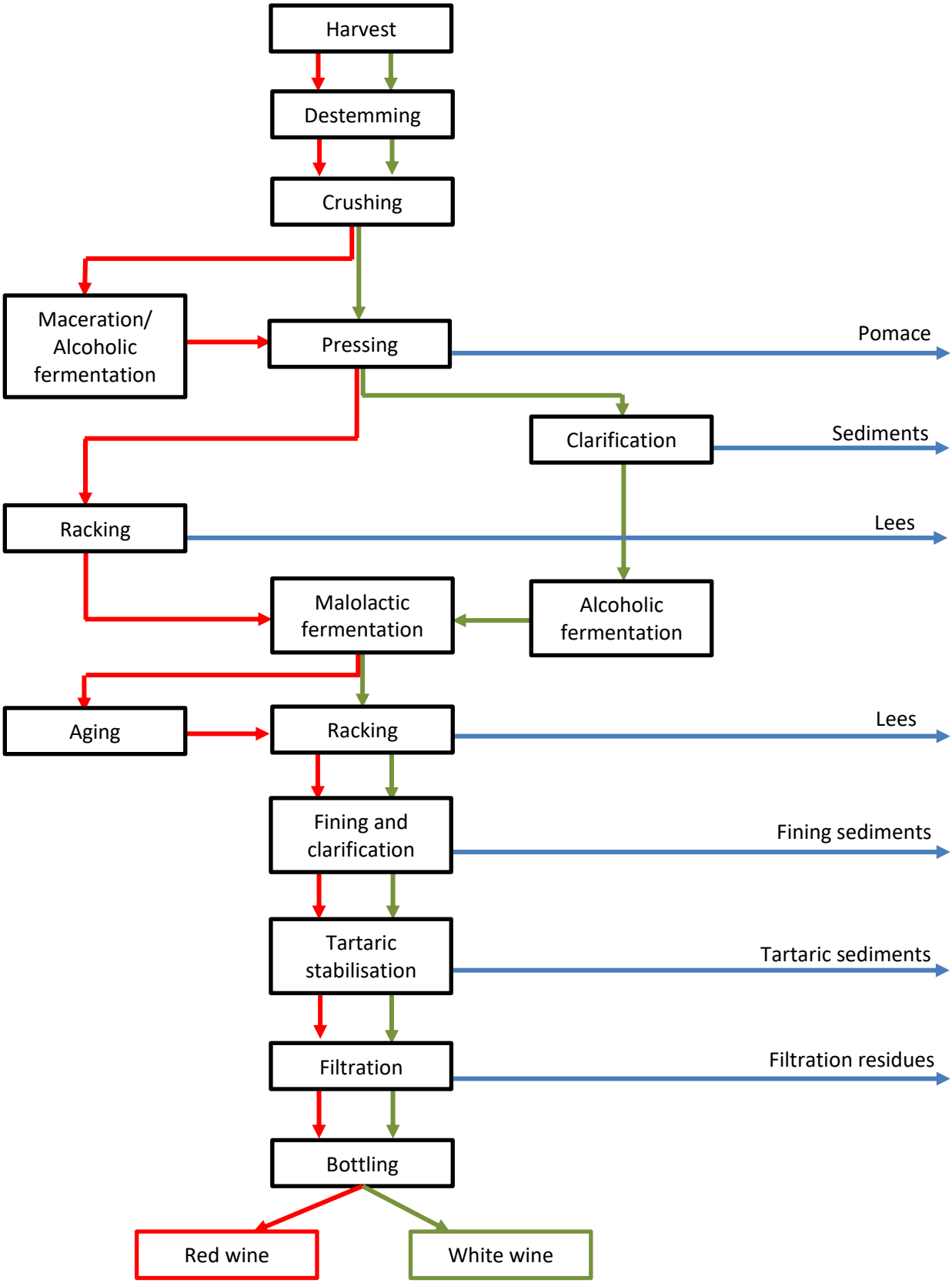


Figure 2.1 Red and white wine making process and their residues generated during process

Adapted from Dávila, Robles, Egüés, Labidi, & Gullón, (2017)

The phenolic compounds in grape pomace are regarded as antioxidant, anti-bacterial, anti-inflammatory agents (González-Centeno *et al*., 2013; Guaadaoui *et al*., 2014; Trost *et al*., 2016). Wine contains between 30 – 35% of the phenolic content of grapes. In addition, grape pomace contains other constituents such as dietary fibre or lipids (García-Lomillo & González-SanJosé, 2017a; Saura-Calixto, 2011).

2.1.1 Grape phenolic compounds and classification

Phenolic compounds are molecules that naturally appear in plants or microbes, comprising of a phenyl ring backbone with a hydroxyl group or other substitutes. These bioactive compounds are secondary metabolites produced by the plant to cope with environmental stress conditions such as UV radiation, infections or wounding (Anastasiadi *et al*., 2012; Naczk & Shahidi, 2004). As a result of these functions, the bioactive compounds are of importance for plants to maintain their "health status". Phenolic compounds in grapes were first discovered in the late 19th century (Kennedy, Saucier, & Glories, 2006). Generally, in grapes, these compounds are divided between non-flavonoid (simple C_6 backbone, hydroxybenzoic acids, hydroxycinnamic acids, volatile phenols and stilbenes), and flavonoid compounds (flavones, flavonols, flavanones, flavanols and anthocyanin). A schematic structure of a ripe grape berry and the distribution of grape phenolics between tissues is illustrated in Figure 2.2.

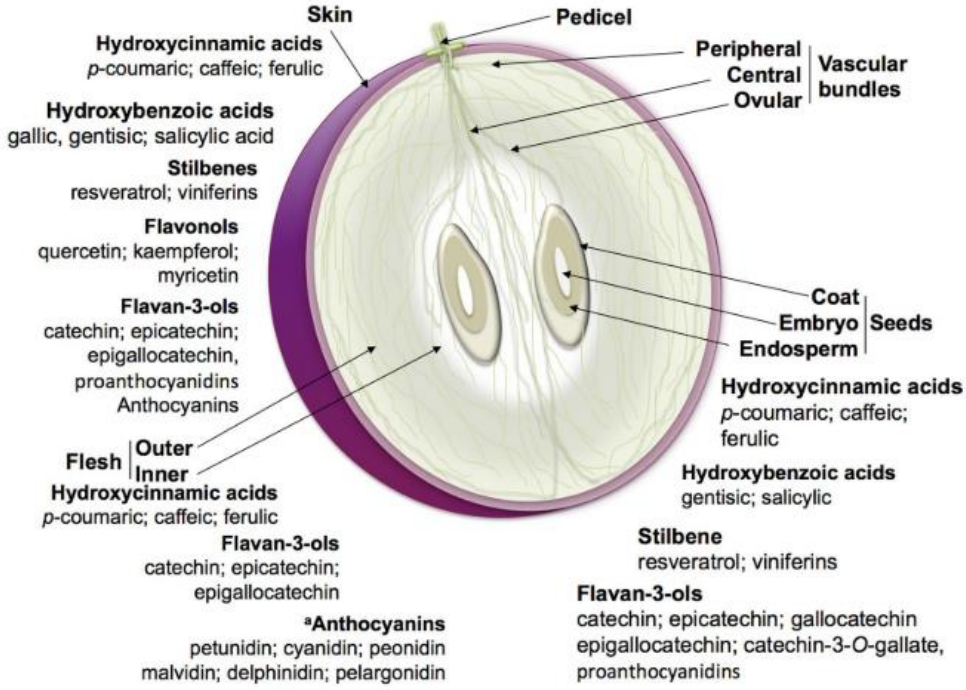


Figure 2.2. Schematic structure of a grape berry and phenolics distribution between organs and tissues

(Teixeira *et al*., 2013a)

The skin of grape consists of layers, the outermost layer - the epidermis - is cutinised making it strong, while the inner layers (the number of layers is dependant on variety) store most of the skin flavonoids. These flavonoids are mainly anthocyanins, proanthocyanins with a smaller amount of flavonols and flavanols. Grape seeds contain flavanols and most of the non-flavonoids of the grape. A detailed explanation of the distribution of phenolic compounds of grape is presented in Table 2.1.

Depending upon the type and the concentration of the phenolics present, each grape variety has a unique colour, aroma and other plant characteristics. The phenolics in each variety also varies due to the environment, agricultural practices and levels of synthesised metabolites.

Table 2.1. Distribution and accumulated of phenolic compounds in grape berry

Compound	Level of synthesis			Location	Berry phenological scale			
	Skin	Flesh	Seed		Blooming	Green stage	Veraison	Ripening
Nonflavonoids								
Hydroxycinnamic acids	++	+++	++	Hypodermal cells and placental cells of the pulp; primarily in the vacuoles of mesocarp cells	+++	+++	+	+
Hydroxybenzoic acids	+	-	++					
Stilbenes	+++	+	++	Skin and seeds	-	+	++	+++
Flavonoids								
Flavonols	++	-	-	Dermal cell vacuoles of the skin tissue and cell wall of skin and seeds	++	+	+++	++
Flavanols	++	+	+++	Specific vacuoles of hypodermal skin cells and seed coat soft parenchyma	+	++	+++	++
Anthocyanins	+++	-*	-	Cell layers below the epidermis; storage confined to the vacuoles and cytoplasmic vesicles named anthocyanoplasts	-	-	+	+++

Very abundant (+++) to not detected (-)

Adapted from (Teixeira et al., 2013)

A number of research publications have indicated that the phenolic content of pomace material left after winemaking may be higher than that recorded in grapes possibly due to a dilution process during winemaking (Fontana *et al*., 2013; Mattos, Tonon, Furtado, & Cabral, 2017; Montealegre *et al*., 2006), or due to the physicochemical processes that occur during the pressing of the grapes (Lingua, Fabani, Wunderlin, & Baroni, 2016b; Teixeira et al., 2014). Research has correlated the total phenolic content (TPC) of fruits to the antioxidant capacity and subsequent health-promoting activities such as anti-cardiovascular and anti-cancer activity (Rockenbach *et al*., 2011; Teixeira *et al*., 2014). In the study of Katalinić et al., (2010), the phenolic profiles of grape skin extracts from fourteen grape varieties (seven red and seven white) were characterised, and the result showed that the average TPC of red varieties was 1851 mg GAE/kg fresh weight (FW) while the corresponding number for white varieties was only 875 mg GAE/kg FW. In terms of varieties, studies have concluded that some cultivars, such as Pinot Noir and Sauvignon Blanc, show significantly higher levels of TPC than the others, such as Zelen and Rebula (Trost *et al*., 2016). Recent studies have reported the presence of twenty

eight major phenolic compounds in grape pomace. These vary in concentrations, mostly being derivatives or sub-units of flavonoid and non-flavonoid compounds (Mattos *et al*., 2017).

2.1.1.1 The non-flavonoid compounds

Phenolic acids and stilbenes are the most significant non-flavonoid compounds in grape pomace. The phenolic acids found in grape pomace are mainly comprised of hydroxybenzoic acids ($C_6 – C_1$ backbone) and hydroxycinnamic acids ($C_6 – C_3$ backbone), with the hydroxybenzoic acids being more prevalent than the hydroxycinnamic acids (Pino-García, González-SanJosé, Rivero-Pérez, García-Lomillo, & Muniz, 2017). The total phenolic acid content ranges from 0.06 to 0.973 g/kg dry weight (DW) for red grape skins and from 0.105 to 0.227 g/kg DW for white grape skins (Kammerer *et al*., 2004). The most common derivatives of hydroxybenzoic acids are gallic acid, gentisic acid, salicylic acid, p-hydroxybenzoic acid, protocatechuic acid, syringic acid and vanillic acid.

Hydroxycinnamic acids are located in the pulp and skins of grapes, with the common derivatives including caffeic acid, p-coumaric acid, ferulic acid and synaptic acid (Teixeira, Eiras-Dias, Castellarin, & Gerós, 2013b). Stilbenes have the skeleton $C_6 – C_2 – C_6$ and are responsible for inhibiting microbial action, responding to UV radiation, fungal, bacterial and viral pathogen attacks (Hogervorst *et al*., 2017). They are mostly located in the skins of the grape with the content varying from 0.011 to 0.123 g/kg DW (Kammerer *et al*., 2004). Some studies have reported that stilbenes have only been found in grape pomace as the *trans* form, not as the *cis* form. This may be explained by assuming that the extraction process takes place in the dark thus preventing exposure to UV radiation, which is the cause of the transformation from *trans*-isomers to *cis*-isomers (Lecce *et al*., 2014). The most well known stilbene subgroup in grape, as well as grape pomace, is *trans*-resveratrol (Teixeira *et al*., 2013b). Gatto *et al*., (2008) investigated the stilbene synthesis of 78 *V. vinifera* varieties and reported that red varieties had

a higher resveratrol content than white/pink varieties and that the family of Pinots (Pinot Noir, Pinot Tete de Negre and Pinot Gris) are the largest resveratrol producers. Recent publications have reported the existence of piceatannol, a tetra-hydroxy stilbene, which has a higher *in vitro* antioxidant capacity than *trans*-resveratrol (Piotrowska, Kucinska, & Murias, 2012).

2.1.1.2 The flavonoid compounds

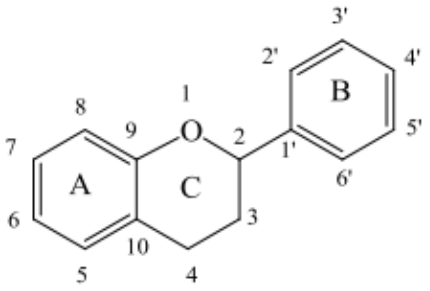


Figure 2.3. Flavonoid structure and numbering system

Flavonoids are the most abundant phenolic compounds in grape pomace; their classes are differentiated by the degree of oxidation of their oxygenated heterocycle (García-Lomillo & González-SanJosé, 2017a). Figure 2.3 illustrates the skeleton structure of flavonoid; it consists of a flavan core which has fifteen carbons with two aromatic rings (A and B), bounded by three carbon atoms which form an oxygenated heterocycle (ring C). Depending upon the oxidation state, and degree of substitution on ring C, flavonoids can be further divided into different subgroups. The major subgroups of flavonoids in grapes and grape pomace include flavonols, flavan-3-ols, anthocyanins, and the minor amounts of flavones and isoflavones.

Flavonols

R = Glucose; glucuronic acid

Kaempferol	R_1 = H, R_2 = H
Quercetin	R_1 = OH, R_2 = H
Myricetin	R_1 = OH, R_2 = OH
Isorhamnetin	R_1 = H, R_2 = OCH_3
Laricitrin	R_1 = OH, R_2 = OCH_3
Syringetin	R_1 = OCH_3, R_2 = OCH_3

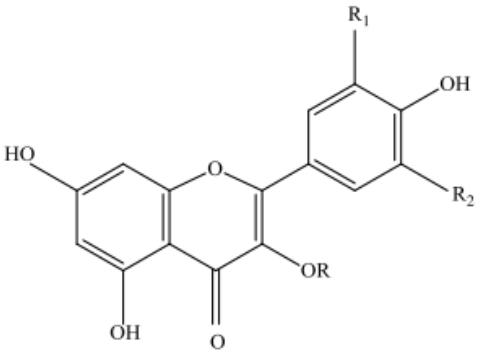
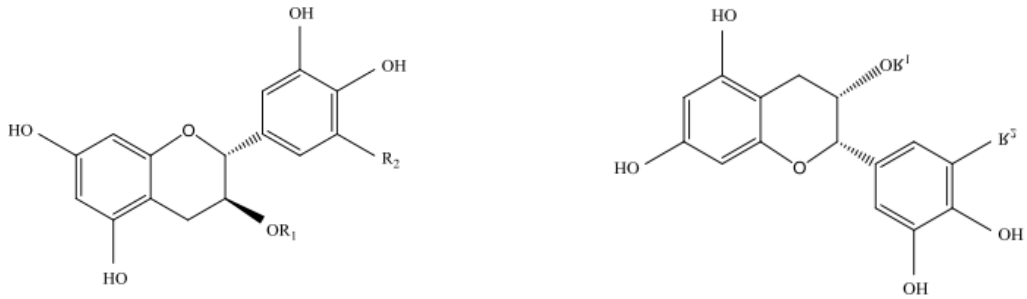


Figure 2.4. The principal flavonols of grape

Adapted from Ky, (2013)

The main flavonols in grape pomace are quercetin, kaempferol, myricetin and isorhamnetin (Ky, 2013), which are presented in Figure 2.4. The content of flavonols in grape skin pomace ranges from 0.3 – 2.6 g/kg DW (Lavelli, Kerr, García-Lomillo, & González-SanJosé, 2017). The flavonol content of the whole grape pomace (seeds and skins) is higher than when considering the separate materials (only skins or seeds), and amounts of flavonols in red grape pomace are higher than in white grape pomace (Teixeira *et al*., 2014).

Flavanols



(+)-Catechin	$R_1 = R_2 = H$	(-)-Epicatechin	$R_1 = R_2 = H$
(+)-Catechin gallate	R_1 = gallyl, $R_2 = H$	(-)-Epicatechin gallate	R_1 = gallyl, $R_2 = H$
(+)-Gallocatechin	$R_1 = H$, $R_2 = OH$	(-)-Epigallocatechin	$R_1 = H$, $R_2 = OH$
(+)-Gallocatechin gallate	R_1 = gallyl, $R_2 = OH$	(-)-Epigallocatechin gallate	R_1 = gallyl, $R_2 = OH$

Figure 2.5. Flavanols structure

Adapted from Ky, (2013)

Flavanols, or flavan-3-ols, are responsible for the sensorial characteristics of wine including astringency, bitterness and structure (Montealegre *et al*., 2006) with the total content range from 0.3 – 2.6 g/kg DW (Kammerer *et al*., 2004; Teixeira *et al*., 2013b). Grape seeds contain the greatest quantity of flavan-3-ol and the highest quantity of galloylated flavanols, which have more efficiency in antioxidant activity than other non-galloylated compounds (Makris, Boskou, & Andrikopoulos, 2007). Monomers of (+)-catechin and (-)-epicatechin are the major flavan-3-ols fractions in both red and white winery pomaces, with the concentrations ranging from 44 – 71% of total flavan-3-ol content (González-Centeno *et al*., 2013). Condensed tannin or proanthocyanidin, another flavan-3-ol sub-unit , accounts for a large part of the grape pomace polyphenols (21% - 52% of dry weight matter) (Rondeau, 2013), and might be present as procyanidin dimers (B1, B2, B3, B4) or procyanidin trimers (C1, C2 and C3) (Teixeira *et al*., 2014). In red grape varieties, the levels of procyanidin dimer B3 (up to 20.5 mg/g DW) is predominant, whereas B1 is the major procyanidin in white varieties (Teixeira *et al*., 2014).

Anthocyanins

R = Glucose; glucuronic acid

Pelargonidin	R_1 = H, R_2 = H
Cyanidin	R_1 = OH, R_2 = H
Delphinidin	R_1 = OH, R_2 = OH
Peonidin	R_1 = OCH_3, R_2 = H
Malvidin	R_1 = OCH_3, R_2 = OCH_3
Petunidin	R_1 = OCH_3, R_2 = OH

R_3 = H, glucose

R_4 = H, acetyl, coumaroyl, caffeoyl

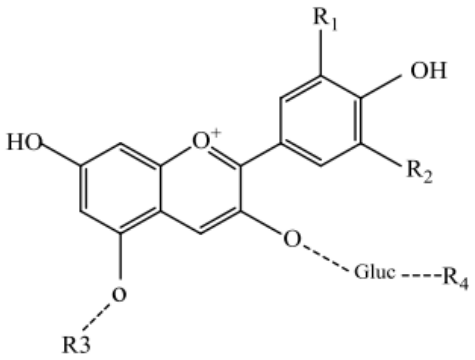


Figure 2.6. Structure of monomer anthocyanins of grape

Adapted from Ky, (2013)

The structure of monomer anthocyanins of grape are presented in Figure 2.6. Anthocyanins are highly soluble phenolic compounds, located in red grape skins but absent from red grape seeds and white grapes (de la Cerda-Carrasco *et al*., 2015; Deng *et al*., 2011). These compounds are responsible for the colour of red grape varieties as well as the transfer of pigments to the wines (Di Lecce *et al*., 2014). Anthocyanins contribute more to the antioxidant capacity of fruits (90%) than flavonols, flavan-3-ols, and phenolic acids (10%) (Teixeira *et al*., 2014). However, anthocyanins are vulnerable to chemical transformation due to the action of agents such as light, temperature, oxygen, pH, and solvents (Beres *et al*., 2017). The total anthocyanin content of grape skins ranges from 2.5 to 132 g/kg DW (Kammerer *et al*., 2004; Ruberto, Renda, Daquino, & Amico, 2007) with the main anthocyanins being the 3-o-glucosides of five common anthocyanins: cyanidin, peonidin, petunidin, delphinidin and malvidin (Lavelli *et al*., 2017). The total phenolic content, and quantity of some main phenolic content obtained from grape pomace cultivated in different regions in the world are shown in Table 2.2.

Table 2.2. Main phenolic compounds and total antioxidant of pomace from grape varieties in different regions

Variety	Ref	Region	Material	TPC (mg GAE/g DW)	Total flavonols	Total flavan – 3 – ols	Total anthocyanins	Total antioxidant capacity
Pinot Noir	(Rockenbach *et al.*, 2011)	Santa Catarina, Brazil	Seeds	16,518 mg CE/100 g DW	n/a	11,187 mg CE/100 g DW	n/a	16,925 μmol TE/100 g DW
			Skins	660 mg CE/100 g DW	n/a	56 mg CE/100 g DW	385.93 mg/100 g DW	1113 μmol TE/100 g DW
	(Reis *et al.*, 2016)	Santa Maria, Brazil	Pomace	90.21 mg GAE/g DW	n/a	n/a	n/a	n/a
	(Cheng *et al.*, 2012)	New Zealand	Seeds	491.9 mg GEA/g extract	0.52 mg/g extract	n/a	n/a	156.8 mg extract/g DPPH
			Skins	45.0 mg GEA/g extract	3.31 mg/g extract	n/a	10.48 mg ME/g extract	1365.7 mg extract/g DPPH
			Pomace	112.5 mg GEA/g extract	1.5 mg/g extract	n/a	6.63 mg ME/g extract	304.0 mg extract/g DPPH
	(Deng *et al.*, 2011)	Oregon, USA	Pomace	21.4 mg GAE/g DW	n/a	42.6 mg CE/g DW	0.29 mg Mal-3-glu/g DW	32.2 mg AAE/g DM
	(Pantelic *et al.*, 2016)	Central Serbia	Seeds	102.98 mg GAE/g DW	16.47 mg/g DW	2244.55 mg/g DW	n/a	863.29 μmol TE/g DW
			Skins	7.21 mg GAE/g DW	25.89 mg/g DW	5.33 mg/g DW	n/a	95.10 μmol TE/g DW
Cabernet Sauvignon	(Rockenbach *et al.*, 2011)	Santa Catarina, Brazil	Seeds	8,249 mg CE/100 g DW	n/a	5312 mg CE/100 g DW	n/a	8281 μmol TE/100 g DW
			Skins	1,065 mg CE/100 g DW	n/a	252 mg CE/100 g	934.67 mg/100 g DW	2032 μmol TE/100 g DW
	(Ribeiro *et al.*, 2015a)	Parana, Brazil	Pomace	≈ 2800 mg GAE/100 g DW	240.77 μg/mL	160.44 μg/mL	65.5 μg/mL	n/a
	(Deng *et al.*, 2011)	Washington, USA	Pomace	26.7 mg GAE/g DW	n/a	42 mg CE/g DW	0.89 mg Mal-3-glu/g DW	39.7 mg AAE/g DW
	(Montealegre *et al.*, 2006)	Castilla la Mancha, Spain	Seeds	n/a	n/a	720 mg/kg FW	n/a	n/a
			Skins	n/a	190 mg/kg FW	63.03 mg/kg FW	n/a	n/a
	(Pantelic *et al.*, 2016)	Central Serbia	Seeds	69.57 mg GAE/g DW	35.85 mg/g DW	2239.06 mg/g DW	n/a	670.86 μmol TE/g DW
			Skins	9.10 mg GAE/g DW	78.44 mg/g DW	24.78 mg/g DW	n/a	97.26 μmol TE/g DW
	(Yi *et al.*, 2009)	Ontario, Canada	Pomace	250 mg/100 g FW	n/a	n/a	131 mg/100g FW	63.3 %
Chardonnay	(Montealegre *et al.*, 2006)	Castilla la Mancha, Spain	Seeds	n/a	n/a	1270 mg/kg FW	n/a	n/a
		Castilla la Mancha, Spain	Skins	n/a	53 mg/kg FW	89 mg/kg FW	n/a	n/a
	(Pantelic *et al.*, 2016)	Central Serbia	Seeds	62.34 mg GAE/g DW	19.16 mg/g DW	557.54 mg/g DW	n/a	873.62 μmol TE/g DW
			Skins	2.04 mg GAE/g DW	12.84 mg/g DW	20.07 mg/g DW	n/a	46.86 μmol TE/g DW

Sauvignon Blanc	(Montealegre *et al.*, 2006)	Castilla la Mancha, Spain	Seeds	n/a	n/a	730 mg/kg FW	n/a	n/a
	(Montealegre *et al.*, 2006)	Castilla la Mancha, Spain	Skins	n/a	25 mg/kg FW	54 mg/kg FW	n/a	n/a
	(Trost *et al.*, 2016)	Vipava Valley, Slovenia	Pomace	n/a	44 mg GAE/G DW		n/a	n/a
	(Pantelic *et al.*, 2016)	Central Serbia	Seeds	54.26 mg GAE/g DW	10.24 mg/g DW	716.87 mg/g DW	n/a	586.11 μmol TE/g DW
			Skins	0.51 mg GAE/g DW	21.84 mg/g DW	3.93 mg/g DW	n/a	30.57 μmol TE/g DW
Merlot	(Montealegre *et al.*, 2006)	Castilla la Mancha, Spain	Seeds	n/a		870 mg/kg FW	n/a	n/a
			Skins	n/a	130 mg/kg FW	96.28 mg/kg FW	n/a	n/a
	(Ribeiro *et al.*, 2015a)	Parana, Brazil	Pomace	≈ 3,200 mg GAE/100 g DW	349.48 μg/mL	153.04 μg/mL	24.52 μg/mL	n/a
	(Deng *et al.*, 2011)	Oregon, USA	Pomace	25 GAE/g DW (P)	n/a	61.2 mg CE/ g DW	1.42 mg Mal-3-glu/g DW	40.2 mg AAE/g DW
	(Pantelic *et al.*, 2016)	Central Serbia	Seeds	77.38 mg GAE/g DW	7.08 mg/g DW	116.15 mg/g DW	n/a	481.69 μmol TE/g DW
			Skins	8.26 mg GAE/g DW	40.57 mg/g DW	23.69 mg/g DW	n/a	97.26 μmol TE/g DW
	(Katalinić *et al.*, 2010)	Vrgorac, Croatia	Skins	1,666 mg GAE/kg FW	n/a	1068 mg GAE/kg FW	739 mg MglcE/kg	n/a

RGP: red grape pomace; WGP: white grape pomace; P: pomace (Seeds and Skins)
GAE: Gallic acid equivalent; TE: Trolox equivalent; CE: catechin equivalent; ME: malvidin equivalent; AAE: ascorbic acid equivalent; DW: dry weight; FW: fresh weight:
n/a: not available

As can be seen in the table, the phytochemical profile of grape pomaces are different based on their varieties and cultivated regions. PN has a broad range of total phenolic content (TPC) from 21.4 mg GAE/g DW for grape pomace in harvested in Oregon, USA to 90.21 mg GAE/g DW of grape pomace from Santa Maria, Brazil. The same trend was observed in other grape varieties and specific phenolic compounds such as anthocyanin, which was observed mainly in red grape pomace. The TPC also reflects the antioxidant capacity of grape pomace, hence the linear correlation between them were shown in the table. Besides, the grape seeds alone tend to contain more phenolics and antioxidant as their content were higher than those of grape skins or grape pomace. For instance, the seed of Cabernet Sauvignon had 54.26 mg GAE/g DW of TPC and 586.11 μmol TE/g DW of antioxidant capacity, while the corresponding numbers of its skins were only 0.51 mg GAE/g DW and 30.57 μmol TE/g DW.

2.1.2 Dietary fibre

In dried grapes, dietary fibre is present at concentrations ranging from 43% to 75% (García-Lomillo & González-SanJosé, 2017a). The main constituents of dietary fibre are cell wall polysaccharides such as lignin. Deng *et al*., (2011) investigated the chemical composition of two white grape skins (Morio Muscat, and Muller Thurgau), and three red grape skins (Cabernet Sauvignon, Pinot Noir and Merlot) and illustrated that red grape pomace was far higher in dietary fibre (51.1 – 56.3%) than white grape pomace (17.3 – 28%). Gül *et al*., (2013) found that grape seeds are richer in fibre than skins, and red grape pomace is richer in fibre than white grape pomace. Table 2.3 illustrates the fibre content of some grape pomaces observed from studies in different regions. Dietary fibres are separated into soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). Dietary fibre contains significant quantities of non-extractable polyphenols including hydrolysable polyphenols and non-extractable proanthocyanidins (Larcher *et al*., 2015), and these possess significant antioxidant and other health-promoting abilities (Saura-Calixto, 2012; Zhu, Du, Zheng, & Li, 2015). Human trials have been conducted to

test the influence of the antioxidant capacity of extractable polyphenols and non-extractable polyphenols (Pérez-jiménez *et al*., 2009), both these studies demonstrated that they increased the plasma antioxidant capacity of subjects. Saura-Calixto, (2011) concluded that dietary fibre acts as a carrier of antioxidants as most bioactive compounds, including vitamins and phenolic compounds are the main dietary antioxidants.

Table 2.3. Composition of grape pomace dietary fibre from different varieties

Composition / Varieties	Protein (% DM)	Fat (% DM)	Soluble sugars (% DM)	Total extractable pectins (mg GUAE/g DM)	IDF (% DM)	SDF (% DM)	TDF (% DM)
Manto Negro	12.2	n/a	3.27 ± 0.10	6.20 ± 0.30	63.7 ± 2.12	10.8 ± 0.30	74.5 ± 2.43
Pinot Noir	12.13	4.74	1.38 ± 0.93	50.6	54.59 ± 1.57	1.72 ± 0.15	56.31 ± 1.47
Carbenet Sauvignon	12.34	6.33	1.71 ± 0.49	~ 53	52.40 ± 0.40	0.81 ± 0.06	53.21 ± 0.38
Merlot	11.26	3.35	1.34 ± 0.92	56.4	49.59 ± 0.34	1.51 ± 0.14	51.09 ± 0.58
Morio Muscat	5.38	1.14	77.53 ± 1.01	32.3	16.44 ± 0.24	0.84 ± 0.07	17.28 ± 0.21
Muller Thurgau	6.54	2.64	55.77 ± 2.12	41.2	27.29 ± 1.46	0.72 ± 0.14	28.01 ± 1.36

IDF: insoluble dietary fibre; SDF: soluble dietary fibre; TDF: Total dietary fibre
DM: dry matter; GUAE: galacturonic acid equivalents
n/a: not available

(Deng *et al*., 2011; Mildner-Szkudlarz, Bajerska, & Zawirska-wojtasiak, 2013)

2.1.3 Protein and carbohydrate

The carbohydrate and protein content of grape pomace were investigated as part of this project. Depending upon the grape varieties and cultivation practices, the protein content of grape pomace has been suggested to vary from 6 to 15% of dry matter (García-Lomillo & González-SanJosé, 2017b). One study examined ten grape varieties and suggested that the carbohydrate and protein content of grape pomace were influenced by cultivar with the carbohydrate concentration ranging from 39.46 g/100g 44.22 g/100g, and the protein content ranging from 12.09 g/100g to 28.13 g/100g (Çetin, Altinöz, Tarçan, & Baydar, 2011). Interestingly, the protein content of grape seeds and grape skins are similar, but the protein content of pomace is slightly higher than the skins or seeds. The protein profile of grape pomace

illustrates that the protein is rich in glutamic acid and aspartic acid, but is low in tryptophan and sulphur-containing amino acids (García-Lomillo & González-SanJosé, 2017a).

2.1.4 Lipids

Most of the lipids in grape pomace come from the seeds, with the content ranging from 14% to 17% of weight (Gül *et al*., 2013). Grape pomace lipids are rich in polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids, and low in saturated fatty acids.

2.1.5 Minerals

Similar to other components, the mineral content of grape pomace may differ due to variety, climate, viticultural practices and the winemaking process. Common minerals found in grapes can be classified into different groups depending on their mobility in the phloem. Calcium, potassium, phosphorus, sulphur and magnesium are the dominant minerals found in both grape seeds and skins (Gül *et al*., 2013; Rogiers *et al*., 2006). Table 2.4 illustrates the content of some most popular minerals found in grape pomace.

Table 2.4. Mineral content of different grape varieties

Cultivars	K (mg/g)	P (mg/g)	Ca (mg/g)	Fe (mg/100g)	Mg (mg/100g)	Zn (mg/100g)
Alphonse Lavallée	5.68	0.74	7.38	0.32	3.74	4.14
Atasansi	6.30	0.81	5.95	0.30	5.12	2.37
Cardinal	5.87	0.08	6.33	0.34	3.00	2.18
Hafizali	5.19	0.42	7.20	0.26	11.12	0.70
Horozkarai	7.02	0.78	7.02	0.42	6.57	2.90
Isabella	5.69	0.49	7.98	0.68	3.18	9.82
Italia	8.23	0.50	10.21	0.26	4.72	1.48
Sultani Cekirdeksiz	6.00	0.84	7.71	0.33	1.94	7.42
Tekirdag Cekirdeksiz	6.29	0.93	7.94	0.44	4.56	5.81
Trakya ˙Ilkeren	6.95	0.62	7.85	0.26	2.58	2.54

All data are mean data

Adapt from (Çetin *et al*., 2011)

2.1.6 Stability of grape pomace compositions

Despite grape pomace containing large quantities of bioactive compounds, the stability of those bioactive compounds is of importance and should be taken into consideration before planning to exploit those valuable sources. Fresh grape pomace has a high moisture content and water activity; this has been found to influence the chemical and microbiological stability of grape pomace negatively. Direct use of fresh grape pomace is also limited due to its high moisture

content; only a few publications have reported the use of wet grape pomace (Di Cagno *et al*., 2007). Since huge quantities of grape pomace are produced in a short period of time during the harvesting season, it is necessary to have effective treatments and preservation methods to stabilise and prevent the degradation of bioactive compounds in grape pomace. Traditionally, protecting grape pomace from exposure to oxygen and storage at a low temperature have been proposed (Da Porto, 2002). Another alternative is using preservative chemicals such as acids or sulphites as effective protection for the antioxidant compounds in grape pomace (Ayed *et al*., 1999; Silva & Malcata, 1998). However, both of these processes demand large scale storage facilities to preserve the huge amount of grape pomace, and the latter may require large quantities of additives that are not preferable in food processing (Vashisth, Singh, & Pegg, 2011). Additionally, the high moisture content is not favourable for the rapid transportation of fresh pomace as it increases the cost of processing (Milczarek, Dai, Otoni, & McHugh, 2011). Hence, dehydration of fresh grape pomace is an important step before applying grape pomace to any further applications. The most common dehydration methods for plant products are oven drying and freeze drying (Barcia *et al*., 2015; Iora *et al*., 2015; Teixeira *et al*., 2014; Teixeira, *et al*., 2014; Trost *et al*., 2016). Despite concerns about the degradation of bioactive compounds and other components in products, the advantages regarding compromising quantity and quality of products, cost-effectiveness, ease of operation and availability makes drying remains the most popular preserved method. Drosou *et al*., (2015) compared the extraction yield of three extraction methods on dried and wet grape pomace and found that the yield of bioactive compounds from dried samples rather than wet samples was higher, mainly due to the influences of the drying process in terms of breaking and destroying walls, opening large cavities and spaces, and allowing the substances to be extracted easily. Most studies recommended drying with a temperature range from 50 – 60 °C to preserve most of the bioactive compounds

(Deng *et al*., 2011; Kammerer *et al*., 2004; Nindo *et al*., 2003; Rockenbach *et al*., 2011; Vashisth, Singh, & Pegg, 2011).

2.2 Extraction of grape pomace bioactive compounds

The general principle for the recovery of bioactive compounds from plant-origin by-products includes five steps: macroscopic pretreatment, macro and micromolecular separation, molecular extraction, isolation and purification and product formation (Galanakis, 2012). Among these steps, the extraction process is the most important as it is responsible for obtaining the specific desired compounds. Numerous research has focused on improving the efficiency of the recovery process. Typical problems of the existing extraction techniques include loss of phenolics due to ionisation, hydrolysis and oxidation as well as being time-consuming and using a large amount of solvent (Fontana *et al*., 2013). Additionally, extraction qualities are impacted by a number of external factors such as natural variety, cultivation practices, storage conditions and facilities (Barba, Zhu, Koubaa, & Sant, 2016). As a plant by-product, grape pomace is a favourite substance to apply intensive extraction techniques to, in order to exploit their abundant bioactive compounds effectively. To date, conventional extraction methods are still dominant in terms of industrial applications, but new emerging techniques propose a prospective future for the development of better solutions (Galanakis, 2012, 2017).

2.2.1 Conventional extraction techniques

As a result of long-standing development, conventional solid-liquid extraction (SLE) is often used in the extraction of bioactive compounds from grape pomace, and they are still the most popular and dominant in the industry (Barba et al., 2016; Hogervorst et al., 2017; Igartuburu et al., 1991). These techniques are based on the principle of mass transfer in which the desired objects from the solid phase migrate into the solvent phase that is in contact with the matrix

(Fontana *et al*., 2013), this stage is also called “leaching”. Common solvents are hydroalcoholic solutions, which strongly induce polyphenols and other compounds to solubilise. However, the use of heat in these techniques causes the degradation of the heat-sensitive compounds, and also raise energy consumption. Additionally, due to the polarity of phenolic compounds in grape pomace, the selection of appropriate solvents is important for extract efficiency and extract quality (Ameer, Shahbaz, & Kwon, 2017; Baranowski & Nagel, 1981). The molecular affinity between solvent and solute, mass transfer, the use of co-solvents, environmental safety, human toxicity and financial feasibility should also be considered when selecting a solvent for bioactive compound extraction (Azmir *et al*., 2013).

2.2.1.1 Maceration

Based on the principle of SLE, maceration is a cheap and easy operating method used in the extraction of oils and bioactive compounds from plant products. Maceration has a large number of industrial applications, for instance, in the extraction of herbal and other food preparations (Baranowski & Nagel, 1981). Maceration is the process of soaking in order to make the matrix soften and leach the compounds. Initially, materials are ground in order to increase the contact area with solvents. Then the ground materials are mixed with solvents and poured into a close vessel. During the process, the macerate is pressed and shaken regularly to maximise the yield (Azmir *et al*., 2013; Cowan, 1999). Manconi *et al*., (2016) successfully combined maceration and homogenisation to increase the concentration of grape pomace polysaccharide associated liposomes, with less negative impacts from the gastrointestinal environment. The researchers concluded that this system is promising to use in pharmaceutical, cosmetic or nutraceutical fields. The main drawbacks of maceration are the large scale consumption of toxic solvents and long processing time, both of which can be solved by combining with other techniques (Hogervorst *et al*., 2017).

2.2.1.2 Soxhlet extraction

This system was developed by von Soxhlet in 1879 and has become a standard extraction technique. In the Soxhlet extraction procedure, the sample is placed in a thimble holder that is continuously filled with fresh solvent from a distillation flask. When the liquid solvent reaches the overflow level, it spills out bringing solutes from the thimble holder to go back to distillation flask. The operation is repeated until the extraction is completed. Initially, it was designed as a batch process to extract lipids, but has been developed to be suitable for large numbers of industrial applications. The advantages of the Soxhlet process are its low cost, simplicity of operation and delivery of high purity extracts. However, the drawbacks are long extraction times, large quantities of solvent are required, degradation of the extracts, and environmental concerns (Castro & Priego-Capote, 2010). To date, Soxhlet is still the standard solid-liquid extraction method, being the reference for other new techniques to be measured against. In grape pomace studies, the vast majority of studies still employ Soxhlet to identify phytochemical profiles. There have been a number of modifications with the assistance of emerging techniques, in order to optimise this method such as high pressure Soxhlet extraction, ultrasound assisted Soxhlet extraction or microwave assisted Soxhlet extraction (Castro & Priego-Capote, 2010).

2.2.2 Emerging extraction techniques

New and emerging techniques have been developed to try and solve the inherent disadvantages of traditional extraction techniques. These novel alternatives have alleviated the problems of traditional extraction processes such as long extraction time, loss of compounds during processing, and reducing environmental harm. As conventional methods of extraction have reached maximum thresholds over the years, emerging technologies are focusing on enhancing the mass transfer rate of intracellular compounds to the extracts to overcome those limits.

2.2.2.1 Supercritical fluid extraction (SFE)

SFE employs the characteristics of supercritical fluids, such as SC-CO_2, which exhibit good solvating power, high diffusivity, low viscosity and marginal surface tension to permit rapid mass transfer in the supercritical phase (Fontana *et al*., 2013). A typical SFE system includes a tank of mobile phase, usually CO_2 (due to the low critical temperature and pressure), a pump to pressurise the gas, a co-solvent vessel and pump, an oven that contains the extraction vessel, a controller to maintain the high pressure inside the system, a trapping vessel, and a range of meters to monitor the system (Azmir *et al*., 2013; Baiano, 2014). The system can generate the supercritical state, in which fluid has a higher diffusion coefficient, lower viscosity and surface tension than a liquid solvent, leading to more penetration of the sample matrix and favourable mass transfer (Azmir *et al*., 2013; Fontana *et al*., 2013). This technique is less time-consuming, and requires lesser organic solvents, than traditional ones and the process can reuse the supercritical fluid and produces pure extract. In the work of de Campos, Leimann, Pedrosa, & Ferreira, (2008), the influence of SFE, conventional SLE, and Soxhlet extraction techniques were studied in relation to the extraction of phenolic compounds from Cabernet Sauvignon. Results showed that SFE, using a mixture of SC-CO_2 and co-solvents, increased the extraction yield by 15%, but the antioxidant activity and TPC were considerably lower than extraction by SLE and Soxhlet processes. Compared to other techniques, SFE shows a high extraction of nonpolar compounds such as fatty acids, thus is suitable for the production of grape seed oils, even at industrial scale (Fiori, 2010).

2.2.2.2 Accelerated solvent extraction (ASE)

ASE is a method based on the correlation between pressure and temperature whose operating principle is illustrated in the Figure 2.8. The boiling point of water is increased under higher pressure. This condition raises the interaction between solvent and matrices, hence improves the speed and efficiency of the extract process (Fontana *et al*., 2013). ASE allows the researcher

to increase the extraction temperature and pressure up to 150 °C and 2000 psi without degrading the quality of phenolics, allowing the solvent to penetrate deeper into the sample matrix, hence leading to a higher yield of phenolic compounds (Fontana *et al*., 2013; Gazzola *et al*., 2014; Guaadaoui *et al*., 2014). Rajha *et al*., (2014) applied ASE in the extraction of wet and dry grape pomace, and their results showed that ASE allowed the extraction of wet pomace doubled the dry matrix in phenolic compounds, as well as increased the extraction efficiency by fifteen times higher compared to that extract gained from the Soxhlet extraction. It has also been reported that a higher catechin yield, greater reproducibility, and increased time efficiency in grape pomace extraction are achieved by using ASE in comparison with ultrasound assisted extraction (Piñeiro, Palma, & Barroso, 2004). Interestingly, water can be used as the unique solvent in ASE with similar extraction efficiency as conventional methods, but the extraction speed was increased.

2.2.2.3 Pulsed electric field (PEF)

The PEF machine consists of a high voltage pulsed generator, a treatment chamber with a suitable fluid handling system, and two electrodes of generator placed inside the chamber. When a liquid food product is put into the chamber, in either batch or continuous mode, it is exposed to electrodes, and the generator creates an electrical pulse which damages the food cell membranes, and intracellular compounds are released as extracts (Barba *et al*., 2016). PEF is reported to be ideal for juice production, increasing the content of valuable components, and has been suggested as an efficient replacement process for methods such as enzymatic maceration. El Darra *et al*., (2013) compared the application of PEF (0.8 – 5 kV/cm, 1 – 100 ms, 42 – 53 kJ/kg) and heat treatment (50^0C, 15 min, 125 kJ/kg) on the recovery of phenolic from Cabernet Franc. PEF gave a better phenolic extraction (anthocyanin and tannin content) by 51 – 62% compared to heat treatment (increased by only 20%). Other researchers have shown

similar results and highlighted the recommendation to use PEF in the selective extraction of anthocyanin (Cholet *et al*., 2014; M. Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008).

2.2.2.4 Ultrasound assisted extraction (UAE)

Compared to PEF, UAE has wider industrial applications either as a stand-alone process or as a part of a stepwise procedure in plant compound extraction (Barba *et al*., 2016). It generates cavitation forces which in turn cause bubbles in the liquid/solid extraction which can collapse explosively, producing localised pressure. This pressure ruptures plant tissue rupture and therefore improves the release of intracellular substances into the solvents (Knorrj, Ade-Omowaye, & Heinz, 2002).

There are two types of device used for the generation of ultrasound, namely bath and probe. Both of these devices employ a transducer to generate ultrasound power. Bath ultrasound is more popular than the probe one as it is cheaper and can handle a large number of samples at the same time. However, bath processing has shortcomings such as low reproducibility and lower ultrasound power than probe ultrasound systems (Chemat *et al*., 2017; Luque-García & Castro, 2003). UAE has been reported to improve the total polyphenols, colour intensity, scavenging activity and especially resveratrol of grape pomace compared to solvent extraction techniques. The most significant benefit of ultrasound is the reduction of extraction time. Da Porto *et al*., (2013) compared UAE and Soxhlet performance in the test on grape seed oil extraction and reported that a 30-minute extraction using UAE yielded similar extraction levels (14 g/100 g) to those observed after 6 h extraction using Soxhlet. UAE uses less solvent and energy and can be integrated easily with already existing devices in production lines (Barba *et al*., 2016; Parniakov *et al*., 2016).

2.2.3 Comparison between extraction techniques

In general, conventional extraction methods are more popular than their rivals among the works of scientists worldwide, not only in grape pomace extraction but also in other plant bioactive compounds extractions. The long-standing history of traditional extraction techniques enable them to be reproduced easily, and the installation and operation have been standardised. The drawbacks such as long extraction times, loss of compounds, instability of product quality and environmental pollution might compromise the acceptable results and reasonable general cost. However, work parameters of classical methods can be adjusted to be compatible with various objects' characteristics, and then broaden their application.

As can be seen in Figure 2.7, either conventional or new methods can be differentiated based on the principles of "leaching" or "cell damage". The leaching group, which have the same requirement of using solvents, includes both conventional and emerging methods. While traditional methods still raise the issue of toxic solvents, emerging technologies (SFE, ASE and US) require fewer solvents and can be more efficient in destroying cell membranes, allowing a more complete extraction.

The development of emerging methods is promising, but current studies of the extraction of bioactive compounds from grape pomace still rely on conventional techniques to achieve reliable outcomes. In fact, apart from ultrasound processing, which has been applied on an industrial scale as a stand-alone process, the majority of the new extraction processes have just been being tested in laboratory (Barba *et al*., 2016). A potential opportunity exists to combine a traditional extraction process with an emerging one. Such a combination has been reported to achieve better results than a conventional method alone (Azmir *et al*., 2013; Baiano, 2014; Nawaz *et al*., 2006). The combination of new techniques with Soxhlet methods has been shown to be a promising potential in the extraction of bioactive compounds (Castro & Priego-Capote, 2010).

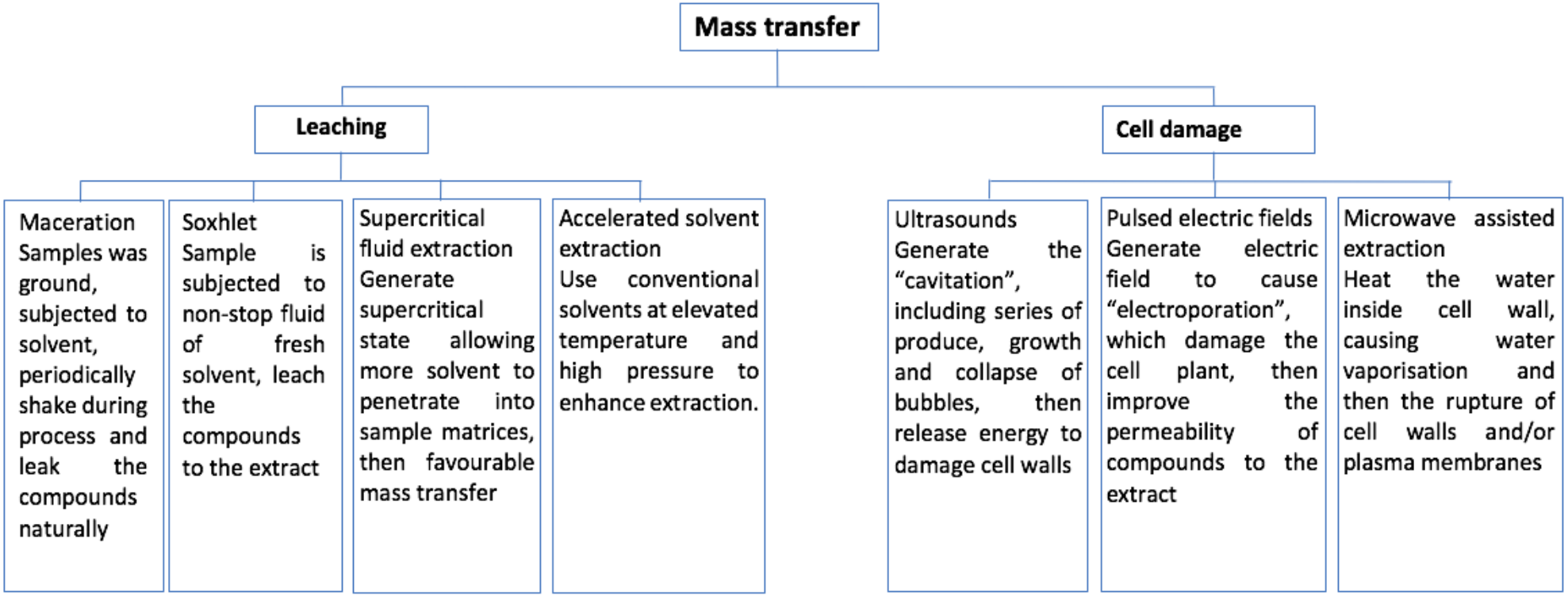


Figure 2.7. Groups of extraction methods

2.3 Application of grape pomace

From the point of consumer acceptance, food quality is defined as taste, aroma and appearance characteristics (Brewer, 2011). To enhance food quality, the food industry has employed synthetic additives as a "panacea" for multiple purposes from food processing to food perseveration for decades. However, the use of artificial chemicals is often linked to health concerns, and detrimental impacts due to long-term consumption have to be clarified. With the rise in consumer demands for food quality and safety, plus the development of science, replacement of synthetic additives by "natural" ones is of the utmost importance.

Grapes (*V. vinifera* L.) are one of the world's largest fruit crop with the yield in 2018 of 77.8 million tonnes, 80% of which is used in the production of wine (Domínguez *et al*., 2017). It means that a huge amount of grape pomace is discarded after the winemaking process annually, causing severe environmental issues. Popular treatments of grape pomace include soil burial, use as a fertiliser or animal feed. However, they all have their risks. Although grape pomace is a potential source that could be used as a nutrient-rich organic soil amendment, the overproduction at a local scale in small geographic areas can lead to inappropriate disposal of the material on agricultural land, and untreated raw material might damage crops due to the release of excessive amounts of phytotoxic polyphenols to soils (Fontana *et al*., 2013). Excessive use of grape pomace also leads to a reduction of digestibility in animals owing to the high content of lignin (Fontana *et al*., 2013). Approximately 60 – 65% of phenolic compounds of grapes are left in the by-products after the winemaking process (Baoshan *et al*., 2001), grape pomace possesses a high quantity of valuable constituents that deserve more proper treatments to take advantage of all their benefits. For decades, scientists have focused their interest on the recovery of valuable components in grape pomace to apply in not only the food industry but also cosmetics or pharmaceuticals. The antioxidant capacity of phenolic rich

extracts can be used as natural additives in food to avoid lipid oxidation or microbial growth (Mattos *et al*., 2017), while antioxidant dietary fibre has been shown to be related to the prevention of cardiovascular diseases (Saura-Calixto, 2011). Commercial products derived from grape pomace are being sold in the marketplace such as grape seed oil in Europe, but promising research is required to achieve a brighter future for the application of these natural resources.

As each component of the grape is comprised of valuable natural components, grape pomace can be used as a whole, skin only or seed only. There are two processing methods of grape pomace firstly extraction using solvents to recover soluble polyphenols, and secondly by drying and micronising the grape pomace into powder which includes non-extractable polyphenols and antioxidant dietary fibre (Lavelli *et al*., 2017). Hence, the product will be in liquid form, called grape pomace extract or powder form, called grape pomace flour. Both can be applied in the same object, or each form can be applied in particular objects for a specific target.

2.3.1 Grape pomace as a value added ingredient

Enrichment of the food matrix with plant compounds has been undertaken for a long time so as to utilise their bioactive ingredients and enhance the overall quality of final products regarding, sensorial and other specific characteristics. Grape pomace products have been applied to enhance food matrices to enrich products with antioxidants. Among the food categories which have been investigated, cereal-based food products have received most interest with most applications having made use of grape pomace powder as illustrated by Table 2.5. In general, bread and cookies are the products which have been utilised the most with the prospect of commercialisation. Cookies, enriched by grape skin powder and whole grape pomace powder, have been shown to obtain higher approval from consumers than seed powder (Acun & Gül, 2014). Increased phenolic content, antioxidant capacity and TDF of products are the typical benefits derived by incorporating grape pomace into food matrices

(Table 2.5). The recommended daily intake of fibre for adults is from 46 - 60 g/day (Saura-Calixto, 2011), but most of the commonly consumed foods are low in fibre content, thus improving the fibre content of foods is a meaningful result for nutrition-based researchers. The addition of fruit pomaces such as grape pomace to foodstuff is clearly beneficial since it can provide additional health benefits. Fibre from fruit pomace in general, and grape pomace in particular, is better than the fibre from cereals because of the high content of associated bioactive compounds such as anthocyanins and tannins (Saura-Calixto, 1998).

Table 2.5. Published works about foodstuff enriched with grape pomace

Category	Food products	Main components	GP type	Added level	Results	Reference
Cereal products	Bread	Wheat flour	Seed powder	5% to 25%	Increase of fat, TPC and TDF Decrease of total protein, wet gluten, water absorption, stability	(Aghamirzaei *et al.*,2015)
		Wheat flour	Seed powder	2.5% to 10%	Increase of TPC Decrease of loaf brightness and volume, increase of hardness and porosity Decrease of consumer acceptance at high addition levels	(Hoye & Ross, 2011)
		Wheat flour	Seed powder	2.5%, 5%, 7.5%	Increase of antioxidant capacity, gallic acid and catechin content Increase of dough development time, extensibility and stability	(Meral & Dogan, 2013)
		Wheat flour	Pomace powder	6%, 10%, 15%	Increase of TPC and antioxidant capacity Negative sensorial properties Degree of influences depend on grape varieties	(Šporin *et al.*, 2017)
	Cookies	Wheat flour	Pomace powder	5%, 10%, 15%	Increase of dough water absorption and development time Decrease of volume, with, thickness and spread ratio Consumer acceptance at 5%	(Kohajdová *et al.*, 2013)
		Wheat flour	Pomace powder	20%, 25%, 30%	Increase of fibre, hardness, brittleness, TPC and antioxidant capacity Decrease in water activity	(Karnopp *et al.*, 2015)
		Wheat flour	Skin powder	10%, 20%, 30%	Increase of DF, TPC and antioxidant capacity Decrease of WA and hardness Acceptable at 10%	(Mildner-Szkudlarz *et al.*, 2013)
		Wheat flour	Defatted seed powder	5%	Increase of TC and antioxidant capacity Increase of moisture, protein, fat and minerals content Increase of width, thickness and spread ratio	(Aksoylu *et al.*, 2015)
		Wheat flour	Pomace, seed and skin powder	5%, 10%, 15%	Increase of TPC, antioxidant capacity and TDF except for skin added samples No change on cookies dimensions	(Acun & Gül, 2014)

					Best sensorial acceptability at 5%	
	Bread, muffin and brownie	Wheat flour	Pomace powder	5%, 10%, 15%, 20% and 25% depend on foodstuff	Increase of TPC, antioxidant capacity and TDF Best sensorial acceptance at low added concentration depended on products	(Walker *et al.*, 2014)
	Noodles, pancakes and bars	All-purpose flour	Seed powder	20% and 30% for noodles 25% and 30% for pancakes 5% for bars	Increase of TPC and antioxidant capacity High consumer acceptance at low addition level	(Soto, *et al.*, 2012)
Dairy products	Fermented milk	Skim milk powder	Pomace extracts	100 mg/L GAE	Phenolic compounds were stable during fermentation Increase of TPC and antioxidant capacity at first, but decreased after storage time	(Aliakbarian *et al.*, 2015)
		Skim milk powder	Pomace powder	10g, 20g and 50 g/L	Increase of TPC and TPC Decrease of pH No influences on probiotic counts	(Frumento *et al.*, 2013)
	Yoghurt	Milk and trim milk	Seed extract	50 mg/mL added to 150g yoghurt	Increase of TPC and antioxidant capacity No obvious influences on yoghurt's consistency, colour and flavour Degradation due to long time storage happened as usual	(Chouchouli *et al.*, 2013)
		Trim milk	Pomace powder and extract	1%, 2% and 3%	Increase of TPC and antioxidant capacity Decrease of pH immediately and then after storage Longer fermentation time for addition > 3% Pomace decreased viscosity, Extract increased viscosity Delayed lipid oxidation during storage	(Tseng & Zhao, 2013)
		Milk	Skin powder	60 g/kg	Increase of TPC and antioxidant capacity Decrease of pH immediately and then after storage Need to reduce addition level to reach consumer acceptance	*(Marchiani et al., 2*016)

Grape pomace has also been added to dairy products with interesting results partly related to the interactions between milk proteins and phenolic compounds (García-Lomillo & González-SanJosé, 2017b). For instance, these interactions between phenolics and milk proteins result in excessive syneresis of yoghurt (Tseng & Zhao, 2013). Other research has discovered that although the addition of grape pomace into milk products increases the TPC and antioxidant capacity content of these products, the phenolic compounds exhibit significant degradation even after a short time of storage due to the high pH environment of yoghurt *(Aliakbarian et al.*, 2015), and may prevent lipid oxidation (Chouchouli *et al.*, 2013). There have been other

attempts to enrich other types of dairy foods with grape pomace such as cheese (Felix da Silva *et al*., 2015) and ice cream (Sagdic, Ozturk, Cankurt, & Tornuk, 2012). However, significant enhancement has not been achieved; for instance, the hydrophobic interactions between proteins and polyphenols may decrease the amount of hydrophobic group in casein, leading to the poor structure and texture of the products. These problems make the application of grape pomace into products other than cereal-based foodstuff difficult from a processing and nutritional viewpoint.

2.3.2 Grape pomace as a source of antioxidants

Along with microbial spoilage, lipid oxidation is the main factor which degrades during shelf life and storage of food products, causing deterioration of the sensory quality, nutrients or even worse, generating toxic effects within the product (Kanner *et al*., 1994). Autoxidation of food can be prevented by one of the following solutions: scavenging species that initiate peroxidation, chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides, quenching singlet oxygen formation, breaking the autoxidative chain reaction or reducing localised oxygen concentration (Brewer, 2011). An antioxidant is a compound or system that has the ability to delay oxidation by inhibiting the formation or propagation of free radicals by one or more of the above mechanisms, and the most effective antioxidants are those that interfere with the free radical chain reaction. Plants are an ideal source of natural antioxidants thanks to the antioxidant systems they have developed since they undergo constant oxidative stress from various sources (Agati, Matteini, Goti, & Tattini, 2007; Brown & Kelly, 2007). In plants, the major antioxidative phytotoxic polyphenols can be divided into four general groups: phenolic acids, phenolic diterpenes, flavonoids and volatile

Table 2.6. Application of grape pomace-derived products to prevent lipid oxidation

Materials	Foodstuffs	Level	Sample processing	Results	Ref
Seed seasoning Skin seasoning GP seasoning	Beef patties	2 g/100 g	Raw and cooked beef patties, refrigerated under high-oxygen atmosphere (70% O_2, 30% CO_2) up to 6 months Raw and cooked beef patties, vacuum packed and frozen at -18^{0}C up to 6 months	All seasoned samples showed higher antioxidant activity, with skin seasoning being the most effective 10 times lower of oxidation degree (TBARS) compared to refrigerated samples GP skin seasoning was the best, with no TBARS in the cooked sample, and 60% lower in raw samples, compared to control samples.	(García-Lomillo *et al*., 2017)
Grape pomace flour Freeze-dried, milled to < 0.5mm	Horse mackerel minced muscle	0%, 2% and 4%	Fish were filleted, mixed with liquid GP, stored at -20^{0}C up to 6 months	The rate of inhibition oxidation was 57.28% and 54.13% for samples with 2% and 4% added GP, compared to 0 % added sample	*(Sánchez-Alonso, et al*., 2006)
Grape skin extract four (TP: 150 g PE/kg)	Precooked pork patties	0.2 g CE/kg	Mincing, mixing with additives, forming, cooking to reach 80°C (internal temperature), cooling, packaging, and storage at 4°C in polyethene bags (OTR > 2000 cm^3/m2d)	Experimented on extracts of grape skin, rosemary, green tea and coffee. Antioxidant efficiency as follow: Rosemary>Grape skin>Tea>Coffee>Reference. The extracts help to prevent degradation of vitamin E	(Nissen, Byrne, Bertelsen, & Skibsted, 2004)
Grape seed extract (TP: 980 g F/kg)	Precooked beef and pork patties	0.1 – 0.2 g CE/kg	Grounding, mixing with other ingredients and patties additives, forming, freezing, thawing, cooking to reach 71°C (internal temperature) cooling, packaging, and storage in polyvinyl chloride bags	Experimented on grape seed extract, oleoresin rosemary extract, water-soluble oregano extract. Grape seed extract exhibited the best antioxidant activity for both beef and pork samples. The higher grape seed extract concentration, the more antioxidant activity	(Rojas & Brewer, 2007)
Grape seed flour (TP: 865 g GAE/kg)	Raw and cooked pork patties	0.05 – 1 g CE/kg	Mincing, mixing with additives, forming, cooking to reach 72°C (internal temperature), cooling, packaging, and storage in barrier film packs (OTR: 3 cm^3/m2d) under 75% O2 and 25% CO_2, at 4°C	Lipid oxidation of raw patties was decreased Antioxidant activity increased in both raw and cooked samples along with the increase of grape seed extract concentration Cooked patties sensory was not affected by the addition of extract	(Carpenter, Grady, Callaghan, Brien, & Kerry, 2007)

oils (Shan, Cai, Sun, & Corke, 2005). Phenolic acids generally act as antioxidants by trapping free radicals, while flavonoids can scavenge free radicals and chelate metals (Damodaran, 1996). These activities allow phenolic compounds to interact with biological systems, preventing degenerative diseases linked to oxidative stress in separate tissues and organic systems (Katalinić *et al.*, 2010; Ky *et al.*, 2014). Since grapes, and grape pomace, appear to be abundant sources of phenolic acids and flavonoids, they are promising possibility to be the source of antioxidants.

Meat, and products from meat, have utilised the antioxidant components from grape pomace to facilitate extended shelf life of products. Table 2.6 mentions results observed from some studies about this application. Meat foods contain a high content of fat and prooxidants such as salt and metals, hence their ability to inhibit lipid oxidation is important to their quality as well as shelf-life storage (García-Lomillo & González-SanJosé, 2017a). Different meat processing techniques such as mincing, grinding and cooking are prone to increasing meat oxidation and as such have gained special interest as the antioxidant compounds can influence the colour, flavour, odour, texture and nutritional value of these products (Fernindez, Pérez-Álvarez, & Fernindez-López, 1997). Grape pomace materials (seeds, skins or whole pomace) in either powder or liquid form have been tested, and in general they all exhibit the ability to prevent oxidation, with the phytotoxic polyphenols content range from 0.03 to 0.86 g/kg of tissue (Carpenter *et al*., 2007; Nissen *et al*., 2004; Rojas & Brewer, 2007; Selani *et al*., 2011). Besides meat and meat products, grape pomace has also been shown to be effective against lipid oxidation in fish, which contain a high quantity of PUFAs. Depending upon the kind of fish, processing methods and storage conditions, the necessary concentration of phytotoxic polyphenols might range from 0.1 g/kg tissue (Pazos *et al*., 2005) to 1.3 g/kg tissue (Özen *et al*., 2011).

2.3.3 Grape pomace as a source of antimicrobials

A large problem facing the food industry is microbial action, which leads to food spoilage (causes off – flavours, colour deterioration or acidification, hence reducing consumer acceptance), and foodborne pathogens (harmful to consumer health). Popular solutions include air-packaging (often CO_2), vacuum packaging or synthetic preservatives (García-Lomillo & González-SanJosé, 2017a). Air packing and vacuum packing cannot inactivate microbes completely as some microorganisms are resistant to CO_2, while the utilisation of synthetic substances raises consumer concerns about their safety.

Similar to other plants, phytotoxic grape polyphenols are functionalised to defend against stress from various external factors such as microbial attack. Thus, grape pomace may be an ideal source of natural products to prevent microbial activity and extend the shelf-life of foodstuffs.

The antimicrobial activity of grape pomace products is ascribed to different phenolic compounds. Mingo, Silván, & Martinez-Rodriguez, (2016) reported that flavan-3-ols, especially epicatechin gallate, were effective against *Campylobacter jejuni* and *Campylobacter coli* with an inhibitory concentration of 10 mg/mL and a bactericidal concentration of 20 mg/mL. The antimicrobial activity of flavan-3-ols is explained by their ability to bind the peptidoglycan membrane disrupting its functionality (Lavelli *et al*., 2017). Other flavonoids like flavonols and flavones also have antimicrobial activities, although their effectiveness is lower than those of flavan-3-ols. Xu, Burton, Kim, & Sismour, (2016) reported the role of quercetin in activity against *Staphylococcus aureus*, but not against Gram-negative bacteria. Whereas, the activities of anthocyanins and non-flavonoids compounds are not clear when different studies show controversial results (Lavelli *et al*., 2017).

Regarding grape pomace components, grape seed extracts show higher antimicrobial activities than extracts of skins (Xu *et al*., 2014), which may be explained by the higher concentration of

flavan-3-ols, especially quercetin and its derivatives (Corrales *et al*., 2010). In addition, Katalinić *et al*., (2010) reported lower activities of white grape skins extracts against Gram bacteria than the extracts of red grape skins. Hence the red grape pomace extracts appear to have greater antimicrobial efficiency.

2.4 Conclusion

This chapter has summarised general knowledge about grape pomace from around the world. Common phenolic profiles of grape pomace with the most abundant and popular compounds have been illustrated and discussed. Based on the data summarised, grape pomace is a valuable source of natural bioactive compounds. Current technology and investment are not yet sufficient to fully exploit this natural potential. For years, researchers and even winemakers have paid attention to the recovery of grape pomace in order to turn it into something more meaningful rather than discarding it into soils or feeding it to animals. These are tough challenges for all involved as scaling up and commercialisation of recovery requires complex approaches depending on a number of factors. With the help of emerging techniques such as UAE or PEF, results from laboratory and small-scale factories show promising results with a significant reduction in extraction time and an increase in the yield of extracts.

It can be concluded that grape pomace can be successfully incorporated into food formulations to produce nutritional foodstuffs. The abundance of bioactive compounds assures the promising future for this kind of application. Published results show that the most appropriate use for grape pomace is to enrich cereal-based products which allows full use of the wide range of nutrients found in grape pomace such as phenolic compounds, dietary fibre, minerals (García-Lomillo & González-SanJosé, 2017b). Despite these advantages, there are not many scientific facts showing how the fortification of grape pomace into food benefits human health, how they change when go through the digestion process, and whether or not the bioactive

compounds can really positively influence to the body. Hence, besides attempting to investigate phenolic profiles of some typical New Zealand grape pomaces, this thesis also focuses on *in vitro* experiments to shed light on these matters.

Chapter 3

Materials and methods

3.1 Materials

3.1.1 Grape pomace powder preparation

Three white grape pomaces (SA, PG and GE) and two red grape pomaces (ME and PN), all belonging to *V. vinifera L.*, were collected from Lincoln University winery during the harvesting season in April 2018. The pomaces (including the seeds, skins, and pulps) were dried for 12 h in an oven (Clayson, Clayson Laboratory Apparatus Ltd., New Zealand) at 60 °C, as can be seen in Figure 3.1 and Figure 3.2.

Figure 3.1. Pomaces being dried in the oven

Dried samples were then ground in a blender (Breville BCG200, Breville Pty. Ltd., Australia) (Figure 3.3) to pass through a 60µm mesh screen. The resulting powders were kept in separate Ziploc bags and stored in a freezer (Simpson Opal H700F – XNZ, Simpson Opal Ltd., Australia) at -18 °C until used.

Figure 3.2. Oven Clayson

Figure 3.3. Blender BCG200

3.2 Extraction of materials

3.2.1 Conventional extraction of grape pomace powder

Extraction was conducted based on the method proposed by Iora *et al*., (2015). One gram of each grape pomace powder was mixed with 20 mL of 40% ethanol solution in a Falcon tube to achieve a proportion of solute/solvent of 1:20 (w/v). Samples were then placed in a shaker (Model: IKA-WERKE RT – 15P, IKA Ltd., Germany) as illustrated in Figure 3.4 for 12 h at room temperature, and then centrifuged at 3,000 g for 10 min in a centrifugation (Heraeus Multifuge X1R, Thermo Fisher Scientific, Germany) (Figure 3.5). Supernatants were collected into new Falcon tubes and stored at -18 °C until analysis.

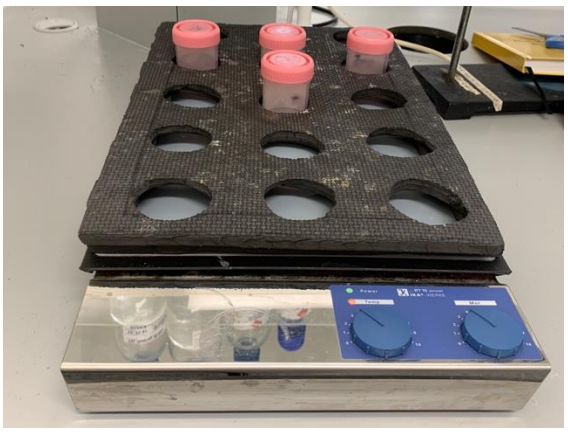


Figure 3.4. Shaker IKA-WERKE RT-15P

Figure 3.5. Centrifugation Multifuge X1R

3.2.2 Ultrasound assisted extraction

A preliminary experiment was conducted with different ratios of ethanol ranging from 20 to 90 %. From this preliminary research an optimum concentration of ethanol was determined at 60 %. Besides, water was also employed as another solvent to compare the extractive effectiveness with the one using ethanol. One gram of each grape pomace powder was mixed with 30 mL of ethanol 60 % in a glass flask to achieve a proportion of solute/solvent of 1:30 (w/v). Samples were then placed in the chamber of ultrasound bath (Elmasonic S300H, Elma Schmidbauer GmbH, Germany) (Figure 3.6), water was placed into the chamber over the pomace in each flask. Extraction parameters were as followed: temperature 50 °C, time 40 min. Supernatants were collected separately, centrifuged at 3,000 g for 10 min in centrifugation, then stored at -18 °C until analysis.

Figure 3.6. Elmasonic S300H

3.2.3 Phenolic extract preparation

Four grams of each grape pomace powder were mixed with 120 mL of ethanol 60 % to have ratio of solute/solvent of 1:30 (w/v), then was extracted by using extraction procedure of ultrasound assisted extraction in section 3.2.2. Supernatants were then collected and evaporated by using evaporator system, which is illustrated in Figure 3.7. The left over materials after the evaporation were then collected, freeze-dried and kept in freezer at -18 ^{o}C until analysis.

Figure 3.7. Evaporation system

3.3 Food samples preparation

3.3.1 Cookie preparation

Cookies were prepared based on AACC Method 10-50.05. Baking Quality of Cookie Flour, which is approved by American Association of Cereal Chemists (AACC, 1999). Ingredients and their quantity are described in Table 3.1.

Table 3.1. Ingredients and their quantity used to make cookies

Ingredients	Quantity
Granulated sugar	130 g
Salt	2.1 g
Sodium bicarbonate ($NaHCO_3$)	2.5 g
Shortening	64 g
Dextrose solution (8.9 g dextrose flour in 150 mL water)	33 mL
Distilled water	16 mL
Whole wheat flour	225 g

Depending on the formulas, wheat flour was partially replaced by grape pomace powder at levels of 5% (11.25 g), 10% (22.5g) and 15 % (33.75 g).

The shortening, sugar and sodium bicarbonate were creamed in a mixer (Delta 500A, Delta Food Equipment) (Figure 3.8) using flat beater at a low speed for 3 min. After mixing, the dextrose solution and distilled water were added, with further mixing for 1 min at low speed, and 1 min at medium speed. The flour was then added and mixed for 2 min at low speed. The dough was placed on a flat chopping board, lightly rolled to achieve a thickness of 5 mm. Cookies were cut from the dough using a 57 mm wire cutter. The cookies were baked in an oven as illustrated in Figure 3.9 (Model: E32M, Moffat Ltd., New Zealand) at 160 °C for 10 min. The final products were cooled at room temperature for at least 6 h before analysis.

Figure 3.8. Mixer Delta 500A

Figure 3.9. Oven Turbofan E32M

3.3.2 Paste preparation

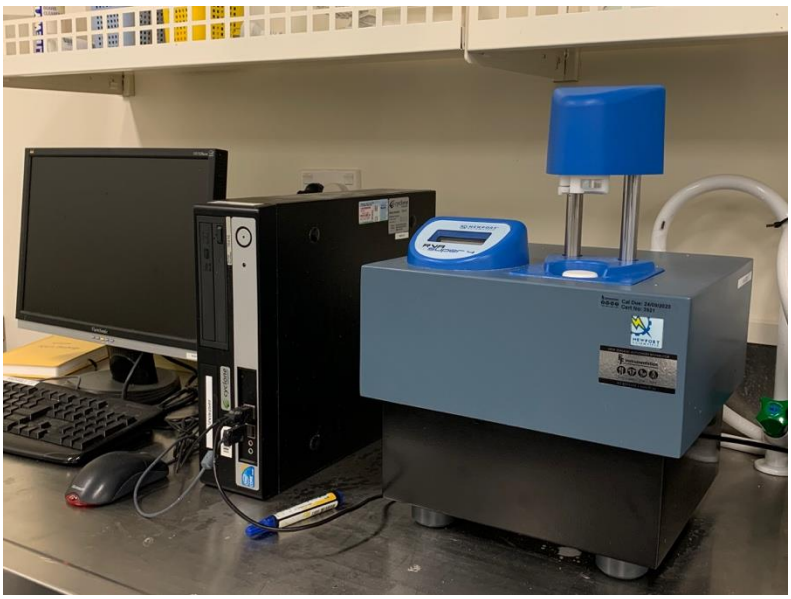


Figure 3.10. RVA Super 4

Pasting samples were prepared based on AACC method 76-21.01 (General pasting method for Wheat or Rye flour or Starch using Rapid visco-analyser) (AACC, 2010). Figure 3.10 presents the an RVA machine (RVA Super 4, Newport Scientific Ltd., Australia), which was used in this experiment. Three g of rice starch or mixture (rice starch and grape pomace powder or pomace extracts) were added to a test canister containing 25 mL of distilled water. Details about how much starch were replaced are presented in Table 3.2. A plastic blade stirrer was used to disperse any lumps by jogging up and down in the canister for 1 min before starting the measurement cycle. Total time for the cycle was 13 min and comprise of the following steps: the temperature was kept at 50 °C for 1 min, then quickly increased to 95 °C over 3.5 min and held at this temperature for 3 min. After that, it was cooled down to 50 °C over 3.5 min and maintained at 50 °C for 2 min.

Table 3.2. Weight of pomace substituted to rice starch

Samples fortified with	5 %		10 %	
	Weight of starch (g)	Weight of pomace (g)	Weight of starch (g)	Weight of pomace (g)
Grape pomace powder	2.85	0.15	2.7	0.3
SA pomace extract	2.9975	0.0025	2.9955	0.0045
PG pomace extract	2.994	0.006	2.988	0.012
GE pomace extract	2.9955	0.0045	2.991	0.009
ME pomace extract	2.9933	0.0067	2.9865	0.0135
PN pomace extract	2.9919	0.0081	2.9838	0.0162

SA: Sauvignon Blanc; PG: Pinot Gris, GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir

3.4 Methods

Some assays mentioned below including total phenolic content determination, antioxidant capacity determination were multi-purpose which were used for various kind of samples. All chemical and reagents were of analytical grade

3.4.1 Moisture content

A 2 g ground sample of each cookie type was placed in a tin and dried overnight in an oven at 105 °C. The dried sample was weighed after being allowed to cool for 1 h in a desiccator. Moisture content was calculated by using the equation 3.1.

$$M(\%) = \frac{M_1 - M_2}{M_1} x100 \quad (3.1)$$

In which M_1: weight of sample before drying

M_2: weight of sample after drying

3.4.2 Fat content

This method is the Soxhlet extraction of crude fat from Feed & Forage, which was developed by the Faculty of Agriculture and Life Sciences, Lincoln University. It is based on the principle that crude fat (total lipids) is determined gravimetrically by Soxhlet extraction with hexane X4. The dried samples (2 g) were placed into a paper thimble and then attached to a collector cup filled

with 80 mL of hexane X4. At the end of the extraction process, all the cups were dried in the oven at 100 °C and reweighed. All samples were extracted in duplicate. The percentage of fat was calculated using the following equation 3.2:

$$\text{Crude fat (as is, \%)} = \frac{\mathrm{fW-eW}}{\mathrm{sW}} \mathrm{x}100 \quad (3.2)$$

fW – final weight of collector cup after process

eW – weight of each empty collector cup

sW – weight of sample

The crude fat based on dried matter was calculated based on equation 3.3

$$\text{Crude fat (DM basis, \%)} = \frac{\text{Crude fat (as is,\%)}}{\mathrm{rDM}} \mathrm{x}100 \quad (3.3)$$

rDM – residual dry matter of the sample, determined on an independent sub-sample using the standard method

3.4.3 Total phenolic content (TPC) determination

Determination of TPC of samples was performed using Folin – Ciocalteau as described by Iora *et al*., (2015). Sample aliquots (0.5 mL) were mixed with 2.5 mL of 0.2 N Folin – Ciocalteau reagent and 7.5% sodium carbonate (Na_2CO_3) solution and incubated for 2 h in the dark. The absorbance of the mixture was measured at 760 nm (V1200, Shimadzu, Maryland, USA). The absorbance reading result was compared to a standard curve of gallic acid and the final results expressed as milligram gallic acid equivalent per gram dry matter (mg GAE/g DM). Depending upon specific cases, samples might need to be diluted by appropriate factors. Except for sample aliquots, all other reagents and solutions were freshly prepared before use. All analyses were conducted in triplicate.

3.4.4 Anthocyanin content determination

Firstly samples were diluted by the appropriate factor, which was determined by diluting the sample with potassium chloride buffer pH 1.0 until the absorbance of the sample at $\lambda_{vis\text{-}max}$ was within the linear range of the spectrophotometer. The final volume was then divided by the initial volume to obtain the dilution factor. In this assay, $\lambda_{vis\text{-}max}$ = 510 nm and the dilution factor was 5 for the white pomaces, 15 for Merlot pomace and 10 for Pinot Noir pomace.

Each sample was diluted separately using potassium chloride buffer and sodium acetate buffer with the dilution factor determined previously. Samples were left to equilibrate for 15 min, then were measured at $\lambda_{vis\text{-}max}$ = and λ = 700 nm, against a blank cell filled with distilled water.

The final absorbance of the sample was calculated using the equation 3.4

$$A = (A_{\lambda vis\text{-}max} - A_{700})_{pH1} - (A_{\lambda vis\text{-}max} - A_{700})_{pH4.5} \quad (3.4)$$

The monomeric anthocyanin pigment concentration in the original sample was calculated using the equation (3.5). Results were expressed as mg Cyanidin-3-glucoside/litre (mg Cya-3-glu/L)

$$M = \frac{A \text{ x } MW \text{ x } DF \text{ x } 1000}{\varepsilon \text{ x} 1} \quad (3.5)$$

In which: M – monomeric anthocyanin pigment (mg Cya-3-glu/L)

A – final absorbance (determined previously)

MW – molecular weight (in this case MW = 449.2 for cyanidin-3-glucoside)

DF – dilution factor

ε - molar absorptivity (in this case ε = 26,900)

3.4.5 Tannin content determination

In this experiment, control samples and pomace samples were tested using different formulas. For the control, 100 µL of the sample was dispensed into a microtube, followed by 200 µL of saturated ammonium sulphate solution and 700 µL of deionised water. For the pomace samples, 100 µL of sample were dispensed into a microtube, followed by 300 µL of methylcellulose, 200 µL of saturated ammonium sulphate solution and 400 µL of deionised water. All solutions were left to react for 10 min at room temperature, then centrifuged for 5 min at 10,000 xG. The absorbance was measured at 280 nm.

Actual absorbance of the grape pomaces were calculated as followed

$$A = A_c - A_s \tag{3.6}$$

In which: A – absorbance of sample

A_c – absorbance of control

A_s – absorbance of sample

The absorbance values were used to calculate tannin content by using the epicatechin calibration curve

The tannin concentration of the grape pomaces was calculated using equation 3.7

$$T = \frac{[\text{Tannin}]_e \text{ x } V_e}{W_h} \tag{3.7}$$

In which: T – final tannin concentration of samples (mg epicatechin equivalent /L, mg epi./L)

$[\text{Tannin}]_e$ = tannin x DF, where DF – dilution factor (in this case DF = 10)

V_e – final volume of extract (litre, L)

W_e – initial weight of powder sample used to extract (mg)

3.4.6 High-performance liquid chromatography (HPLC)

The detection of different phenolic compounds was done by using a systems included an Agilent HPLC with quaternary pump, photodiode array detector and fluorescence detectors. To start, 10 µL of sample were injected into an Ace® 5 column (Advanced Chromatography Tecnologies, Aberdeen, Scotland) with the dimension of 250 x 4.6 mm. Table 3.3 shows the solvent gradient used for separation. After the separation was initiated, the chromatograms were recorded at 280, 320 and 360 nm in photodiode array detector to detect and quantify the compounds. The corresponding chromatogram was then scan from 220 nm to 600 nm in flouresence detectors. All samples were tested in triplicate. The data were processed and compared with the pure standard compounds in the specific wavelength.

3.4.7 Antioxidant capacity

3.4.7.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) determination

The DPPH (2,2-diphenyl-1-picrylhydrazyl) is used to determine the antioxidant capacity of samples. The assay used here has been described by Hossain, Brennan, Mason, Guo, & Brennan, (2017). An aliquot (0.5 mL) of the sample was mixed with 1 mL of 0.1N DPPH solution and 1.5 mL of methanol and then incubated for 30 min in the dark. The absorbance of the mixture was measured at 530 nm. The results were compared to a standard curve of Trolox and the final results were expressed as milligram Trolox equivalent per gram dry matter (mg TE/g DM).

Table 3.3. The solvent gradient used for seperation

Time	Solvent A (%)	Solvent B (%)	Solvent C (%)
0	100	0	0
2	100	0	0
5	93.6	6.4	0
17	2.8	11.2	86
22	3.6	14.4	82
29.5	4.2	16.8	79
55	6.6	26.4	67
70	10	40	50
75	10	40	50
78	36	64	0
81	36	64	0
86	100	0	0
90	100	0	0

Solvent A: $NH_4H_2PO_4$ (0.05 M, pH = 2.6)

Solvent B: 100% acetonitrile

Solvent C: H_3PO_4 (0.2 M, pH = 1.5)

3.4.7.2 Ferric ion reducing antioxidant power (FRAP) determination

The FRAP assay used in this study is based on the assay described by (Ky *et al*., 2014). Water (1 mL) and 80 µL of the sample were placed in 4 mL plastic cuvette. Then 600 µL of FRAP reagent was added to the cuvette and briefly inverted to mix the solution. After 4 min of incubation, the absorbance was measured 593 nm. Results were compared to a standard curve of Trolox or Ferrous sulphate, and final results were expressed as micromole Trolox equivalent per gram dry matter (µmol TE/g DM), or micromole ferrous per gram dry matter (µmol Fe^{2+}/g DM).

3.4.7.3 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) determination

The ABTS assay used in this study is based on the assay described by (Ky *et al*., 2014) with some modification. ABTS radical reagent (3 mL) and 300 µL of sample or standard solution were mixed in a 4 mL plastic cuvette. After 6 min of incubation, the absorbance was measured at at 734 nm. Results were compared to a standard curve of Trolox, and final results are expressed as micromole Trolox equivalent per gram dry matter (µmol TE/g DM). Except for sample aliquots, all other solutions were freshly prepared before use. All analysis were done in triplicate.

3.4.8 Dietary fibre determination

Dietary fibre content was determined based on AOAC method 991.43 "Total, Soluble and Insoluble dietary fibre in foods" and AACC method 32-07.01 "Determination of soluble, insoluble, and total dietary fibre in food and food products" (Lee, Prosky, & Vries, 1992)

A sample of 1 g of each pomace powder was weighed into a beaker (each sample had two samples weighed out as at the end of the analysis one of the residues was used for ash determination and the other for protein determination). Then 40 mL MES – TRIS blend buffer solution and 50 µL α-amylase solution were added, it was covered in foil and incubated at 100 °C for 30 min in a shaking water bath. Then, 100 µL of protease were added, and the beaker was incubated at 60 °C for another 30 min. Finally, 5 mL of 0.561 N HCl solution and 200 µL amyloglucosidase were added, and the beaker was incubated one more time at in shaking bath at 60 °C for 30 min.

Determination of IDF

The solutions derived from above were filtered through fritted crucibles, prepared with a bed of 1 g of Celite, using suction generated by two-stage vacuum pump SPX15031, Robinair, Michigan, USA (Figure 3.11). After filtration, the liquid obtained was kept for SDF analysis. The

residues in crucibles were washed twice with 95 % ethanol and acetone, then dried overnight in an oven at 103 °C. The following day, crucibles were cooled in a desiccator for 1 h before being weighed. Residue weight was obtained by using equation 3.8

$$W_R = W_1 - W_2 \quad (3.8)$$

In which: W_R – weigh of residue;

W_1 – weight of crucible, Celite and residue after drying;

W_2 – weight of crucible and Celite before filtration

For ash analysis, the crucible was heated for 5 h at 525 °C in a furnace, then cooled in a desiccator and weighed. The weight of ash was determined by subtracting the crucible weight and the Celite weight.

Protein analysis was done in different laboratory of Lincoln University, New Zealand. It followed Kjeldahl method with the conversion factor was 6.25.

Determination of SDF

Four volumes of 95 % ethanol preheated to 60 °C were added to the liquid residue resulting from IDF extraction. The precipitate was allowed to form for 60 min. The solution was filtered through fritted crucibles, which contained 1 g of Celite. Residues obtained were washed twice with two 15 mL portions of 78 % ethanol, 95 % ethanol and acetone.

Ash and protein were determined as described for IDF determination

Determination of total dietary fibre (TDF)

The TDF was determined by the total of SDF and IDF

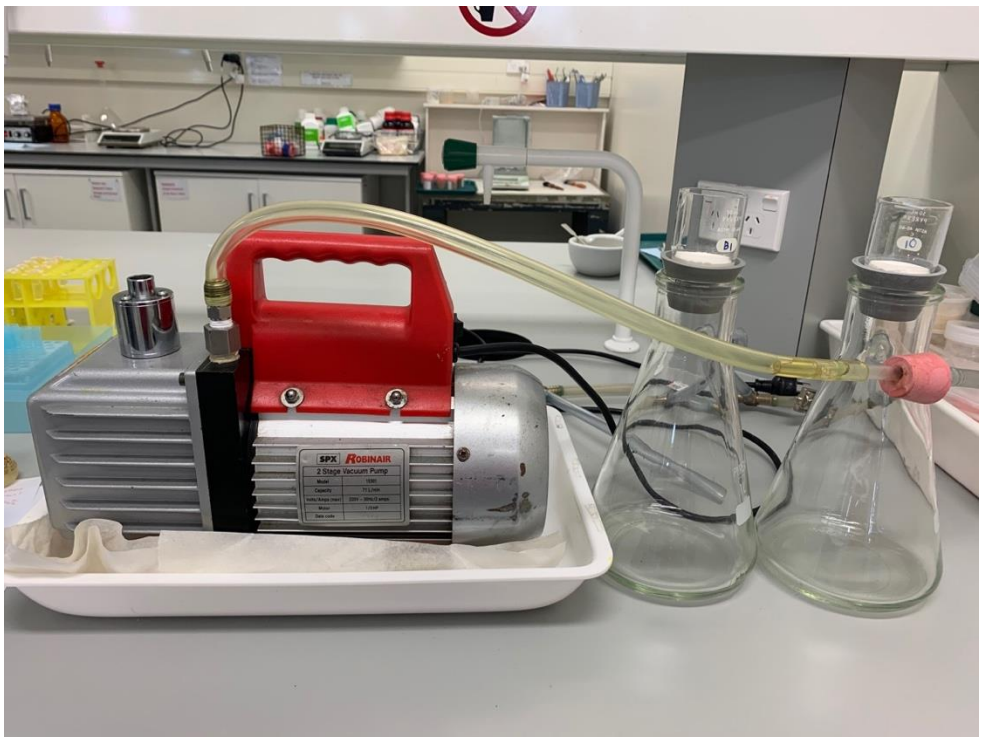


Figure 3.11. System for dietary fibre analysis

3.4.9 Cookies sensory analysis

3.4.9.1 Colour measurement

Three parameters of cookie colours including *L** (brightness), *a** (redness) and *b** (yellowness) were determined by measuring the surface of cookies using a tristimulus colour analyser (Minolta Chroma Meter CR 210, Minolta Camera Co., Japan) (Figure 3.12). The instrument is calibrated using a standard white plate (*L**= 98.03, *a**= -0.23, *b**= 0.25).

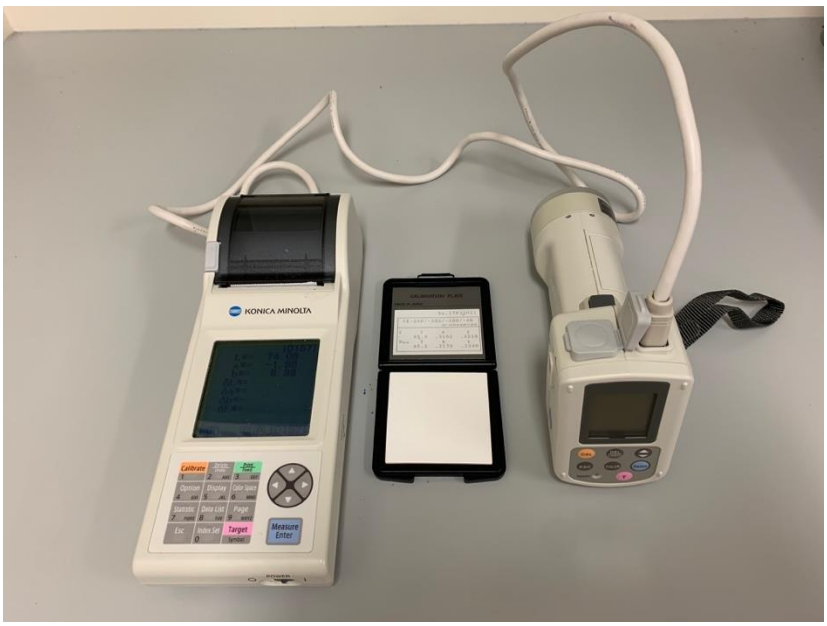


Figure 3.12. Minolta Chroma Meter CR400

3.4.9.2 Texture analysis

Figure 3.13 illustrates a texture analyser (TA.XT plus Texture analyser, Stable Micro Systems, Godalming, UK), which was used to determine the textural properties of the cookies. A blade was installed at the top of the cookie and the breaking process was set up by lowering the blade. The Small three-point bend rigs programme was used with parameters set up as follows: pre-test speed: 2 mm/s, test speed: 5 mm/s, post-test speed: 5 mm/s, distance: 10 mm, load cell: 50 kg. Each cookie was placed on a support ring; when the measurement was initiated, the probe moved down until it the cookie was completely broken. The breaking force was expressed as maximal breaking strength (kg)

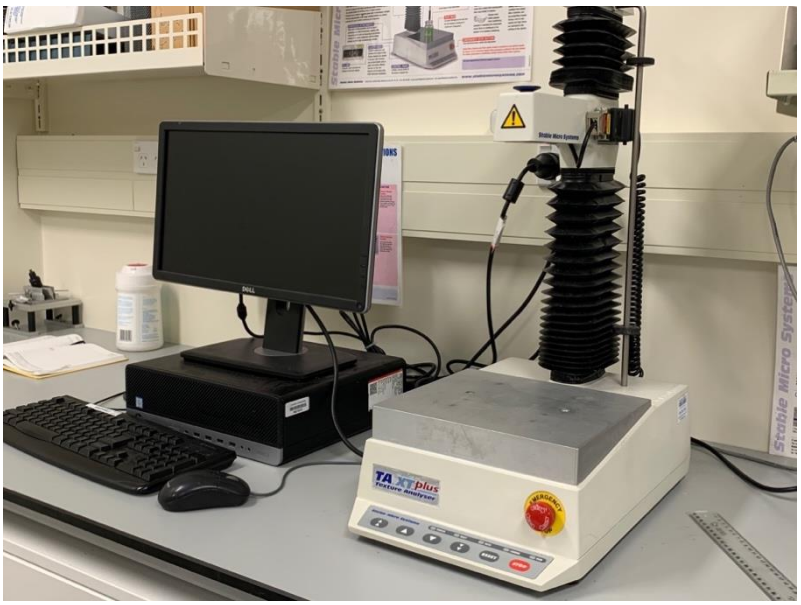


Figure 3.13. Texture analyser TA.XT.Plus

3.4.10 *In vitro* gastrointestinal

The *in vitro* digestion described by Hossain *et al*., (2017) was employed to discover the glycaemic glucose equivalents. This assay measured the amount of free reducing sugars released during enzyme hydrolysis. An amount of ground sample was placed in a pot and suspended with 30 mL of RO water. It was placed on a magnetic heated stirring block (Figure 3.4), set at 37 °C and a constant stirring was maintained for the duration of the experiment. Gastric digestion was mimicked by adding 0.8 mL of 1M HCl and 1 mL of 10 % pepsin (Sigma Aldrich, USA), in 0.05 M HCl. After 30 min incubation, the gastric digestion was stopped by adding 2 mL of 1M sodium bicarbonate. A 1 mL aliquot was then transferred into a Falcon tube containing 4 mL of ethanol and this sample was labelled as “Time 0” and was stored at 4 °C until analysis.

Then 0.1 mL of amyloglucosidase was added to each sample pot in order to prevent end-product inhibition of pancreatic α-amylase. To simulate the small intestine digestion, added 5 mL of 2.5% pancreatin solution (which was prepared in 0.1 M sodium maleate buffer pH6 immediately before use)and the timer was started. Distilled water was added to each pot to make to volume. A 1 mL aliquot was removed at 20, 60 and 120 min and pipetted into each

tube containing 4 mL of ethanol. They were labelled as “Time 20, Time 60 and Time 120” and stored at 4 °C until analysis.

A 50 µL aliquot of either sample, RO water, solution of 5 mg/mL glucose standard or solution of 10 mg/mL glucose standard was added to a glass test tube. Then 0.25 mL of enzyme solution (1 % invertase + 1 % amyloglucosidase in acetate buffer pH 5.2) was added and left to digest for 20 min at room temperature. DNS mixture (0.75 mL) was added to the tube then, it was covered with foil and heated in a boiling water bath for 15 min. Cooling was expedited by placing in cold water for 10 min before 4 mL of RO water was added to the tubes. Absorbance was read at 530 nm. Reducing sugars released were calculated as mg/g sample and plotted against time, then the area under the curve (AUC) was calculated by dividing the graph into trapezoids. Apparatus, chemicals, buffers and enzymes.

3.4.11 α-amylase inhibitory activity

Phenolic compounds were extracted from grape pomace powder using UAE as described in 3.2.2.1, then the samples were freeze-dried until analysis. On the day of the experiment, dried extracts were dissolved in 0.01 M phosphate buffer to a concentration of 2 mg/mL.

In each well of a 96 well-plate, 25 µL of sample or buffer (for control) were mixed with 50 µL α-amylase and then incubated at 37 °C for 10 min. Then 50 µL of starch solution were added and incubation was continued for another 10 min at 37 °C. After incubation, 25 µL of 1M HCl was added to stop the reaction. Then 100 µL of 1 % iodine-potassium solution was added to each well, the absorbance was read at 630 nm. Results were expressed as mM of acarbose equivalent (mmol ACE/g extraction).

3.4.12 Statistical analysis

All experiments were done in triplicate unless stated otherwise. Results were analysed using one way analysis of variance (ANOVA) and Tukey's comparison (T test, $p < 0.05$). SPSS version 20.0 and Prism version 8.0 were the two software employed to conduct the analysis.

Chapter 4

Characterisation of grape pomace and optimisation of phenolic extraction

4.1 Introduction

During the winemaking process, only 30 – 40 % of the polyphenols from the fruit of the grape are extracted into wine, depending on the grape varieties, vineyard sites, and winemaking techniques used. The rest (up to 70 %) of the phenolic compounds are discarded along with the grape pomace (Fontana *et al*., 2013; Ky, 2013). Hence, grape pomace has the potential to become a valuable source of phenolic compounds and other bioactive components including dietary fibre. Trost *et al*., (2016) observed the TPC of Slovenian red grape pomaces and showed that they ranged from approximately 35 to 60 mg GAE/g dry matter, while the content of white grape pomaces ranged from 30 to 45 mg GAE/g dry matter. Also, the antioxidant capacity of various grape pomaces has been investigated with red grape pomaces having higher values (0.25 to 0.6 mmol TE/g dry matter), while white grape pomaces were 0.3 to 0.45 mmol TE/g dry matter. However, there has been little published research focused on the profile of bioactive compounds from New Zealand grape pomace. Cheng et al., (2012) investigated the profile of two New Zealand grape pomaces (Pinot Noir and Pinot Meunier) and obtained TPC ranging from 37.3 to 469.9 mg GAE/g extract, depending upon the parts of the grape (seeds, skins or whole pomaces).

The profiles of grape pomace bioactive compounds have been shown to vary depending on a number of factors, in which the extraction method plays a crucial role. The extraction step decides the quality of recovery, isolation and identification of compounds before utilisation of extracts. However, various methods have been employed depending on the aim of the study, the research objectives, and the available facilities. This is a result of the fact that there is no

standard method for extraction. Long used methods or “traditional methods” contain some disadvantages such as being time-consuming, using large amounts of solvent, having the possibility of thermal decomposition of compounds as heating is required, loss of compounds due to hydrolysis, ionisation and oxidation during the extraction process (Wang & Weller, 2006). The most common technique used for traditional extraction of grape pomace compounds is solid-liquid extraction, which can be defined as “a phenomenon of mass transport in which the analytes contained in a solid matrix migrate into a solvent phase that is in contact with the matrix” (Fontana *et al*., 2013). In solid-liquid extraction, the solvent is the most important factor and methanol, ethanol, acetone and water have been reported as the favourable ones. *Iora et al*., (2015) tested the extraction of grape pomace using ethanol/water with concentration ranged from 0 – 100 % and concluded that optimum extract condition was achieved with a solution of 40 % ethanol for proportion solute/solvent of 1:20. Despite the drawbacks mentioned above, traditional extraction methods are still largely used thanks to their availability, low cost, ease of combination with other techniques to boost the extraction and sustainability of the process (Fontana *et al*., 2013; Wang & Weller, 2006).

To overcome the disadvantages of traditional extraction methods, a number of novel extraction methods have been introduced in recent years. Among all, the most cited methods include microwave assisted extraction, supercritical fluid extraction, accelerated solvent extraction and ultrasound assisted extraction (UAE). Although these assays work in different mechanisms and principles, all of them try to reduce the extraction time and increase extraction efficiency. UAE rely on the waves of sound with frequencies higher than 20 kHz to create vibrations that travel in liquid, causing expansion and compression during travel. The expansion creates bubbles in liquid with negative pressure. Bubbles form, grow and explode to generate compression of gases and vapours, consequently generating high temperature and pressure. Those increased

temperatures and pressures in turn facilitate the penetration of extraction solvent into solid matrices and hence, improve the exchange between solid matrices and liquid phase. Those activities favour the leaks of compounds inside solid resulting in the increase of yield of extraction (Luque-García & Luque De Castro, 2003; Vinatoru *et al*., 1997). Da Porto, Porretto, & Decorti, (2013) compared the efficiency of UAE and Soxhlet method for extracting oil and polyphenols from grape seeds and observed a similar yield of oil and phenolic compounds. However, while the Soxhlet method required 6 h of extraction, UAE only took 30 min to produce the same production.

Extraction temperature is an important parameter that should be considered in any method used to extract compounds from plants and plant products. A growth in temperature leads to the decrease of viscosity and surface tension, and an increase of vapour pressure, which results in more solvent vapours to enter the bubble cavity, hence reduce the pressure when bubbles collapse, decrease the ultrasound effects (Santos, Lodeiro, & Capelo-Martínez, 2009). Some authors reported extraction temperature should be in between of 30 – 50 °C to achieve better yield (Chemat *et al*., 2017; Zhang, Yang, Zhao, & Wang, 2009).

This chapter aimed to investigate the compositions of New Zealand grape pomaces obtained from five typically local grape varieties. Sauvignon Blanc (SA) is the most popular variety which is cultivated in the largest areas with the yield has been increasing from 224,412 tonnes in 2011 to 326,058 tonnes in 2020. Pinot Noir (PN) comes next with the yield was 7 times to 10 times lower than SA during the same time. Meanwhile, Pinot Gris (PG), Merlot (ME) and Gewürztraminer (GE) are ranked at 3th, 5th, and 9th position, respectively (New Zealand Winegrowers, 2020). Besides, the comparison between different extraction methods were made to find out the most appropriate one which gives the better extraction results regarding the yield and extraction time.

4.2 Materials and methods

4.2.1 Grape pomace preparation

As described in section 3.1.1

4.2.2 Conventional grape pomace extraction

As described in section 3.2.1

4.2.3 Ultrasound assisted extraction

As described in section 3.2.2 with some changes. The extraction time included different periods: 5, 10, 15, 20, 30, and 40 min. Supernatants of each period were collected separately, centrifuged at 3,000 g for 10 min in centrifugation, then stored at -18 °C until analysis.

4.2.4 Total phenolic content determination

As described in section 3.4.3

4.2.5 Anthocyanin content and tanin content determination

As described in section 3.4.4 and 3.4.5

4.2.6 Antioxidant capacity determination

As described in section 3.4.7.2 for FRAP and 3.4.7.3 for ABTS

4.2.7 Dietary fibre content and High-performance liquid chromatography (HPLC)

As described in section 3.4.8 and 3.4.6

4.2.8 Analysis of data

As described in section 3.4.12

4.3 Result and discussion

4.3.1 Selection of extract method

Since grape pomace is well known for the abundance of phenolic compounds, it is essential to recover as much as possible those compounds plus other valuable components from this by-product. Determining the quantity and quality of compounds recovered are important factors to evaluate the efficiency of extracted methods. Other factors which should be taken into consideration include how the methods help to shorten the extraction time, reduce organic solvent consumption and increase sustainability. In recent years, UAE has been studied and widely proposed as an ideal technique for the extraction of phenolics from grape pomace. It is essential to determine optimal parameters when employ UAE to extract grape pomaces in this study. As a "new" method which has appeared in recent times, optimal working parameters of UAE on specific extracted objectives require specific study to thoroughly understand. The following parts of this section will discuss what is the best time duration and how the concentration of solvent (ethanol) affects the extraction. The time and yield of UAE extraction will also be compared with conventional extraction, which is widely use in this lab, to find out which technique is better. In this section, all analysed results are presented for comparison between extraction methods. Details about the differences between time of extraction regarding TPC, anthocyanin content, tannin content and antioxidant capacity of each assay are described in the Appendix.

4.3.1.1 Total phenolic content

Table 4.1 illustrates the TPC of five grape pomaces extracted by using ultrasound assisted extraction. The two methods applied in the extraction of the samples differed in the solvent used. The first one used ethanol with a concentration of 60 %, while the second one employed water as the solvent. Samples extracted by 60 % ethanol showed an increase in TPC associated with the length of extraction time. For instance, the extraction time of 40 min gave the highest TPC, while the lowest contents were obtained after 5 min (the analysis of results for differences between time periods for each method are presented in the Appendix A).

Table 4.1. Total phenolic content of grape pomaces extracted by different methods

	Conventional extraction				
	SA	**PG**	**GE**	**ME**	**PN**
	11.68 ± 0.26^{b}	31.28 ± 0.28^{b}	26.49 ± 0.09^{b}	32.19 ± 0.41^{c}	48.53 ± 0.81^{d}
	Ultrasound assisted extraction using 60 % ethanol				
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	15.70 ± 0.87^{c}	44.77 ± 2.67^{c}	35.61 ± 1.37^{c}	49.31 ± 0.65^{c}	62.50 ± 2.75^{e}
10’	15.90 ± 0.96^{c}	46.40 ± 1.06^{cd}	36.54 ± 0.72^{cd}	56.29 ± 0.86^{e}	63.31 ± 0.57^{e}
15’	16.19 ± 0.88^{c}	47.72 ± 2.32^{d}	37.90 ± 1.31^{cd}	57.18 ± 0.82^{e}	65.35 ± 3.59^{ef}
20’	16.52 ± 2.60^{c}	48.58 ± 0.88^{de}	38.56 ± 1.06^{de}	56.05 ± 1.40^{e}	66.32 ± 2.04^{ef}
30’	16.38 ± 0.26^{c}	48.71 ± 0.97^{de}	40.75 ± 1.48^{ef}	61.53 ± 3.07^{f}	67.35 ± 0.34^{f}
40’	16.65 ± 0.65^{c}	51.51 ± 1.59^{e}	42.29 ± 1.32^{f}	62.05 ± 0.19^{f}	68.52 ± 0.68^{f}
	Ultrasound assisted extraction using water				
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	6.37 ± 0.33^{a}	23.30 ± 0.45^{a}	15.19 ± 0.54^{a}	19.31 ± 1.07^{a}	31.32 ± 0.38^{a}
10’	6.77 ± 0.17^{a}	23.25 ± 0.62^{a}	15.26 ± 0.27^{a}	21.47 ± 0.17^{ab}	29.08 ± 0.28^{a}
15’	6.73 ± 0.25^{a}	24.28 ± 0.11^{a}	15.88 ± 0.36^{a}	22.83 ± 0.27^{b}	32.73 ± 0.62^{ab}
20’	6.96 ± 0.19^{a}	24.96 ± 0.73^{a}	15.38 ± 0.27^{a}	21.78 ± 0.33^{b}	33.42 ± 0.51^{bc}
30’	7.49 ± 0.33^{a}	24.96 ± 0.39^{a}	16.93 ± 0.41^{a}	22.50 ± 0.34^{b}	37.09 ± 0.63^{c}
40’	7.94 ± 0.39^{a}	25.60 ± 0.31^{a}	17.18 ± 0.21^{a}	24.05 ± 0.79^{b}	36.26 ± 0.22^{c}

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir
All values are expressed as milligram gallic acid equivalent per gram dried matter (mg GAE/g DM)
Data are expressed as mean ± SD (n = 3)
Results with different superscripts in the same column are significantly different ($p < 0.05$)

However, SA samples showed no significant difference ($p > 0.05$) in TPC among periods of extraction, indicating that the extraction time had no significant impact on the recovery of phenolic compounds from this pomace. By contrast, the other four grape varieties showed significant differences ($p < 0.05$) during extraction times, especially between 5 and 40 min of extraction. Hence, the extraction time influenced the content of phenolic gained by using UAE. In terms of varieties, the levels of TPC from red grape pomace, including ME and PN, were always higher than the three white grape pomaces when the same extraction conditions were used. The highest value gained at 40 min were 68.52, 62.05, 51.51, 42.29 and 16.65 mg GAE/g DM for PN, ME, PG, GE and SA, respectively. When samples were extracted using water as the solvent, the level of TPC recovered decreased by a half to two-thirds in comparison to samples that were extracted using ethanol as the solvent.

The highest content of TPC extracted using water as a solvent for each pomace were as follows: SA (7.94), GE (17.18), PG (25.60), ME (24.05) and PN (37.09), mg GAE/g DM. Similar to ethanol extracts, all samples extracted by UAE using water had the lowest and highest yield at 5 min and 40 min, respectively. The only one exception happened to PN when the content obtained at 30 min was higher than the value obtained at 40 min (37.09 and 36.26 mg GAE/g DM, respectively). However, the variations in TPC values showed no consistent trends with levels decreasing and then increasing over time and extraction processes. Regarding grape varieties, PN yielded the highest TPC, followed by PG, whose concentration was higher than ME at all time of extraction. The two lowest TPC values were obtained from GE and SA, respectively.

Table 4.1 also presents the TPC of five grape pomaces extracted by conventional extraction methods. The term “conventional extraction” used in this thesis refers to assays which have been being widely utilised and confirmed for long time by researchers. The highest TPC value was observed from the PN pomace (48.53 mg GAE/g DM), which was significantly higher than ME (32.19 mg GAE/g DM) and PG (31.38 mg GAE/g DM). The lowest content was obtained from the grape pomace extraction of SA (11.68 mg GAE/g DM). In comparison to the two UAE methods, the phenolic yields gained from the conventional method were significantly lower than UAE using 60 % ethanol but higher than UAE using water. The values also showed the same trend with the new methods in that PN, ME and PG were higher in TPC values than the other grape varieties.

The dominance in TPC of red grape pomaces over white grape pomaces in terms of the levels of TPC has been observed before. For instance, Deng *et al*., (2011) investigated the chemical compositions of three red grape skins (Cabernet Sauvignon, ME and PN) and two white grape pomace skins (Muller Thurgau and Morio Muscat) and illustrated that the red ones had a TPC value that ranged from 21.4 to 26.7 mg GAE/g DM, while the white skin varieties had a TPC

value ranging from 11.6 to 15.8 mg GAE/g DM. Another comprehensive research on red grape pomace (Pinot Noir) and white grape pomace (Pinot Meunier) harvested and collected in New Zealand also supported this facts when the TPC of seeds, skins and pomaces from PN were far higher than those of Pinot Meunier (Cheng *et al*., 2012a)

4.3.1.2 Anthocyanin content

Table 4.2 describes the anthocyanin content gained from the three extraction assays. The content of anthocyanin extracted by using water extraction was much lower than the content extracted by ethanol, while the content from conventional assay lay in between the other values. In the group of extracts gained from water, the recovered contents of three white grape pomaces were very low with the highest values of 0.018 and 0.016 mg malvidin-3-o-glucoside equivalent (M3OG)/g DM being recorded for SA at 40 min and GE at 15 min. The highest content of anthocyanin obtained from PG was only 0.011 mg M3OG/g DM. Indeed, anthocyanin was not detected in the extracts from SA at 5, 10 and 15 min, and were not observed in the extracts of GE from 5, 10 and 30 min intervals. In contrast, the anthocyanin contents of red grape pomaces were much higher than white group. The anthocyanin content of PN increased along with the increase of extraction time and ranged from 0.488 to 0.648 mg M3OG/g DM, while the content of ME ranged from 1.911 to 2.520 mg M3OG/g DM. In terms of red grape pomaces, differences between extraction time were significant ($p < 0.05$).

Higher levels of anthocyanin were observed in ethanol UAE extracted white grape pomace samples compared with extraction by water. The highest yields could be seen in GE extracts with the peak of 0.092 mg M3OG/g DM at 40 min. Although UAE using ethanol helped to

Table 4.2. Anthocyanin content of grape pomaces extracted by different methods

Conventional extraction					
	SA	PG	GE	ME	PN
	nd	nd	nd	2.33 ± 0.09[ab]	1.02 ± 0.16[d]
Ultrasound assisted extraction using 60 % ethanol					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5'	0.006 ± 0.027[a]	0.034 ± 0.012[ab]	0.083 ± 0.004[b]	3.852 ± 0.012[c]	1.446 ± 0.056[e]
10'	0.028 ± 0.003[a]	0.045 ± 0.011[b]	0.089 ± 0.004[b]	4.371 ± 0.011[cd]	1.449 ± 0.025[e]
15'	0.024 ± 0.009[a]	0.063 ± 0.009[bc]	0.084 ± 0.015[b]	4.311 ± 0.009[cd]	1.448 ± 0.078[e]
20'	0.034 ± 0.016[a]	0.068 ± 0.005[bc]	0.063 ± 0.011[b]	4.471 ± 0.005[d]	1.476 ± 0.045[e]
30'	0.026 ± 0.008[a]	0.067 ± 0.006[bc]	0.087 ± 0.008[b]	4.654 ± 0.006[d]	1.451 ± 0.049[e]
40'	0.030 ± 0.003[a]	0.072 ± 0.032[c]	0.092 ± 0.008[b]	4.431 ± 0.032[d]	1.490 ± 0.067[e]
Ultrasound assisted extraction using water					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5'	nd	0.010 ± 0.004[a]	nd	1.911 ± 0.043[a]	0.488 ± 0.020[a]
10'	nd	0.004 ± 0.001[a]	nd	2.229 ± 0.026[ab]	0.539 ± 0.010[b]
15'	nd	0.009 ± 0.004[a]	0.016 ± 0.010[a]	2.329 ± 0.123[ab]	0.556 ± 0.035[bc]
20'	0.003 ± 0.004[a]	0.011 ± 0.001[a]	0.013 ± 0.006[a]	2.382 ± 0.040[ab]	0.598 ± 0.018[bc]
30'	0.006 ± 0.003[a]	0.011 ± 0.005[a]	nd	2.542 ± 0.017[b]	0.646 ± 0.009[c]
40'	0.018 ± 0.005[a]	0.008 ± 0.004[a]	0.008 ± 0.003[a]	2.520 ± 0.078[b]	0.648 ± 0.016[c]

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir
All values are expressed as milligram Malvidin-3-o-glucoside equivalent per gram dried weight (mg malvidin-3-o-glucoside/g DM)
Data are expressed as mean ± SD (n=3); nd: not detected
Results with different superscripts in the same column are significantly different ($p < 0.05$)

increase the content of anthocyanin extracted from white grape pomaces, these values are quite very low in comparison with red grape pomaces. In PN, extraction time had no significant impact on anthocyanin yields using UAE with ethanol. For ME, the anthocyanin content was at least twice as high as those of PN at the same extraction time.

In the extracts obtained from conventional techniques, the content of monomeric anthocyanin of white grape pomaces was not detected, while the content for ME was 2.33 mg M3OG/g DM and for PN was 1.02 mg M3OG/g DM. For samples extracted by the conventional method, anthocyanin can be only detected in red grape pomace, with the content of ME double the content of PN (2.33 mg M3OG/g DM compare to 1.02 mg M3OG/g DM).

The anthocyanin content of ME from water UAE techniques were similar to other methods. However, the anthocyanin content extracted from the grape pomace of PN was higher in conventional methods than the UAE using water, but significantly lower than UAE using ethanol.

4.3.1.3 Tannin content

The tannin content obtained from the three extraction methods is illustrated in Table 4.3. The content extracted by using ethanol was far higher than the tannin content obtained from water extraction techniques. In the group of ethanol extracts, the concentration of tannin extracted for all grape pomaces increased gradually along with the increases of time. Generally, extraction time made significant changes ($p < 0.05$) on the tannin contents of grape pomaces, apart from those observed from SA, which showed no significant differences among all periods of extraction. The grape pomace from PN yielded the highest concentration at all times with values ranging from 62.84 mg EE/g DM at 5 min to 76.64 mg EE/g DM at 40 min, followed by ME and PG. The tannin content extracted by the conventional method showed that the grape pomace of PN yielded higher values (54.51 mg epi./g DM) compared to the other extracts. ME, PG and GE gave relatively similar tannin contents with the values being 34.57, 32.96 and 32.47 mg epi./g DM, respectively. Similar to the observations made for the levels of TPC and anthocyanin, the amount of tannin gained from conventional were significantly higher ($p < 0.05$) than the UAE technique using water but were significantly lower than the UAE technique using 60 % ethanol. Between varieties, PN had the highest content of tannin (54.51 mg epi./g DM), while ME, PG and GE gave a similar amount of tannin (34.57, 32.96 and 32.47 mg epi./g DM, respectively).

Table 4.3. Tannin content of grape pomaces extracted by different methods

	Conventional extraction				
	SA	**PG**	**GE**	**ME**	**PN**
	13.21 ± 0.42^{b}	32.96 ± 0.57^{b}	32.47 ± 0.44^{b}	34.57 ± 0.64^{b}	54.51 ± 1.23^{b}
	Ultrasound assisted extraction using 60 % ethanol				
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5'	17.13 ± 1.59^{c}	41.75 ± 1.36^{c}	43.62 ± 2.73^{b}	50.97 ± 2.94^{c}	62.84 ± 4.17^{b}
10'	18.30 ± 1.57^{cd}	46.35 ± 1.27^{cd}	45.81 ± 3.62^{b}	57.82 ± 3.21^{cd}	66.04 ± 0.28^{bc}
15'	18.41 ± 0.14^{cd}	49.26 ± 2.60^{de}	46.79 ± 1.38^{cd}	60.91 ± 1.96^{de}	70.92 ± 4.36^{cd}
20'	18.45 ± 0.99^{cd}	49.80 ± 4.30^{de}	50.23 ± 3.16^{cd}	61.21 ± 3.80^{de}	71.88 ± 2.29^{cd}
30'	19.57 ± 1.56^{cd}	51.27 ± 2.35^{de}	51.17 ± 2.76^{d}	62.14 ± 0.68^{de}	74.82 ± 2.94^{d}
40'	20.85 ± 1.68^{d}	52.85 ± 3.93^{e}	52.87 ± 4.76^{d}	67.36 ± 3.51^{e}	76.64 ± 2.25^{d}
	Ultrasound assisted extraction using water				
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5'	4.14 ± 0.10^{a}	15.91 ± 1.18^{a}	9.85 ± 0.81^{a}	12.32 ± 1.05^{a}	15.15 ± 2.60^{a}
10'	4.26 ± 0.15^{a}	16.94 ± 0.50^{a}	10.92 ± 0.35^{a}	13.12 ± 2.98^{a}	16.38 ± 0.96^{a}
15'	4.63 ± 0.44^{a}	17.41 ± 2.74^{a}	11.45 ± 0.80^{a}	13.78 ± 2.17^{a}	18.31 ± 2.76^{a}
20'	4.69 ± 0.08^{a}	17.04 ± 0.95^{a}	11.02 ± 0.57^{a}	14.45 ± 1.24^{a}	19.18 ± 3.89^{a}
30'	4.54 ± 0.11^{a}	17.31 ± 0.40^{a}	11.78 ± 1.51^{a}	16.35 ± 1.71^{a}	22.54 ± 1.10^{a}
40'	4.94 ± 0.14^{a}	18.08 ± 1.06^{a}	12.62 ± 0.60^{a}	18.11 ± 2.43^{a}	22.74 ± 3.59^{a}

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir
All values are expressed as milligram Epicatechin equivalent per gram dried weight (mg EE/g DM)
Data are expressed as mean ± SD (n=3);
Results with different superscripts in the same column are significantly different ($p < 0.05$)

4.3.1.4 Antioxidant capacity

The antioxidant capacity of the grape pomace extracts obtained by UAE were examined in order to determine the most active grape pomace extracts among the five varieties. Different methods should be used in order to accurately evaluate the ability of grape pomace as antioxidant measurements can be related to the capacity of extracts to directly transfer hydrogen to a radical (ABTS) or to act as competitors for the peroxyl radical (FRAP) (Huang, Ou, & Prioir, 2005). Therefore, ABTS and FRAP were employed to evaluate the radical scavenging capacities of grape pomaces extracted by UAE using 60 % ethanol and water solvent and their results are illustrated in Table 4.4 and Table 4.5. In the group extracted using 60 % ethanol, the ABTS assay showed that the extracts from PN grape pomaces had the highest antioxidant capacity (ranging from 758.67 µmol TE/g DM at 5 min to 862 µmol TE/g DM), and that significant

differences ($p < 0.05$) were observed between extraction times. ME extracts yield the next highest antioxidant levels, followed by PG, GE and SA. The result obtained from the FRAP assay showed a similar trend as that of ABTS, in that the antioxidant capacity value for PN was 130.03 µmol TE/g DM at 5 min and 302.78 µmol TE/g DM, while SA exhibited the weakest FRAP values ranging from 38.31 µmol TE/g DM at 5 min to 50.01 µmol TE/g DM.

The scavenging capacities of samples extracted by water were far lower than ethanol extraction, with some minor differences in order. PN and SA extracts were still observed to yield the highest and least antioxidant capacities respectively, but values of PG were slightly higher than ME. FRAP data for water extracted samples showed the same trend to the ones extracted by ethanol. In general, samples extracted by UAE using 60 % ethanol solvent showed higher antioxidant capacity than the ones extracted by water. This result is in agreement with the content of TPC and tannin analysed above, which indicated that UAE using 60 % ethanol gave higher content of those compounds compared to water extraction only. The antioxidant capacity of the grape pomaces extracts obtained from conventional assays were also tested using ABTS and FRAP and the results are shown in Table 4.7. In both two tests, data showed significant differences ($p < 0.05$) between grape varieties. The scavenging capacity of the extracts obtained from PN were the highest in both ABTS (302.91 µmol TE/g DM) or FRAP (89.01 µmol TE/g DM) compared to the other grape pomace extracts. However, there were fluctuations between ME and PG in that the values obtained using the ABTS assay method showed that PG yielded higher values than ME (197.45 µmol TE/g DM compares to 181.05 µmol TE/g DM), while the FRAP data showed the reverse trend (ME: 61.38 µmol TE/g DM and PG: 53.81 µmol TE/g DM).

The antioxidant capacity of extracts of grape pomace from conventional extraction techniques showed the same overall trend, in that PN had the highest value (302.91 µmol TE/g DM for ABTS

and 89.01 µmol TE/g DM for FRAP). The extract obtained from ME had a higher value according to the FRAP assay (61.38 µmol TE/g DM) but a lower ABTS value (181.05 µmol TE/g DM) in comparison with PG (53.81 and 197.45 µmol TE/g DM, respectively). GE and SA had the least capacity in both test assays. Compared to UAE, conventional extracts showed significantly higher capacity than the one using water UAE, but significantly lower capacities than the extracts obtained when using UAE with 60 % ethanol. These results are in accordance with the content of TPC and tannin content mentioned above.

Table 4.4. Antioxidant capacity of grape pomaces extracted by ultrasound assisted extraction determined by ABTS assays

	Conventional extraction				
	SA	**PG**	**GE**	**ME**	**PN**
	61.73 ± 2.48^{ab}	197.45 ± 2.27^{a}	153.05 ± 0.23^{ab}	181.05 ± 2.44^{a}	302.91 ± 2.81^{a}
	Ultrasound assisted extraction using 60 % ethanol				
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	137.63 ± 7.78^{c}	491.93 ± 10.02^{c}	354.15 ± 14.46^{c}	487.11 ± 5.88^{d}	758.67 ± 9.69^{d}
10’	159.30 ± 4.24^{d}	509.33 ± 12.81^{cd}	378.22 ± 13.10^{cd}	509.70 ± 15.77^{de}	759.41 ± 11.18^{d}
15’	160.59 ± 1.79^{d}	518.96 ± 6.51^{d}	383.04 ± 10.96^{d}	523.78 ± 9.88^{ef}	795.70 ± 8.98^{e}
20’	171.33 ± 6.31^{de}	525.26 ± 11.18^{d}	385.26 ± 15.77^{d}	536.0 ± 14.44^{f}	804.59 ± 10.96^{e}
30’	171.15 ± 7.58^{de}	538.59 ± 12.49^{de}	396.37 ± 7.23^{d}	560.81 ± 1.28^{g}	852.74 ± 5.59^{f}
40’	183.37 ± 2.25^{e}	553.41 ± 9.25^{e}	487.48 ± 12.24^{e}	605.63 ± 7.14^{h}	862.37 ± 3.39^{f}
	Ultrasound assisted extraction using water				
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	51.18 ± 2.75^{a}	213.56 ± 7.33^{a}	127.16 ± 1.39^{a}	188.36 ± 3.02^{ab}	293.52 ± 6.35^{a}
10’	53.58 ± 5.33^{a}	216.76 ± 3.02^{a}	145.56 ± 7.20^{ab}	196.76 ± 1.39^{ab}	299.92 ± 6.04^{a}
15’	56.18 ± 0.92^{ab}	229.16 ± 4.21^{ab}	146.36 ± 4.85^{ab}	211.16 ± 1.39^{b}	315.92 ± 9.70^{a}
20’	60.78 ± 8.31^{ab}	236.76 ± 5.23^{ab}	148.76 ± 1.83^{ab}	211.96 ± 4.85^{b}	352.72 ± 1.39^{b}
30’	61.98 ± 3.12^{ab}	239.96 ± 2.50^{b}	149.96 ± 2.50^{ab}	241.56 ± 2.078^{c}	371.92 ± 9.10^{b}
40’	65.98 ± 1.51^{b}	260.36 ± 7.33^{b}	161.16 ± 7.86^{b}	254.36 ± 12.60^{c}	419.92 ± 11.34^{c}

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir
All values are expressed as micromole Trolox equivalent per gram dried matter (µmol TE/g DM)
Data are expressed as mean ± SD (n=3)
Results with different superscripts in the same column are significantly different ($p < 0.05$)

Table 4.5. Antioxidant capacity of grape pomaces extracted by ultrasound assisted extraction determined by FRAP assays

	Conventional extraction				
	SA	**PG**	**GE**	**ME**	**PN**
	25.54 ± 0.40^{a}	53.81 ± 0.80^{a}	31.63 ± 0.58^{a}	61.38 ± 1.35^{a}	89.01 ± 0.56^{a}
	Ultrasound assisted extraction using 60 % ethanol				
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	38.31 ± 4.42^{b}	121.61 ± 5.41^{d}	126.91 ± 1.54^{c}	143.53 ± 9.52^{c}	167.53 ± 4.58^{d}
10’	40.71 ± 1.67^{b}	144.91 ± 2.72^{e}	133.41 ± 4.96^{cd}	174.53 ± 8.11^{d}	211.53 ± 6.24^{e}
15’	40.21 ± 3.45^{b}	153.31 ± 6.71^{e}	137.31 ± 6.26^{cd}	215.78 ± 7.16^{c}	238.28 ± 8.64^{f}
20’	43.61 ± 5.28^{bc}	160.21 ± 9.66^{ef}	139.11 ± 9.70^{de}	227.28 ± 9.38^{cd}	254.53 ± 9.26^{g}
30’	43.81 ± 3.06^{bc}	162.21 ± 9.12^{ef}	145.01 ± 6.92^{de}	233.03 ± 7.23^{d}	265.53 ± 5.62^{g}
40’	50.01 ± 5.10^{c}	172.11 ± 8.55^{f}	151.51 ± 2.27^{e}	261.28 ± 5.53^{e}	302.78 ± 3.97^{h}
	Ultrasound assisted extraction using water				
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	17.51 ± 1.42^{a}	79.01 ± 1.25^{b}	56.31 ± 3.16^{b}	75.53 ± 3.75^{ab}	130.03 ± 3.12^{b}
10’	19.61 ± 0.17^{a}	79.51 ± 3.70^{bc}	57.11 ± 3.48^{b}	87.28 ± 1.15^{b}	131.03 ± 3.97^{bc}
15’	20.01 ± 3.34^{a}	84.41 ± 0.76^{bc}	58.21 ± 0.62^{b}	90.78 ± 1.15^{b}	133.03 ± 1.73^{bc}
20’	20.51 ± 1.14^{a}	86.31 ± 1.82^{bc}	59.61 ± 2.40^{b}	94.03 ± 1.15^{b}	145.53 ± 4.88^{c}
30’	21.01 ± 0.46^{a}	88.61 ± 1.35^{bc}	59.81 ± 1.51^{b}	101.28 ± 5.10^{b}	153.28 ± 4.13^{c}
40’	22.71 ± 1.37^{a}	94.91 ± 1.51^{c}	60.91 ± 1.42^{b}	104.03 ± 0.75^{b}	163.03 ± 3.70^{c}

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir
All values are expressed as micromole Trolox equivalent per gram dried matter (µmol TE/g DM)
Data are expressed as mean ± SD (n=3)
Results with different superscripts in the same column are significantly different ($p < 0.05$)

Based on the results analysed above, conventional extraction was better than UAE using water but less efficient than UAE using 60 % methanol in terms of TPC, anthocyanins, tannins and antioxidant capacity yields. The differences may due to the solvent used. Fontana *et al*., (2013) found that in most studies, researchers tried to achieve as much content of phenolic compounds as possible prior to further analysis, and the most recommended solvents were mixtures of methanol/water or ethanol/water. The presence of water may increase the permeability of cell tissue, thus lead to better mass transfer by molecular diffusion and the recovery of water-soluble compounds. Besides, Bonfigli, Godoy, Reinheimer, & Scenna, (2017) found that conventional extraction may obtained a similar yield with UAE if prolong the extraction time. Since the conventional method in this study was conducted overnight, it is

reasonable that its yield lied between the UAE using water and methanol. Besides, the two tests show that 40 min is the ideal extraction time as it help to gain the extracts with high bioactive compounds and radical scavenging capacity.

4.3.2 Grape pomace phenolic compounds characterised by HPLC

The contents of the individual phenolic compounds of five grape pomace samples are presented in Table 4.6. Catechin was the predominant compound in all of the extracts from the grape pomaces, with the highest content found in the extracts from PN (231.18 ppm), followed by PG (194.49 ppm), while the extracts from SA yielded the lowest value (13.05 ppm). The second most abundant phenolic was epicatechin. The extracts obtained from the PN grape pomace also contained the largest amount of this compound (85.2 ppm), while SA yielded the lowest concentration (9.49 ppm). Malvidin-3-O-glucoside was detected in ME (28.17 ppm) and PN (5.59 ppm) but could not be found in SA, PG and GE. This is explained by the fact that this phenolic belongs to the anthocyanin group, which appears in red but not white grape pomace. It is also consistent with the result mentioned above when the anthocyanin content of ME doubled the one of PN. In contrast, hydroxybenzoic acid was only found in SA (2.02 ppm) and PG (1.31 ppm) while could not be detected in others. Other phenolics had significantly lower content ranging from 0.01 ppm to a few ppm.

Table 4.6. Polyphenols of grape pomaces determined by HPLC

Samples	SA (ppm)	PG (ppm)	GE (ppm)	ME (ppm)	PN (ppm)
Caffeic acid	1.61 ± 0.03[c]	0.03 ± 0.01[a]	0.01 ± 0.01[a]	0.75 ± 0.01[b]	nd
Caftaric acid	3.18 ± 0.08[c]	0.51 ± 0.004[a]	0.53 ± 0.03[a]	0.50 ± 0.004[a]	0.72 ± 0.04[b]
Catechin	13.05 ± 0.03[b]	194.49 ± 1.70[d]	61.93 ± 0.03[c]	40.29 ± 0.07[b]	231.18 ± 4.93[e]
Epicatechin	9.49 ± 0.03[a]	39.68 ± 0.004[d]	15.90 ± 0.07[b]	29.32 ± 0.07[c]	85.20 ± 2.46[e]
Ferulic acid	nd	nd	0.07 ± 0.01[a]	0.06 ± 0.01[a]	0.05 ± 0.04[a]
Gallic acid	2.18 ± 0.003[a]	4.68 ± 0.01[b]	5.10 ± 0.05[c]	5.20 ± 0.02[d]	7.08 ± 0.05[e]
Hydroxybenzoic acid	2.02 ± 0.02	1.31 ± 0.43	nd	nd	nd
Malvidin-3-O-glucoside	nd	nd	nd	28.17 ± 0.10	5.59 ± 0.19
p-Coumaric acid	nd	0.15 ± 0.04[b]	0.53 ± 0.002[d]	0.46 ± 0.004[c]	0.03 ± 0.02[a]
Protocatechuic acid	nd	1.28 ± 0.004[a]	1.14 ± 0.07[a]	0.99 ± 0.03[a]	1.26 ± 0.08[a]
Quercetin	nd	nd	0.39 ± 0.002[a]	3.01 ± 0.05[b]	0.44 ± 0.03[a]
Resveratrol	nd	nd	nd	0.15 ± 0.002	0.16 ± 0.02
Rutin	nd	nd	0.62 ± 0.54	0.13 ± 0.23	nd
Syringic acid	1.16 ± 0.04[b]	0.99 ± 0.30[b]	0.50 ± 0.17[a]	2.33 ± 0.01[c]	4.68 ± 0.04[d]

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir
Data are expressed as mean ± SD (n = 3), nd: not detected
Results with different superscripts in the same row are significantly different ($p < 0.05$)

4.3.3 Proximal analysis of the different grape pomaces

The proximal composition of all the grape pomaces was determined to see if variations in starch, fat or fibre components were observed between the grape pomaceS obtained from the different varieties. The results of Table 4.11 illustrate that starch content and fibre content varied between grape pomaces and were related to the colour of the grape. For instance, the TDF was high in red grape pomaces and the starch content was low, while for white grape pomaces the starch content was significantly higher and the TDF was lower than red grape pomaces. In particular, the IDF of PN was 55.73 % and of ME was 50.42 %, which were significantly higher ($p < 0.05$) than the other pomaces from the white grapes (PG: 34.9 %, GE: 28.98 % and SA: 19.5 %). In addition, the two red grape pomaces had more than 10 % of SDF, far higher than GE (7.19%), PG (7.08 %) and SA (3.18 %). In contrast, the starch content of the white grape pomaces was significantly higher than red grape pomaces. SA had the highest content (63.74 %), followed by GE (50.97 %) and PG (41.56 %), while ME and PN had similar

content of starch (26.98% and 23.92%, respectively). Deng, Penner, & Zhao, (2011) assessed TDF of five grape skins and observed a similar trend as the TDF content of red grape pomaces (PN and ME) were 56.31 % and 51.09 %, which were far higher than white grape pomaces (Morio Muscat: 17.28 % and Muller Thurgau: 28.01 %). However, the authors observed that the content of sugar in red grape skins were trivial (ME: 1.38 % and PN: 1.34%). Bravo & Saura-Calixto, (1998) observed similar results when the content of soluble sugars of grape skins were approximate to 3 % (dry matter basis).

Table 4.7. Proximate composition of grape pomaces produced in New Zealand (% dry matter)

Samples	SA (%)	PG (%)	GE (%)	ME (%)	PN (%)
Starch	63.74 ± 3.53^{b}	41.56 ± 2.17^{c}	50.97 ± 1.65^{c}	26.98 ± 1.16^{a}	23.92 ± 1.91^{a}
Fat	2.25 ± 0.05^{a}	3.52± 0.04^{b}	5.73 ± 0.15^{c}	5.38 ± 0.03^{c}	5.37 ± 0.03^{c}
Ash	8.36 ± 0.24^{a}	5.61 ± 0.54^{b}	5.40 ± 0.20^{c}	5.82 ± 0.07^{b}	4.64 ± 0.13^{c}
IDF	19.50 ± 0.71^{a}	34.90 ± 0.12^{b}	28.98 ± 1.11^{c}	50.42 ± 1.72^{d}	55.73 ± 1.11^{e}
SDF	3.18 ± 0.04^{a}	7.08± 0.32^{b}	7.19 ± 0.09^{b}	10.17 ± 0.14^{c}	10.48 ± 0.21^{c}
TDF	22.68 ± 0.75^{a}	41.97 ± 0.19^{c}	36.17 ± 1.02^{b}	60.59 ± 1.86d	66.21 ± 1.32^{e}

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir
IDF: insoluble dietary fibre; SDF: soluble dietary fibre: TDF: total dietary fibre
Data are expressed as mean ± SD (n = 3).
Results with different superscripts in the same row are significantly different ($p < 0.05$)

These pomaces also contained remarkable amount of minerals, with the lowest concentration was attributed to PN (4.64 %) and the highest content was observed at SA (8.36 %), the other three pomaces had relatively similar values. On the other hand, the highest fat content was recorded at GE (5.73 %), followed by ME and PN with no significant differences ($p < 0.05$) between the three pomaces, and SA had the lowest fat concentration (2.25 %).

4.4 Conclusion

The results presented in this chapter demonstrate that UAE is a far more efficient method in the extraction of compounds from grape pomace than “conventional” methods. In the same extraction conditions, while the conventional assay took 10 - 12 h to extract samples, UAE took only 40 min to finish the process. In addition, the content of bioactive compounds gained from

UAE were far higher than the ones derived from the conventional method. Hence, using UAE would benefit research and the food industry in terms of saving time, budget, and product quality. These conclusions may encourage future studies and even mass production to employ UAE as the mean of extraction to achieve better results.

The phenolic profiles of five New Zealand grape pomace were elucidated in this study. While the values are in agreement with previous studies, it is novel to observe the specific trends in that red grape pomace showed higher phenolic content and composition when compared to white grape pomace (Kammerer, Kammerer, Valet, & Carle, 2014; Trost *et al*., 2016). Along with phenolic compounds, the antioxidant capacity of each of the grape varieties was tested and PN and ME were observed to yield higher antioxidant capacity values compared to the other samples. In white grape pomace, PG exhibited the highest antioxidant capacity values while SA normally had the lowest content. Considering these findings and earlier studies, it can be concluded that pomaces from the SA, PG, GE, ME and PN wine grape varieties have significantly different phenolic profiles and antioxidant capacity. Those differences are likely due either to differences in the availability of proximal compounds among varieties or to differences in the extraction processes used in red and white winemaking. The differences in fibre and starch composition between the grape pomaces was of significant interest as it is likely that the phenolic compounds of the grapes are intrinsically linked to the fibre components of the grapes. In addition, the fibre components may exhibit antioxidant activities themselves. Accordingly, grape pomace represents a diverse and potentially important source of polyphenols, which could be used advantageously for either nutritional or pharmacological purposes

Chapter 5

The effect of replacement of wheat flour by grape pomace powder on the physiochemical characteristics of cookies

5.1 Introduction

Cookies are one of the most popular snacks in the world with the global production growing by more than two per cent annually from 2013, and it is predicted to reach 24 million tonnes in 2023 (Statista.com) as shown in Figure 5.1.

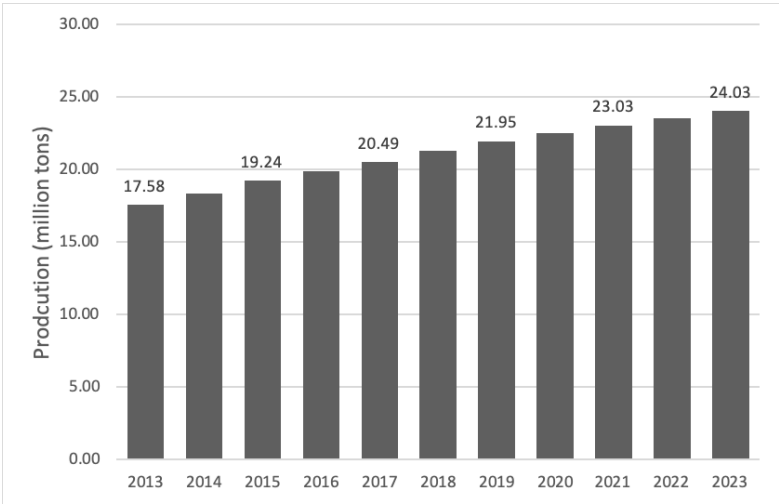


Figure 5.1 Cookies and crackers production worldwide 2013 - 2023

Source: Statista.com (accessed 10 am, 09/03/2020)

The popularity of cookies can be attributed to their convenience, ease of processing and long shelf-storage. Consumption of cookies keeps rising and is predicted to reach 3 kg/capita/annum in 2023 (Figure 5.2). Common ingredients of cookies include cereal flour, sugar, fat, water and other added ingredients for particular purposes. The quality of cookies is dependent on the physicochemical characteristics of doughs, whose quality is defined by components mentioned above and cooking parameters (Manohar & Rao, 1997, 2002). One problem of popular cookie recipes is that they use a high amount of sugar (27 %) and flour (47 %), which consists mainly

of starch. The high starch content of cookie doughs is responsible for the high energy content of cookies and also the high glycaemic impact the of final products. Hence, a high intake of cookies is similar to eating other high-sugar-containing food products and may lead to many human health problems such as obesity, diabetes or cardiovascular diseases. In recent years, along with consumer awareness of health problems caused by unhealthy eating habits, researchers have paid attention to reducing the glycaemic impact of such food items, which is directly related to the degradation of starch. Although various factors might influence starch degradation such as the type of carbohydrate, food form or processing of food, fortification of cereal foods with plant or fruit material rich in dietary fibre content is one of the most effective ways to reduce and manipulate the glycaemic effect of cookie digestion (Brennan & Samyue, 2004).

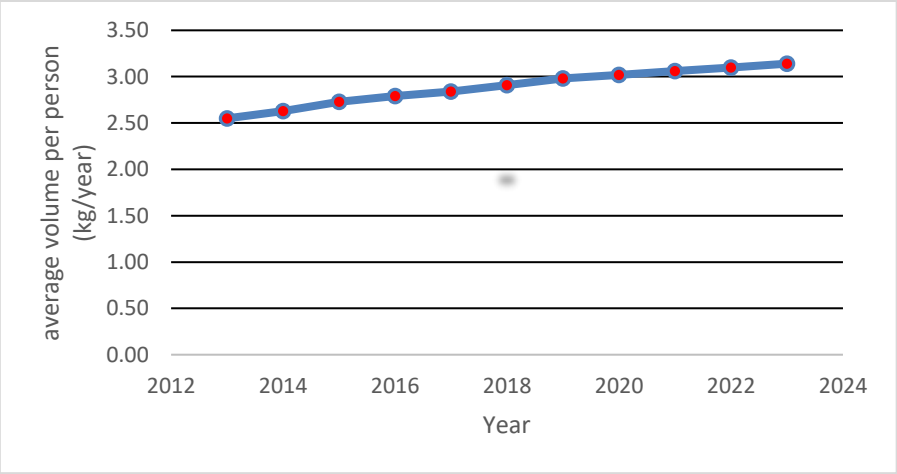


Figure 5.2 Worldwide annual consumption of cookies and crackers 2013 – 2023

Source: Statista.com (accessed 10 am, 09/03/2020)

The simplicity of the cookie recipe allows for the potential to enrich the cookie with various ingredients, allowing the creation of many types of cookie. The idea of fortified cookies with plant and fruit by-products has been investigated for decades with a number of promising results being published. For instance, Ajila *et al*., (2008) replaced wheat flour by 2.5, 5, 7.5, 10,

15 and 20 % of mango peel powder to make cookies and observed an increase in antioxidant capacity when the IC_{50} of samples fell from 250 mg (control) to 10, 4.9 and 4.3 mg for samples with 10 %, 15 % and 20% added powder, respectively. Also, the TDF content of cookies which had mango peel added to them rose by twofold to threefold compared to the control sample. Apple pomace powder has been used to fortify cookies at an industrial scale and obtained increases in TPC, dietary fibre content, flavonoid content and antioxidant capacity while the gluten content decreased with the increasing of pomace powder addition (Zlatanović *et al*., 2019). The researchers also illustrated that in terms of sensorial quality, including appearance, structure, taste, odour and chewiness, the highest overall score was attributed to the sample with 25 % added powder, while the 50% fortified sample was rated as "very good". An interesting finding in this research was that coarse powder (average particle size of 0.5 mm) performed better than fine powder (average particle size of 0.16 mm) in both chemical properties and sensorial properties of the cookies. Grape pomaces have been added to cereal foods as different components such as grape seed powder or grape pomace powder (Samohvalova, Grevtseva, Brykova, & Grigorenko, 2016), white grape skin powder (Mildner-Szkudlarz *et al*., 2013), and red grape skins, seeds and whole pomace (Acun & Gül, 2014). Such studies observed similar results in that incorporation of grape pomaces was associated with a rise of TPC and antioxidant capacity in cookies, but the physical properties varied differently. In particular, researchers found that as 10% red grape seed powder had a similar antioxidant activity as 20 % of mango peel powder, while the white grape skin powder had an equal antioxidant activity to mango peel. However, there is little research focused on the *in vitro* digestibility of foods enriched with grape pomace. Other fruits have been used to manipulate the digestibility of starch, for instance blackcurrant pomace has been applied to barley, oat or whole wheat flour products (Hossain *et al*., 2017). Banana powder has been employed to substitute wheat flour by 15 %, 30% and 50 % in cookies recipe and showed a decreasing trend

in starch hydrolysis percentage along with a proportional increase of banana powder substitution (Agama-Acevedo *et al*., 2012). Apple pomace is another favourable compound for decreasing the release of reducing sugars from starch products. A study by Alongi, Melchior, & Anese, (2019) showed that partial replacement of flour with apple pomace (10 % and 20 %) significantly reduced the overall glycaemic index of cookies despite the fact that apple pomace itself provided a large amount of sugar to cookie samples. The authors explained that the dietary fibre of the apple pomace attenuated the glucose released the SDF and IDF binding to the starch granules. Dietary polyphenols are another factor that participates in the prevention of starch released during digestion. Barros, Awika, & Rooney, (2012) reported that tannin extracted from sorghum interacted with amylose and the linear fragments of amylopectin to form resistant starch, hence significantly reducing the digestibility of high amylose starch. Other work on black and green tea polyphenol extracts reported their effectiveness in reducing the hydrolysis of wheat, corn, potato and rice starches by binding starch and inhibiting digestion enzymes (Guzar, Ragaee, & Seetharaman, 2012). A few studies have evaluated grape polyphenols, and these have shown that the polyphenols can decrease sugar release and that the polyphenols from grapes may be more effective than the polyphenols of lingonberry and cranberry (Quek & Henry, 2015). Although grape pomace has been proved to be a rich source of dietary fibre, have higher TPC and antioxidant capacity than apple pomace, orange peel pomace and a number of other fruit pomaces (Deng *et al*., 2011), its ability to reduce the rate of starch hydrolysis has yet to be thoroughly studied. Hence, this study aimed to investigate the possibilities of partial replacement of grape pomace powder into cookies and its ability to slow the degradation of starch during *in vitro* digestion. the influences of different grape varieties on the physical and chemical properties of cookies were also investigated.

5.2 Materials and methods

5.2.1 Grape pomace preparation

Described in section 3.1.1

5.2.2 Cookies preparation

Described in section 3.3.1

5.2.3 Moisture content and Texture analysis

Described in sections 3.4.1 and 3.4.9.2

5.2.4 Colour determination

Described in section 3.4.9.1

5.2.5 Total phenolic content determination

Described in section 3.4.3

5.2.6 Antioxidant capacity determination

Described in sections 3.4.7.1 and 3.4.7.2

5.2.7 *In vitro* starch digestibility

Described in section 3.4.10

5.3 Results and discussion

5.3.1 Change of cookies parameters after baking

Table 5.1 illustrates the changes in the thickness, diameter and spread ratio of cookies which were fortified with different grape pomace materials. It can be seen that replacement of wheat flour with grape pomace powder significantly changed the physical dimensions of the cookies ($p < 0.05$).

Table 5.1. Change of cookie parameters after baking

Samples	Increase in thickness (%)	Increase in diameter (%)	Spread ratio
Control	97.72 ± 2.99^{a}	9.39 ± 0.42^{a}	5.26 ± 0.10^{a}
SA5	84.63 ± 7.92^{ab}	9.14 ± 1.20^{a}	5.62 ± 0.30^{ab}
SA10	80.74 ± 6.95^{b}	$8.84 \pm .017^{a}$	5.73 ± 0.21^{ab}
SA15	53.31 ± 8.51^{c}	5.69 ± 0.60^{bc}	6.56 ± 0.4^{bc}
PG5	72.76 ± 2.05^{b}	7.02 ± 0.32^{b}	5.89 ± 0.07^{b}
PG10	71.50 ± 1.53^{b}	6.79 ± 0.55^{b}	5.92 ± 0.03^{b}
PG15	51.41 ± 4.14^{c}	5.23 ± 0.77^{bc}	6.61 ± 0.14^{c}
GE5	88.37 ± 1.03^{ab}	7.20 ± 0.93^{b}	5.41 ± 0.06^{a}
GE10	81.33 ± 8.41^{b}	7.00 ± 0.02^{b}	5.61 ± 0.26^{a}
GE15	33.52 ± 3.62^{d}	5.52 ± 0.19^{bc}	7.51 ± 0.21^{d}
ME5	91.54 ± 5.76^{ab}	7.70 ± 0.77^{ab}	5.34 ± 0.12^{a}
ME10	69.72 ± 8.22^{b}	6.92 ± 0.18^{b}	5.99 ± 0.31^{ab}
ME15	40.15 ± 3.42^{cd}	4.62 ± 0.37^{c}	7.09 ± 0.19^{cd}
PN5	96.31 ± 1.15^{a}	6.67 ± 0.92^{b}	5.16 ± 0.04^{a}
PN10	67.78 ± 1.42^{b}	6.30 ± 1.15^{b}	6.02 ± 0.02^{ab}
PN15	52.78 ± 1.84^{c}	4.02 ± 0.40^{c}	6.47 ± 0.09^{c}

SA5, SA10, SA15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Sauvignon Blanc pomace powder;

PG5, PG10, PG15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Gris pomace powder;

GE5, GE10, GE15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Gewürztraminer pomace powder;

ME5, ME10, ME15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Merlot pomace powder;

PN5, PN10, PN15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Noir pomace powder;

Results are expressed as mean value ± standard deviation, n = 3;

Different superscripts in same column indicate a significant difference ($p < 0.05$).

In general, as grape pomace inclusion levels increased there was an observable decrease in both the thickness and diameter of the cookies when compared to the control samples. The smallest increase in thickness was observed with 15% replacement of the GE powder (33.52 %), while the greatest increase in thickness was observed with 5 % replacement of the PN powder (96.31 %). Similar differences have been observed using the pomace of blackcurrant fruits and were explained by the incorporation of the pomace into cookies increasing the water absorption capacity of wheat flour dough and causing a lower expansion of cookies (Hossain *et al*., 2017). The red grape pomace showed significant differences in the rate of increase of the thickness of the samples with 5 %, 10 % and 15 % replacement of wheat flour. However, the white grape pomaces showed significant differences only with 15 % replacement of the pomace. Cookie diameters decreased, but significant differences were observed only at 15% incorporation of all pomace groups. Samples fortified with SA powder showed the largest increase in diameter rates, while replacement of PN powder resulted in the smallest increase of diameter. The spread ratios were in parallel with the increase of replacement with grape pomace powder. The spread rate of diameters was far lesser than the increase in the cookie thickness. Changes in the thickness and the diameter of cookies were related to the nature of the gluten in the cookie dough, and when the gluten (starch-protein) interface) is disrupted, then the gluten matrix of the dough was disrupted (Ajila *et al*., 2008). Samohvalova *et al*., (2016) also reported that grape pomace had a high water absorption capacity (3 times higher than that of flour). This leads to another possibility that the replacement of grape pomace resulted in the high viscosity of cookie doughs, hence the lower rate of expansion. The results of this chapter are in agreement with the observations made by Tańska *et al*., (2016), who enriched cookie dough with 20 % four fruit pomace powder (rosehip, rowanberry, blackcurrant and elderberry) and observed an approximately 12 % increase in diameter and 30 % increase in thickness of the cookies.

Table 5.2 presents the changes of moisture content and hardness of cookies when increase the substitution levels of grape pomaces. The moisture content of the cookies did not alter significantly with grape pomace powder addition. The hardness of all samples significantly decreased ($p < 0.05$), but there was no difference between red grape pomace powders and white grape pomace powders. The softening of the cookies might be due to the increase of water absorption of the dough (Mildner-Szkudlarz *et al*., 2013). The decrease in hardness may also be associated with the non-significant increase in moisture content, in that the grape pomace may be absorbing and retaining moisture more than the flour control cookies.

Table 5.2. Moisture content and hardness of cookies

Samples	Moisture content (%)	Hardness (kg)
Control	6.00 ± 0.87^{a}	7.74 ± 0.19^{a}
SA5	6.83 ± 0.58^{a}	7.35 ± 0.34^{a}
SA10	7.00 ± 0.50^{a}	6.50 ± 0.20^{b}
SA15	7.17 ± 0.29^{a}	6.33 ± 0.24^{bc}
PG5	6.17 ± 0.29^{a}	7.34 ± 0.49^{a}
PG10	6.50 ± 0.50^{a}	6.18 ± 0.53^{bc}
PG15	6.67 ± 0.29^{a}	5.90 ± 0.14^{bc}
GE5	6.33 ± 0.58^{a}	6.39 ± 0.13^{bc}
GE10	6.50 ± 0.87^{a}	6.10 ± 0.04^{bc}
GE15	6.83 ± 0.76^{a}	5.66 ± 0.27^{c}
ME5	6.17 ± 0.76^{a}	7.62 ± 0.20^{a}
ME10	6.67 ± 0.58^{a}	7.31 ± 0.44^{ab}
ME15	7.00 ± 0.50^{a}	6.27 ± 0.22^{b}
PN5	6.67 ± 0.29^{a}	7.53 ± 0.29^{a}
PN10	6.83 ± 0.29^{a}	7.00 ± 0.38^{ab}
PN15	7.17 ± 0.29^{a}	6.65 ± 0.31^{b}

SA5, SA10, SA15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Sauvignon Blanc pomace powder;

PG5, PG10, PG15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Gris pomace powder;

GE5, GE10, GE15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Gewürztraminer pomace powder;

ME5, ME10, ME15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Merlot pomace powder;

PN5, PN10, PN15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Noir pomace powder;

Results are expressed as mean value ± standard deviation, n = 3;

Different superscripts in same column indicate significant difference ($p < 0.05$).

Table 5.3 shows the change in colours of the cookies when different proportions of grape pomace powder were added. Significant differences ($p < 0.05$) were observed, in which increasing addition levels resulted in lower *L** and *b** values and higher a^* value. All samples exhibited significantly higher redness value (*a**) than the control sample.

Table 5.3. Colour profile of the surface of the cookies

Sample	*L**	*a**	*b**
Control	77.13 ± 0.44^{a}	0.37 ± 0.06^{f}	24.96 ± 0.22^{a}
SA5	67.10 ± 0.72^{b}	3.14 ± 0.21^{de}	22.84 ± 0.34^{b}
SA10	63.24 ± 0.40^{c}	3.80 ± 0.53^{d}	21.46 ± 0.46^{c}
SA15	54.10 ± 0.35^{f}	7.33 ± 0.27^{b}	20.15 ± 0.40^{cd}
PG5	60.38 ± 0.37^{c}	5.02 ± 0.24^{d}	20.61 ± 0.42^{c}
PG10	58.19 ± 0.35^{d}	6.15 ± 0.2^{c}	19.64 ± 0.12^{de}
PG15	48.34 ± 0.28^{i}	10.15 ± 0.1^{a}	18.77 ± 0.61^{e}
GE5	59.74 ± 0.22^{c}	6.27 ± 0.43^{c}	22.19 ± 0.32^{bc}
GE10	53.31 ± 0.40^{f}	7.39 ± 0.26^{b}	21.74 ± 0.37^{c}
GE15	51.98 ± 0.24^{g}	7.74 ± 0.13^{b}	20.09 ± 0.21^{d}
ME5	55.42 ± 0.18^{e}	2.72 ± 0.35^{e}	12.97 ± 0.42^{g}
ME10	50.47 ± 0.49^{h}	3.26 ± 0.39^{de}	9.87 ± 0.53^{h}
ME15	43.03 ± 0.36^{j}	5.06 ± 0.25^{cd}	7.39 ± 0.26^{i}
PN5	53.27 ± 0.19^{f}	$4.31 \pm 0{,}46^{d}$	14.09 ± 0.20^{f}
PN10	47.62 ± 0.34^{i}	5.79 ± 0.24^{c}	13.59 ± 0.28^{fg}
PN15	43.11 ± 0.16^{j}	6.41 ± 0.24^{c}	12.60 ± 0.23^{g}

SA5, SA10, SA15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Sauvignon Blanc pomace powder;

PG5, PG10, PG15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Gris pomace powder;

GE5, GE10, GE15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Gewürztraminer pomace powder;

ME5, ME10, ME15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Merlot pomace powder;

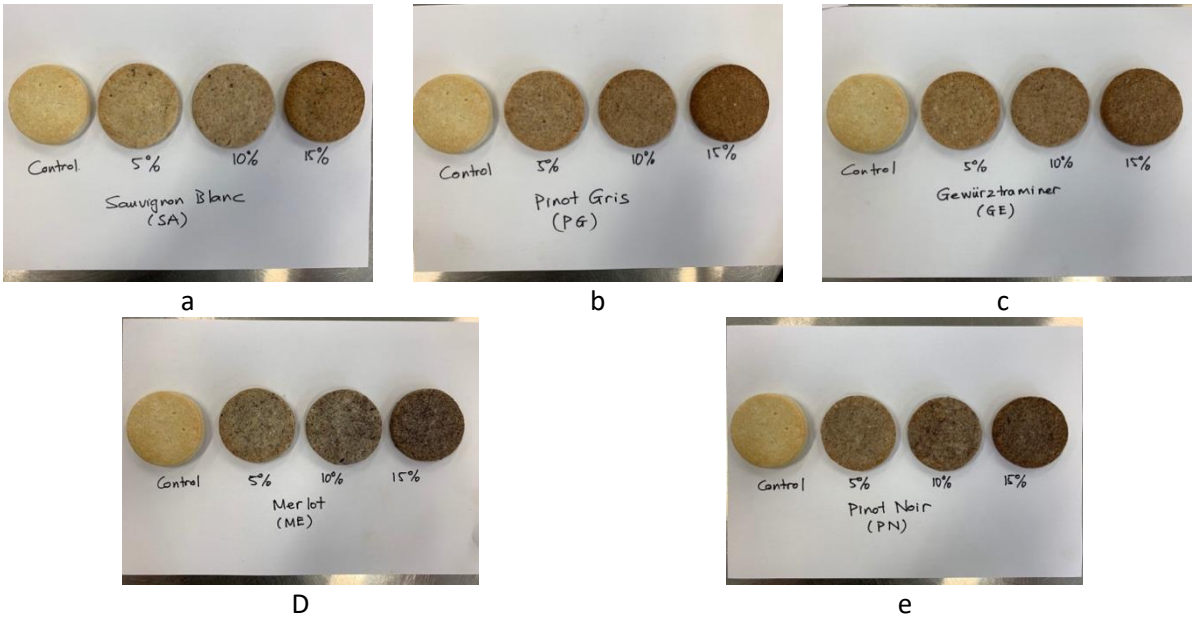
PN5, PN10, PN15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Noir pomace powder;

Results are expressed as mean value ± standard deviation, n = 3;

Different superscripts in same column indicate significant difference ($p < 0.05$).

During the baking process, the polyphenols in the grape pomace may experience oxidation and polymerisation to form brown pigments (Rapeanu, Loey, Smout, & Hendrickx, 2006). In addition, the Maillard reaction and caramelisation occur during the cooking process and these

might cause a lower brightness and blueness value (Mildner-Szkudlarz, Siger, Szwengiel, & Bajerska, 2015). Samples enriched with red grape pomace powder exhibited the lowest brightness value (*L**) compared to the ones with white grape pomace powder. Significantly lower *b** ($p < 0.05$) indicates that the red grape pomace powder tends to turn the colour of the cookies blue, while white grape pomace powders keep this value close to the value of the control sample. Figure 5.3 reflects the actual colour of cookies, and illustrates that with higher addition levels of grape pomace powder, the colours of samples darkened. These appearances might be explained by the higher TPC, and anthocyanin content, which exist only in red grape.



Cookies with different replaced level (5%, 10%, 15%) of pomace powders;
a) Sauvignon Blanc, b) Pinot Gris, c) Gewürztraminer, d) Merlot, e) Pinot Noir

Figure 5.3. Cookies fortified with grape pomaces with different levels of replacement

In general, the TPC values of the samples rose linearly with increases in the replacement levels of pomace powder, as shown in Table 5.4. In each group of pomace powder, different additions caused significant changes ($p < 0.05$) in the TPC of the cookies. For example, samples fortified with red grape pomace powder showed significantly higher phenolic contents than the white ones, mainly due to red grape pomace having a higher TPC.

Table 5.4. Total phenolic content and antioxidant capacity of cookies

Sample	TPC (mg GAE/g DM)	DPPH (µmol TE/g DM)	FRAP (µmol Fe^{3+} /g DM)
Control	0.60 ± 0.02[a]	0.52 ± 0.04[a]	0.83 ± 0.03[a]
SA5	0.58 ± 0.05[a]	1.14 ± 0.01[b]	1.92 ± 0.01[b]
SA10	0.78 ± 0.04[b]	1.25 ± 0.01[c]	3.02 ± 0.06[c]
SA15	1.06 ± 0.04[c]	1.34 ± 0.01[d]	5.32 ± 0.13[d]
PG5	0.66 ± 0.02[a]	1.15 ± 0.01[b]	4.13 ± 0.10[e]
PG10	1.02 ± 0.05[c]	1.26 ± 0.01[c]	8.40 ± 0.25[f]
PG15	1.34 ± 0.03[d]	1.32 ± 0.01[d]	13.05 ± 0.20[g]
GE5	1.13 ± 0.03[e]	1.21 ± 0.02[c]	3.73 ± 0.05[ce]
GE10	1.67 ± 0.02[f]	1.33 ± 0.01[d]	7.73 ± 0.20[f]
GE15	2.17 ± 0.04[g]	1.36 ± 0.01[d]	9.68 ± 0.39[h]
ME5	2.00 ± 0.04[g]	1.14 ± 0.01[b]	5.45 ± 0.27[d]
ME10	2.66 ± 011[h]	1.23 ± 0.01[c]	11.77 ± 0.14[i]
ME15	3.00 ± 0.09[i]	1.27 ± 0.01[c]	17.61 ± 0.33[j]
PN5	1.64 ± 0.11[f]	1.20 ± 0.01[c]	7.05 ± 0.12[f]
PN10	1.97 ± 0.06[g]	1.32 ± 0.01[d]	14.37 ± 0.77[k]
PN15	2.34 ± 0.04[j]	1.37 ± 0.01[d]	21.47 ± 0.74[l]

SA5, SA10, SA15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Sauvignon Blanc pomace powder;

PG5, PG10, PG15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Gris pomace powder;

GE5, GE10, GE15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Gewürztraminer pomace powder;

ME5, ME10, ME15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Merlot pomace powder;

PN5, PN10, PN15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Noir pomace powder;

Results are expressed as mean value ± standard deviation, n = 3;

Different superscripts in the same column indicate a significant difference ($p < 0.05$).

mg GAE/g DM: milligram gallic acid equivalent per gram dried matter

µmol TE/d DM: micromole Trolox equivalent per gram dried matter

The highest level of TPC in the cookies was observed in the cookies made with a 15 % addition of ME pomace powder (3 mg GAE/g DM), followed by 15% addition of PN powder (2.34 mg GAE/g DM), and this was five times and four times higher than the TPC of the control cookie. The sample with 5 % SA powder exhibited a lower TPC value (0.58 mg GAE/g DM) than the control sample, although this was not significantly different. Previous research has reported on the heat treatment and baking of cereal-based cookies in terms of phenolic content (Mahloko,

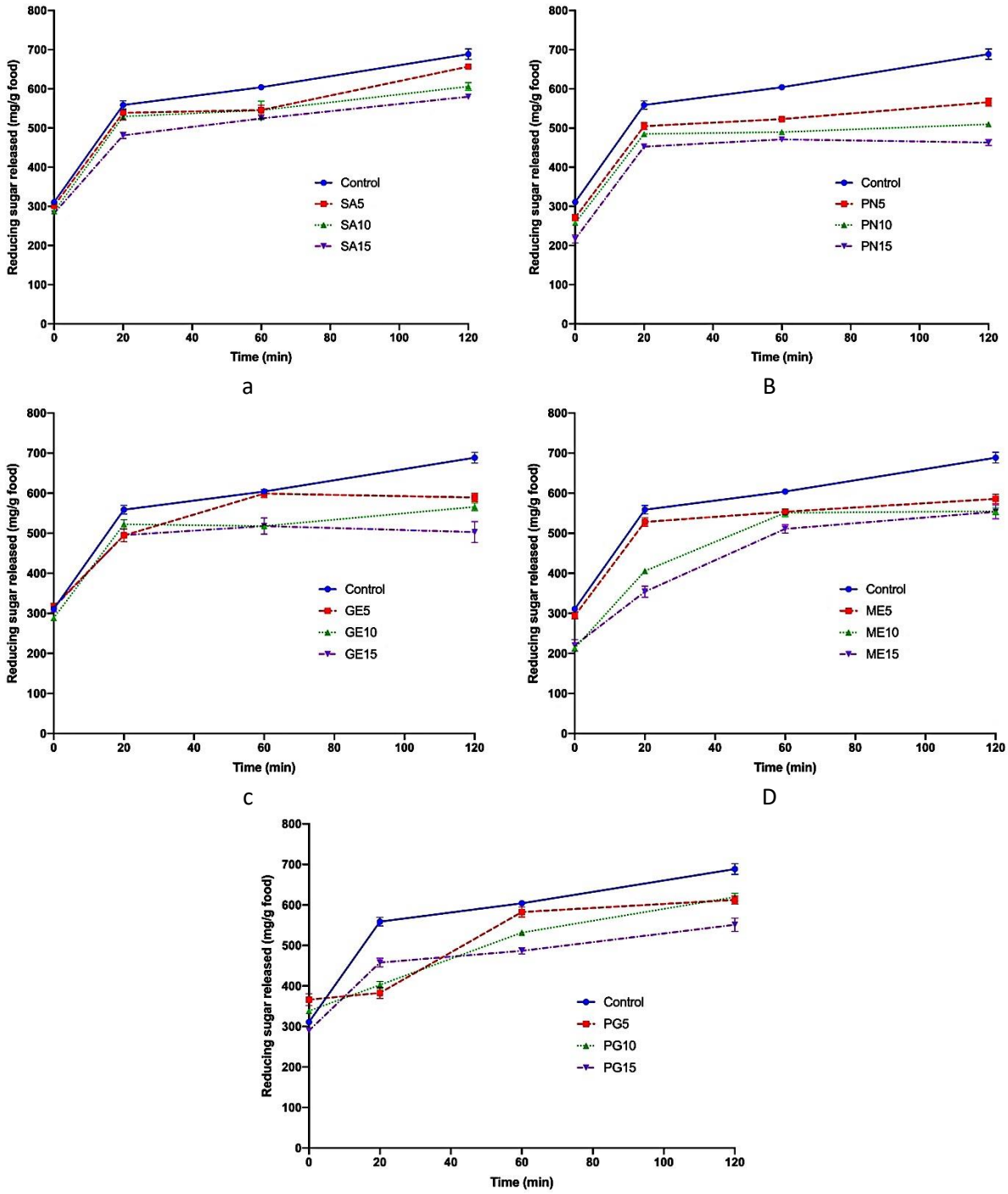
Silungwe, Mashau, & Kgatla, 2019) and explained that the processing of cereals results in the loss of phenolic content and other bioactive compounds (Abdel-Aal & Rabalski, 2013; Krystyjan, Gumul, Ziobro, & Korus, 2015).

The TPC of the cookies increased with grape pomace addition; however, such increases did not necessarily affect the antioxidant activities of the cookies. Hence, the antioxidant activity of all the cookies was determined using the DPPH and FRAP methods and the results are presented in Table 5.4. The two assays showed that a significant increase in the antioxidant capacity of cookies occurred as the level of enrichment increased ($p < 0.05$). In regards to the DPPH assay, increasing levels of grape pomace powder increased the antioxidant capacity of samples by two to three times compared to the control, but no significant differences were observed between grape varieties themselves. Almost all the samples with the 15 % fortification level showed the highest antioxidant capacity value, which ranged from 1.32 µmol TE/g DM of PG to 1.37 µmol TE/g DM of PN, although these values were not significantly different between grape varieties except for ME which had a lower antioxidant capacity at 15% fortification level ($p < 0.05$).

The values obtained from the FRAP assays showed significant differences in antioxidant capacity ($p < 0.05$) between the pomaces of the different grape varieties when applied to cookies. Although each addition level of each variety made a significant change to the samples, red grape pomace powders gave a much higher antioxidant capacity than the white grape pomace powders. Cookies fortified with the pomace from SA showed the smallest increase in FRAP values, ranging from 1.14 µmol Fe^{3+} to 5.32 µmol Fe^{3+}, while the antioxidant capacity of the cookie samples fortified with PN powder showed the greatest increase, ranging from 7.05 µmol Fe^{3+} to 21.47 µmol Fe^{3+}.

5.3.2 *In vitro* starch digestibility

The nutritional quality of cookies fortified with grape pomace was investigated using an *in vitro* starch digestion experiment to evaluate the ability of grape pomace to manipulate the reducing sugar release during a simulated digestive process. Figure 5.4 shows that all of the cookie samples enriched with grape pomace powder exhibited less starch degradation than the control cookie sample. This shows that the inclusion of grape pomace powder helps to reduce the rate of sugars which can be derived from each food matrix. Chapter 4 illustrated that the grape pomaces were rich in dietary fibre, adding grape pomace into cookies increased the content of the dietary fibre and phenolic content of the cookies. Previous research has found that polyphenols are able to reduce starch digestibility by binding to starch granules, thus inhibiting the access of enzymes to starch (Hargrove, Greenspan, Hartle, & Dowd, 2011). In combination with starch, polyphenols alter other characteristics such as dough rheology, starch gelling, retrogradation and gelatinisation. All of these characteristics are important in limiting the extent and rate of starch digestion in food systems. The degree of starch gelatinisation is key for manipulating the initial digestibility of starch by α-amylase, as the catalytic sites are exposed during the gelatinisation process (Guzar *et al*., 2012). In addition, the microstructure of starch changes during the binding interaction with polyphenols to form resistant starch and consequently delays the starch digestion (Sun & Miao, 2020). The results of this chapter are in agreement with previous works that employed other fruit and plant polyphenols in an attempt to reduce starch digestion *in vitro*, such as blackcurrant *(Hossain et al*., 2017) and tea (Sun, Gidley, & Warren, 2018) The polyphenols of grape pomace have the ability to decrease the rate of starch degradation during the digestion process. Another possibility was proposed by Brennan & Samyue, (2004), who suggested that dietary fibre helps to prevent α-amylase from accessing starch granules in the food matrix. This may be due to the mechanism of dietary fibre whereby it forms a “coat” around the starch granule, hence preventing it from being accessed



a) Sauvignon Blanc, b) Pinot Gris, c) Gewürztraminer, d) Merlot, e) Pinot Noir

Figure 5.4. Amount of reducing sugars released (mg/g food) during in vitro digestion

by α-amylase (Tudorică, Kuri, & Brennan, 2002). The SDF is able to increase the viscosity of food during the digestive process, and form gels which entrap the starch granules, limiting the exposure of the starches to the activity of the digestive enzymes, which then results in the reduction in the reducing sugars released. The IDF helps to reduce transit time in the small

intestine and it is believed to reduce the risks of type 2 diabetes (Brennan, 2005; Sun & Miao, 2020).

Standardised AUC values of all samples are presented in Figure 5.5. At a 5% replacement level, all the grape pomace powder significantly ($p < 0.05$) decreased the reducing sugars the sugar released in comparison to the control sample. The amount of reducing sugars released decreased gradually as the levels of enrichment increased, with the samples enriched with 15% of pomace powder releasing the least amount of reducing sugars. The samples PN15 and ME15 had reducing sugar release values of 398.97 mg/g and 412.21 mg/g, respectively. In general, PN gave the best results in reducing the level of starch degradation and hence sugar release during *in vitro* digestion process, even at 5%, 10% or 15% of replacement. It is worth noting that the rate of starch degradation not only depends on the quantity of polyphenols but also the types of polyphenols present. Quek & Henry, (2015) studied the influences of three fruit polyphenol powders on the *in vitro* digestibility of rice and illustrated that red grape had a much better ability in decreasing reducing sugar release than lingonberry and cranberry. The authors observed that the anthocyanins in lingonberry and cranberry had little impact on the inhibition of α-amylase, while red grape anthocyanins proved effective. Besides the impact of anthocyanins, McDougall *et al*., (2005), reported that tannin extracted from fruits also plays an effective role in the inhibition of α-amylase and α-glucosidase. Based on the findings in Chapter 4, (red grape pomace has a higher dietary fibre and total phenolic content, especially anthocyanin and tannin content than white grape pomace), it is possible to predict that the ability of red grape pomace to decrease reducing sugar released during *in vitro* digestive process is related to these proximal components.

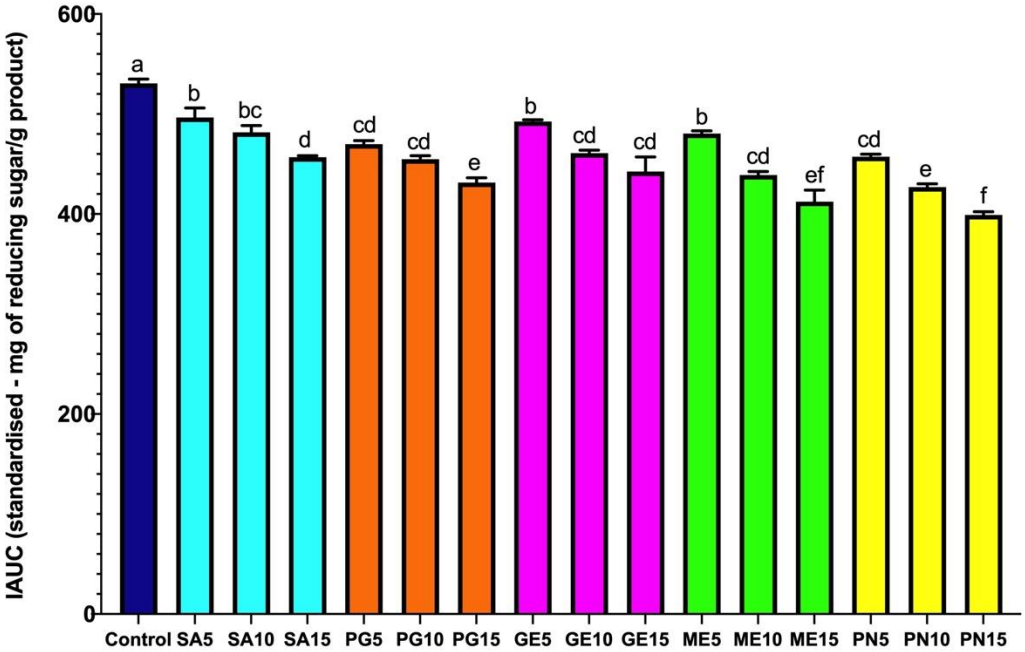


Figure 5.5 Reducing sugar of cookie samples after 120 minutes

SA5, SA10, SA15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Saubignon Blanc pomace powder;

PG5, PG10, PG15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Gris pomace powder;

GE5, GE10, GE15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Gewürztraminer pomace powder;

ME5, ME10, ME15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Merlot pomace powder;

PN5, PN10, PN15: cookies with 5 %, 10 % and 15 % replacement of wheat flour with Pinot Noir pomace powder;

5.4 Conclusion

Results obtained from experiments in this chapter proved that the addition of grape pomace into cookies helped to enhance the chemical and nutritional properties of the final product. Cookies fortified with red grape pomace powder had a higher phenolic content, higher antioxidant activity and lower reducing sugar release than control samples. The decrease in sugar released during *in vitro* digestibility suggests that red grape pomace could play a role in controlling chronic diseases such as obesity or cardiovascular disease.

The addition of grape pomace powder altered the physical properties of the cookies. In general, increasing the proportion of grape pomace powder decreased the expansion and firmness values of the cookies and increased the darkness. Further work is needed to evaluate the acceptance of customers for individual samples using sensory evaluation trials of cookies fortified with grape pomace.

This study is one of the first to investigate the effect of grape pomace powder in reducing sugar release during *in vitro* digestion. Results show that grape pomace has a similar ability to attenuate starch digestion in comparison with other fruit pomaces. Although PN showed somewhat better efficiency, there was no clear difference between grape varieties in decreasing the rate of starch digestion.

Chapter 6

The effect of grape pomace powders on the physiochemical properties and *in vitro* digestion of rice starch paste

6.1 Introduction

Starch is one of the most abundant carbohydrate polymers in the cereal grain (Sulaiman, 2011). It appears, daily, in meals of families all around the world in various forms of food such as bread, cookies and cooked rice. In Asia, rice is the most commonly cultivated and consumed cereal grain (Thuengtung, Niwat, Tamura, & Ogawa, 2018). Apart from starch, rice is reported to contain a number of valuable components such as proteins (20 – 31 g/kg cooked rice, 65 – 75 g/kg raw rice), minerals (P, K, Na, Ca, Mg, Fe, Zn, Cu, and Mn) (Chmiel, Saputro, Kusznierewicz, & Bartoszek, 2018), and phenolic compounds (Qiu, Liu, & Beta, 2010; Setyaningsih, Saputro, Palma, & Barroso, 2015; Zaupa *et al*., 2015). In the food industry, rice flour has a wide range of applications thanks to its reasonable price and gluten-free composition. However, the content of carbohydrate in rice ranges from 71.99 – 79.27% (Ghanghas, Sharma, & Prabhakar, 2020) which leads to a high glycaemic index. It is this fact that raises the concern about increasing health problems for individuals who consume too much carbohydrate. Yu *et al*., (2013) conducted a study on 117,366 Chinese women and men whose carbohydrate intake from rice were over 65% and concluded there was an association between coronary heart disease and long term high carbohydrate intake. Another concern is the possibility of increasing the risks of type 2 diabetes from the overconsumption of carbohydrates (Austin, Ogden, & Hill, 2011; Sakurai *et al*., 2016).

Recent studies have paid attention to the reducing sugars released from cereal-based food products. Among those efforts, plants and plant bioactive components have become attractive to researchers as they are natural products, rich in bioactive compounds, easy to collect and

easy to process. The most common practice is to replace traditional starchy flours with natural plant parts or plant extracts to achieve changes in the physicochemical properties, proteins and bioactive content of the final products. Results obtained from previous research have been promising since plants and fruits have been proved to reduce the glycaemic index of starchy food (Kamble, Singh, Rani, & Pratap, 2019; Phimolsiripol *et al*., 2017). However, the number of research publications dealing with the application of fruit pomaces to reduce the glycaemic index is still low. Cobb *et al*., (2018) fortified corn-based extruded products with 2.5, 5 and 10% of grape pomace and observed a significant reduction in glucose released during a gastric digestion process, alongside an increase in dietary fibre content of extruded products. It is worth paying attention to this kind of waste product as they contain large amounts of bioactive compounds and are abundantly available. Besides the reduction of the glycaemic index, the changes of TPC and antioxidant capacity of food products after digestion are another matter that should be taken into account. However, there are few research publications on this topic, and even rarer for the application of fruit pomaces. In the study of Ng & See, (2019), twelve functional plant-based foods were examined for their TPC and antioxidant capacity before and after *in vitro* digestion. The results varied significantly with eight of the samples showing a reduction in TPC and total flavonoid content, while the other four samples caused an increase. The antioxidant capacity of samples varied between the assays employed (DPPH, FRAP, and TEAC), but in general, the majority of samples increased antioxidant capacity. Since the evaluation of food bioactivity before and after digestion might inform the importance of this process in modulating their potential therapeutic effect (Ng & See, 2019), it is worth including this experiment to comprehensively test the effects of grape pomace powder to the food matrix.

To measure functional properties, including pasting and viscosity of starch and starch-based products, several types of equipment have been developed. One of the first instruments was a Consistometer, but this machine was complicated to operate and not economically viable. The next generation of instruments, such as the Ottawa Starch Viscometer or Brabender Visco-Amylograph have been used since the 1930s. The RVA was developed in the 1990s and has now become the major instrument used to measure viscosity. Balet, Guelpa, Fox, & Manley, (2019) summarised the advantages of the RVA to explain why it is popular and reliable compared to other instruments: able to handle small sample size; standard profile completes in 13 min or less; durability and ease of operation; versatility of test procedure and direct demonstration of starch application in foods; parameters recorded are similar to other instruments and cheap to purchase.

Standard tests operated by RVA last for 13 min and comprise five stages: initial, heating, holding, cooling and final stage. In the initial stage, the sample is mixed with water in the test canister, allowing water to move into the interior of the starch granules and also bind with other constituents like proteins. Once the heating stage begins, the increasing temperature causes starch gelatinisation and expansion due to hydration, and the starch granules start to swell, leading to the leaching of amylose and formation of a paste. The paste consists of a molecular dispersion of dissolved starch molecules and a discontinuous phase of swollen granules and granule fragments (Balet *et al*., 2019). The pasting process leads to an increase in viscosity and the highest viscosity value is generally reached when the majority of starch has become swollen at the end of the heating stage, peak viscosity. During the holding stage, viscosity decreases as the crystalline regions of starch granules start to melt, allowing faster movement of water into the granule. The lowest viscosity recorded at this stage is the holding strength; the difference between the peak viscosity and holding strength is the breakdown viscosity. The next stage is

the cooling stage or setback, in which amylose and amylopectin go through a re-association process. The setback viscosity is defined as the difference between the peak viscosity and final viscosity. At the final stage, the temperature stays constant while the viscosity keeps increasing until reaching the final viscosity at the end of the pasting cycle. Important parameters of the pasting profile include peak viscosity, breakdown, final viscosity, setback and pasting temperature. They are measured and recorded by the RVA computer software, making the RVA easy to operate and analyse the data.

This chapter aimed to investigate the effect of New Zealand grape pomace powder on the physicochemical characteristics of rice starch, a different food matrix to cookies which were investigated in the previous chapter. However, since the cookies are the complex food matrix with the appearance of numbers of individual participants such as sugar, salt, and wheat flour, the paste matrix is more simple with only rice starch. Therefore, in this chapter and next chapter, only the replacement ratios of 5 % and 10% were conducted as they were sufficient to cause a significant change in either physical or nutritional values of pastes. The changes in viscosity during the cooking process were fully examined. Another important objective was to test how grape pomace powder affected the *in vitro* digestibility of starch in regards to the nutritional quality the release of reducing sugars.

6.2 Materials and methods

6.2.1 Grape pomace preparation

As described in section 3.1.1

6.2.2 Paste preparation

As described in section 3.3.2

6.2.3 Total phenolic content determination

As described in section 3.4.3

6.2.4 Antioxidant capacity determination

As described in section 3.4.7.2 and 3.4.7.3

6.2.5 In vitro gastrointestinal

As described in section 3.5.10

6.2.6 Data analysis

As described in section 3.5.12

6.3 Results and discussion

6.3.1 Pasting properties of rice starch fortified with grape pomace powder

The pasting profiles of starch enriched with different grape pomace powder at different levels, as measured by RVA, are illustrated in Table 6.1.

Table 6.1. Pasting properties of rice starch fortified with different levels of grape pomace powder

Samples	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Pasting temperature (°C)
RS	2522.67 ± 29.50^{a}	512.33 ± 25.42^{a}	3090.00 ± 18.68^{a}	1079.67 ± 14.98^{a}	75.60 ± 0.43^{c}
SA5	2485.33 ± 69.02^{a}	351.67 ± 9.50^{c}	2777.00 ± 29.46^{b}	530.00 ± 5.29^{cd}	78.62 ± 0.49^{b}
SA10	2160.33 ± 37.11^{b}	252.33 ± 16.07^{d}	2414.00 ± 36.00^{c}	508.00 ± 7.00^{d}	92.00 ± 0.70^{a}
PG5	2434.33 ± 31.66^{a}	332.67 ± 10.69^{c}	2763.67 ± 18.04^{b}	645.33 ± 13.20^{c}	79.68 ± 0.60^{b}
PG10	2124.67 ± 43.82^{b}	261.33 ± 19.66^{d}	2440.33 ± 23.59^{c}	568.33 ± 8.74^{c}	91.00 ± 0.44^{a}
GE5	2486.00 ± 14.53^{a}	352.67 ± 13.43^{c}	2775.67 ± 26.08^{b}	671.67 ± 26.27^{bc}	77.88 ± 0.35^{bc}
GE10	2177.33 ± 8.02^{b}	325.0 ± 17.35^{c}	2451.67 ± 31.90^{bc}	603.33 ± 13.32^{c}	91.70 ± 0.62^{a}
ME5	2399.33 ± 102.30^{a}	428.67 ± 7.51^{b}	2618.67 ± 181.92^{b}	704.00 ± 20.88^{b}	77.63 ± 0.28^{bc}
ME10	2177.33 ± 15.01^{b}	418.0 ± 18.03^{b}	2361.33 ± 12.50^{c}	633.00 ± 32.19^{c}	93.05 ± 2.23^{a}
PN5	2409.33 ± 20.60^{a}	403.67 ± 14.57^{bc}	2748.33 ± 11.37^{b}	703.33 ± 9.02^{b}	77.20 ± 0.88^{bc}
PN10	2092.00 ± 32.92^{b}	369.00 ± 17.35^{c}	2335.33 ± 28.22^{c}	652.33 ± 27.97^{bc}	90.77 ± 1.59^{a}

RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %
GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %
PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Results are expressed as mean value ± standard deviation, n = 3;
Different superscripts in the same column indicate a significant difference ($p < 0.05$).

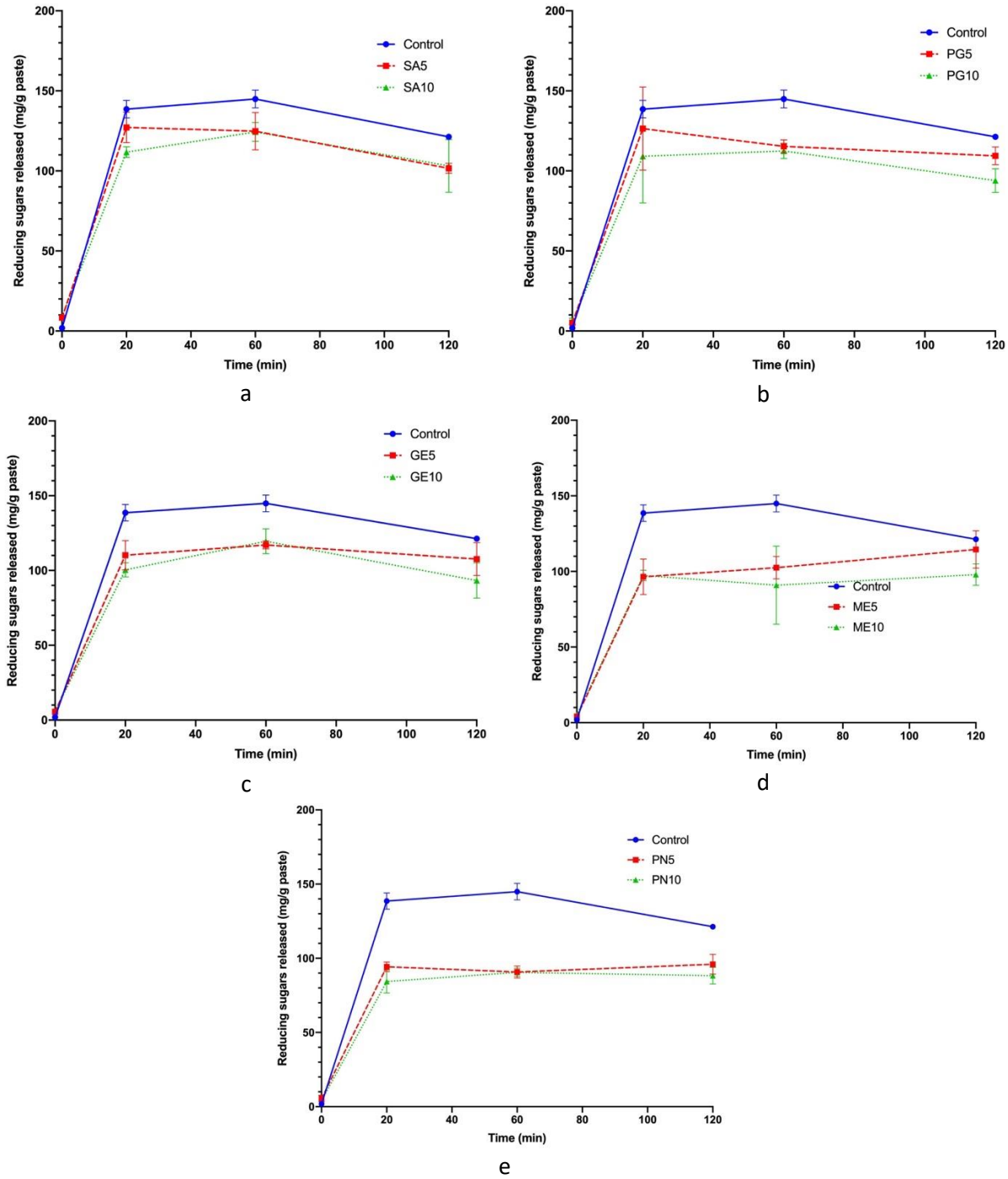
The addition of pomace powder made significant changes to the characteristics of the rice starch pastes ($p < 0.05$). Increasing the addition of pomace resulted in significant decreases of break down, final viscosity and setback in all samples compared to the control ($p < 0.05$). Significant reduction in peak viscosity can be seen at samples with 10% inclusion. The pasting temperature was the only parameter which showed an increase ($p < 0.05$) in all samples with increasing addition. This may be related to the fibre and polyphenols in the pomace binding with the starch and inhibiting starch gelatinisation. No significant differences were seen between red and white grape pomace powder.

Sulaiman, (2011) found that three physicochemical properties of native starch influence the pasting curves; firstly the ratio of amylose to amylopectin; secondly the molecular structure (size, shape, crystallinity, amylopectin branch chain length, and molecular weight); thirdly minor constituents (lipids, protein and phosphate). Of these three properties, the ratio of amylose to amylopectin appears to be the most important in governing the pasting profile of starch. Starch gelatinisation occurs when starch is heated in the presence of water. When starch granules absorb water and swell, some of their components start to leach out and solubilise. As the temperature, and water absorption, keep increasing the granules rupture causing the disordering of chain organisation. After gelatinisation occurs, and starch is cooled down, the disordered chains start to re-associate through hydrophobic interactions and hydrogen bonding in a process called retrogradation. Starch with high amylopectin content causes a high peak viscosity, while high amylose content causes an increase in setback values (Pham, Maeda, Fujita, & Morita, 2007). Amylopectin plays a key role in the swelling of the starch granules, while amylose and lipid restrict swelling (Tester & Morrison, 1990). Hence the replacement of grape pomace powder into starch is likely the amount of starch in comparison with the control samples, hence may change the pasting properties of rice starch pastes. However, researchers

have reported various changes caused by different varieties of vegetables and fruits added to pastes. For instance, Ahmad et. al, (2016) enriched wheat flour with carrot powder (10%, 15% and 20%) and found that samples fortified with 120 mesh carrot powder resulted in a decrease in the peak viscosity, trough, setback, final viscosity and pasting temperature, while only the breakdown increased along with the increase of carrot powder. The authors also reported that carrot powder with bigger granules size (120 mesh) gave more uniform results, while the pasting curves of particles with smaller sizes (70 mesh) were not uniform. Mir *et al*., (2017) studied the influence of apple pomace on the pasting properties of rice flour and Khosar flour and found similar pasting properties to those recorded in this chapter. Another observation was that the pasting values of rice flour (peak, holding, setback and final viscosity) were higher than those of Khosar flour, which was explained by the higher dietary fibre content of Khosar flour. This finding is in agreement with the results of Pham, Maeda, Fujita, & Morita, (2007), in which white waxy flour with a higher starch content, and lower dietary fibre content, had higher pasting properties than those of whole waxy wheat.

6.3.2 *In vitro* starch digestibility

Figure 6.1 shows the amount of reducing sugar released from pastes during *in vitro* digestion. The rate of reducing sugar release from all samples rose dramatically in the first 20 min and then plateaued. The control released more reducing sugars than any of the paste samples with added grape pomace powder. This reveals that the substitution of rice starch with grape pomace powder significantly decreased the amount of reducing sugars released from starch in a simulated digestion process. This result is consistent with other research on cereal-based food products, including the previous chapter of this thesis. This decline might be attributed to some factors.

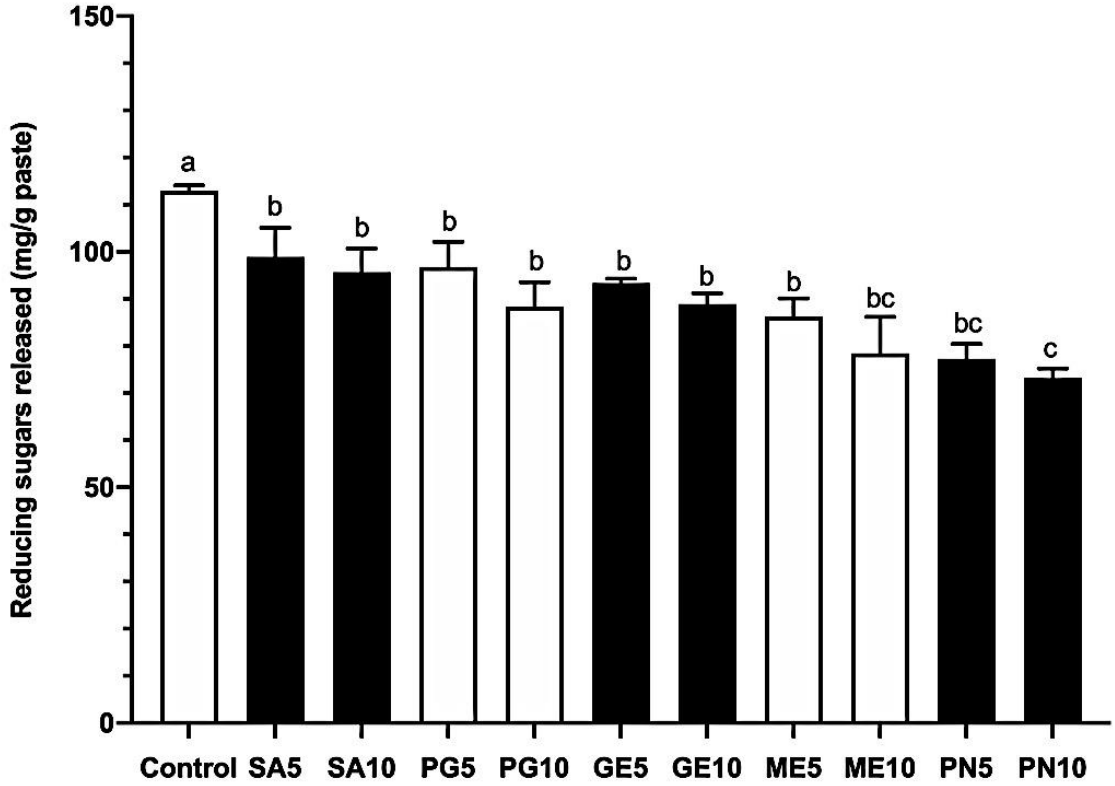


a) Sauvignon Blanc, b) Pinot Gris, c) Gewürztraminer, d) Merlot, e) Pinot Noir

RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3)

Figure 6.1. Amount of reducing sugars released (mg/g product) of pastes fortified with grape pomace powder during *in vitro* digestion

A reduction in reducing sugars released would be expected in line with the replacement of starch by pomace. However, as can be seen in Figure 6.2, which shows the standardised AUC



RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3).
Results with different superscripts are significantly different ($p < 0.05$)

Figure 6.2. Reducing sugar of paste fortified with grape pomace powder after 120 minutes

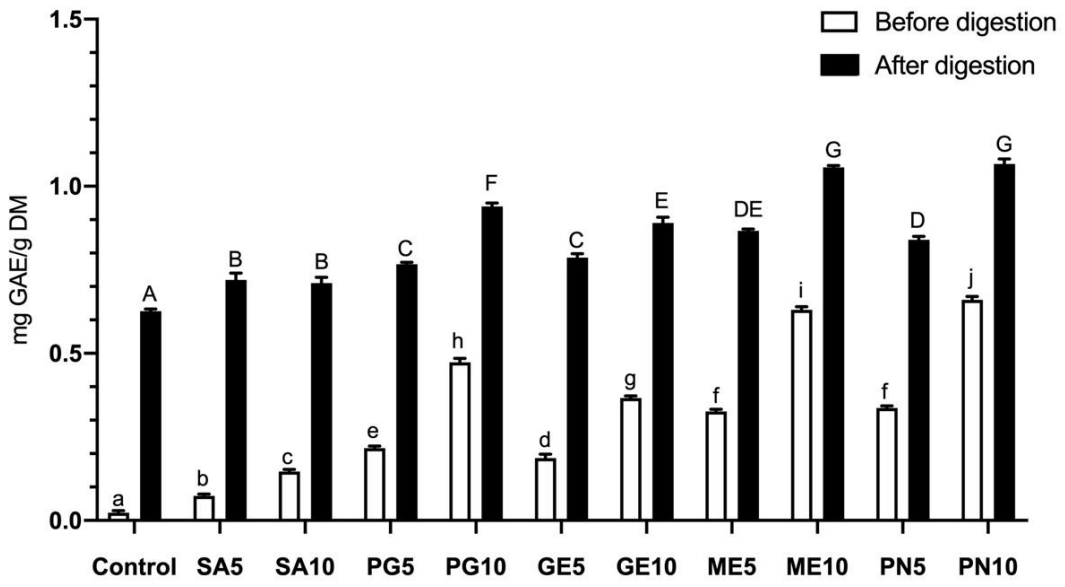
for reducing sugars released, for some samples including PN5, PN10 and ME10, the reductions were more than the expectation. The reason for this greater than expected decrease may be due to the synergistic effect of fibre or phenolic compounds. There are some studies which have focused on the digestibility of rice starch pastes mixed with fruit pomaces. For instance, the experiment conducted by Kamble *et al*., (2019) enriched pasta with soy-okara, a by-product of soybean processing, and observed a reduction in the glycaemic indexes of the pasta samples from 27.41 to 12.38 which were in parallel with the increase of added okara from 0 to 50%. One explanation was that the dietary fibre in okara imbedded rapidly digestible starch in their matrix resulting in the formation of resistant starch, hence affecting the digestion and absorption of

starch. This theory is largely accepted and confirmed by others (Brennan & Samyue, 2004; Moser, 2016; Tudorică *et al*., 2002).

Figure 6.2 clearly illustrates the effect of the grape pomace powders on the starch pastes during the simulated *in vitro* digestion process. As expected, a higher addition of grape pomace powder led to a reduction in the AUC value and the amount of starch being degraded into sugar during digestion. In either 5% or 10% added samples, the degree of reduction was as follow: SA > PG > GE > ME > PN. However, the pastes containing grape pomace showed no significant difference to each other ($p < 0.05$), in reducing sugars released, except for 10% PN sample . There is a non-significant trend that the red grape pomace powders had a greater effect in reducing the AUC values of the pastes compared to the white grape pomace powders. This trend might be due to the richer bioactive compound profiles of ME and PN.

6.3.3 Total phenolic content of pastes

The TPC of the pastes was determined before and after *in vitro* digestion to discover whether digestion affected the release of phenolic compounds. The results are shown in Figure 6.3. It is clear that increasing the grape pomace powder significantly increased the TPC of the paste before digestion, compared to the control. The control sample showed the lowest TPC before digestion of 0.06 mg GAE/g DM, while the highest content was recorded for pastes with 10% enriched of PN. As the rate of addition doubled for each grape pomace powder (5% and 10%), the TPC of the samples also doubled. The results in Chapter 4 showed that the TPC values of grape pomaces followed the order: PN > ME > PG > GE > SA, hence the replacement of grape pomace powder into paste shows the same trend where the samples with red grape pomace powder have a significantly higher TPC than the white grape pomace powder ($p < 0.05$).



RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3)
Results with different superscripts are significantly different ($p < 0.05$)

Figure 6.3. Total phenolic content of pastes fortified with grape pomace powder before and after *in vitro* digestion

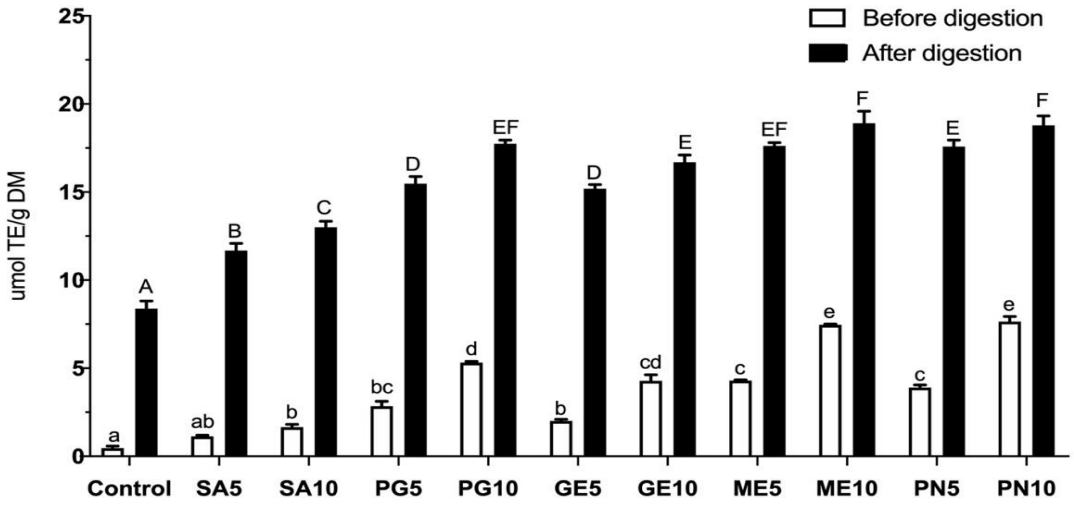
After the *in vitro* digestion process, the TPC values increased significantly ($p < 0.05$). Interestingly, the greatest change was observed in the control sample (an increase from 0.02 to 0.62 mg GAE/g DM). Also of note was the fact that after digestion the TPC of 5 % and 10 % SA samples were the same as each other and SA samples showed the smallest increase in TPC compared to the other samples containing pomace. All other pastes showed significant increases ($p < 0.05$) of TPC from 5% to 10% samples. After digestion, PN and ME had relatively similar contents of phenolics, with no significant differences among their samples of 5 or 10% addition, and their TPC values were still higher than those of the white grape pomace powder fortified pastes. Among the white grape pomace powder added samples, 10% PG had the highest TPC, followed by 10% GE, although no significant difference was observed between 5% PG and 5% GE samples ($p > 0.05$). These results are in agreement with previous research which showed a similar change in TPC of samples before and after digestion. Ti *et al*., (2015) examined the phenolic content of raw, cooked and digested rice and observed that the phenolic of rice

decreased after cooking, but rose again and reached the highest content after digestion. The similar influence of the cooking process was reported previously when it caused the decrease of free phenolic content by 16 – 91 % depending on rice cultivars (Min, McClung, & Chen, 2014). Hydrothermal processing during cooking has been reported to cause changes in the phenolic content of different plants; however, the results varied depending upon plant variety, plant parts or cooking conditions. Some researchers have observed a decrease in phenolic content (Aguilera *et al*., 2011; Fares, Platani, Baiano, & Menga, 2010; Finocchiaro *et al*., 2007), while others have reported an increase (Chandrasekara & Shahidi, 2011; Dewanto, Wu, & Liu, 2002) during digestion. The initial reduction of phenolic content may be due to the heat treatment degrading phenolic compounds in samples compared to the initial raw materials used. The increase might be explained by the disruption of cellular components under heat which promotes the leaching of polyphenols.

The increase of TPC after digestion might be attributed to incomplete digestion of the food matrix. For instance, during the digestion process, a large number of bound phenolics in the food matrix are in a β-conjugated form, which allows them to pass through the stomach and small intestine to reach the colon, where colon bacteria regulate the fermentation, this in turn, results in the release of more bioactive compounds (Ti *et al*., 2015). Furthermore, digestive enzymes might break the chemical bonds in phenolics and proteins, especially phenolic acids, which also raise the TPC content.

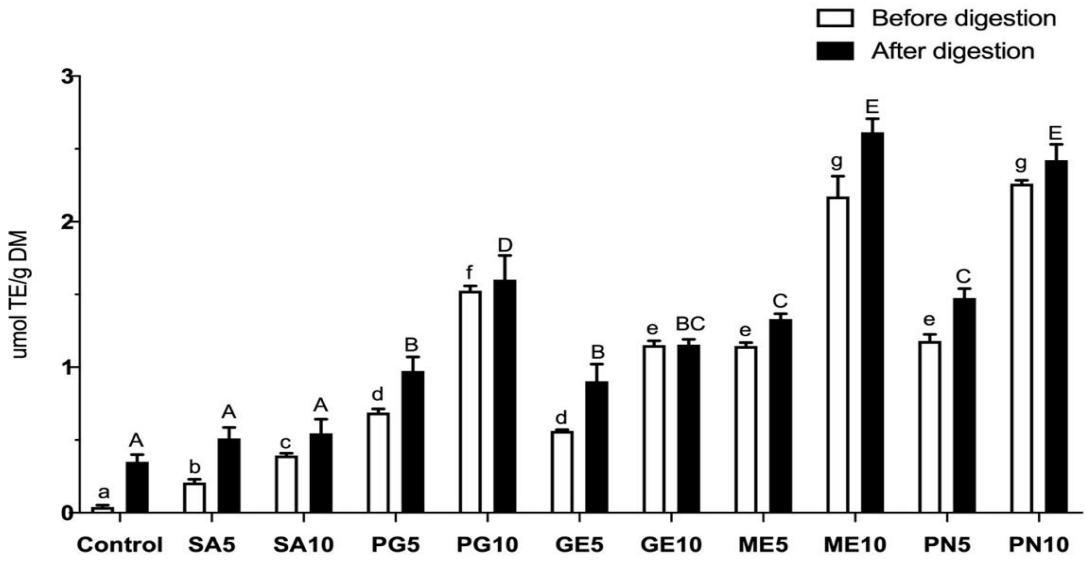
6.3.4 Antioxidant capacity of paste

Two assays were used to determine the antioxidant capacity of paste samples before and after digestion. Figure 6.4 shows the results obtained from the ABTS assay, while Figure 6.5 shows the results of the FRAP assay.



RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %;
PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %;
ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3)
Results with different superscripts are significantly different ($p < 0.05$)

Figure 6.4. Antioxidant capacity (ABTS assay) of pastes fortified with grape pomace powder before and after in vitro digestion



RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %;
PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %;
ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3)
Results with different superscripts are significantly different ($p < 0.05$)

Figure 6.5. Antioxidant capacity (FRAP assay) of pastes fortified with grape pomace powder before and after *in vitro* digestion

In Figure 6.4, it is clear that the inclusion of grape pomace powder significantly increased the antioxidant capacity of all the samples. Before digestion, all the 10% samples exhibited much

higher antioxidant capacity values compared to the 5% samples, except for SA. Red grape pomace powder samples had higher antioxidant capacity capacities compared to white grape pomace powder.

After digestion, the antioxidant capacity of the samples rose sharply, especially the ones which had been fortified with white grape pomace powder. The control sample had the lowest antioxidant capacity values, however, the antioxidant capacity values in the control samples increased by nearly 20 times after digestion, from 0.47 to 8.38 µmol TE/g DM, and this was the largest increase in all the samples. However, the highest antioxidant capacity value was observed in ME10 with 18.91 µmol TE/g DM, closely followed by PN10 (18.78 µmol TE/g DM) and PG10 (17.73 µmol TE/g DM). Except for ME, all other the grape varieties showed a significant difference ($p < 0.05$) between the 5 % and 10 % samples. It is worth noting that the ABTS results showed a similar trend to the TPC results, indicating a strong correlation between TPC and antioxidant capacity.

The FRAP assay was used to measure the reducing potential of an antioxidant reacting with the ferric complex. Ferric reduction is often used as an indicator of electron-donating activity and can be strongly correlated with other antioxidant properties (Benzie & Strain, 1996). In this study, the FRAP assay was performed using Trolox as the standard for ease of comparison with other antioxidant determination assays. Similar to the pattern of ABTS, the results for the FRAP assay showed a significant increase ($p <0.05$) of antioxidant capacity as the fortification level rose from 5% to 10%. In addition, the antioxidant capacity values of pastes made with red grape pomace powder were far higher than the ones made with white grape pomace powder. Before digestion, the lowest value was observed in the control sample (0.04 µmol TE/g DM), which was significantly lower than the other samples. However, after digestion the control sample showed similar values to SA5 and SA10. Another noticeable point is that the values of PG10 and GE10

after digestion were closely similar to their values before digestion. This might be due to the influences of some factors such as gastric acid hydrolysis and pepsin enzymatic hydrolysis, which acted on the plant structure and converted the bound phenolic compounds to free phenolics (Su *et al*., 2019). The FRAP assay also confirmed that red grape pomace was more effective than white grape pomace at increasing the antioxidant activity of pastes.

It is well-documented that TPC contributes to the antioxidant capacity of foods, but there are few research publications which have investigated the antioxidant capacity of grape pomace powder before and after the *in vitro* digestion in foods. The results in this Chapter are consistent with the results from the previous chapters of this thesis which stated that red grape pomace has a higher antioxidant capacity than the white grape pomace. The increase of antioxidant capacity after digestion is strongly related to the high TPC content that is also recorded after digestion. Recent studies have shown variations in the values of antioxidant capacity of fruit parts after *in vitro* digestion. In the study of Su *et al*., (2019), the TPC, total flavonoid content and antioxidant capacity of lychee pulp samples increased significantly after digestion with simulated gastric fluid, but decreased after digestion with simulated intestinal fluid. The increase after the first stage was suggested to be due to the exposure of phenolic compounds to a low pH environment resulting in the release of ellagic and gallic acids. In the second stage, interactions of other components such as carbohydrate and protein may inhibit the release of, or even rebind, bioactive compounds, hence reducing the antioxidant capacity. Chen *et al*., (2016) examined the antioxidant benefits of eleven fruit seeds through simulated *in vitro* gastrointestinal, and observed both significant increases and decreases in selected seeds after digestion, in which seeds of two *V. vinifera* Linn (black and red) had the highest antioxidant values both before and after digestion. The effects of pH were confirmed, alongside other factors such as surrounding conditions, mineral constituents, dietary fibre, all of which may

influence phenolic solubility and availability. The chemical structure of phenolics also should be taken into account as the free radical scavenging activity is mainly dependent on the number and position of the hydrogen donating hydroxyl groups on the aromatic rings of the phenolic molecules (Rammohan *et al*., 2020; Rice-Evans, Miller, & Paganga, 1996).

6.4 Conclusion

In a starchy food matrix, the starch, as well as other food components, will influence its pasting properties (Klunklin & Savage, 2018; Sahin, Zannini, Coffey, & Arendt, 2019). Results of this research confirm this, as the pasting properties of rice starch were significantly affected by the bioactive compounds of grape pomace powder. This knowledge is useful in terms of the orientation of future research as well as the development of cereal-based food products fortified with grape pomace. This study also showed that the replacement of rice starch with grape pomace significantly decreased the reducing sugars released. In terms of varieties, red grape pomace gave a greater decrease in the reducing sugars released compared to white grape pomaces. This decrease indicates that grape pomace is a promising ingredient to use in the fight against type 2 diabetes and heart chronic disease. It is well known that pre-process and heat treatment of cooking is the main reason for the loss of bioactive compounds in food matrices and the inclusion of natural plants and plant parts is an option to compensate for such a loss (Abreu *et al*., 2019; Perez-Hernandez *et al*., 2020). The sharp increase in TPC of all samples fortified with grape pomace powder in comparison with the control shows the potential positive effect of this by-product in enhancing the physicochemical values of food items. The antioxidant activity after the digestion process may help to maintain the cellular redox balance and prevent gastrointestinal diseases (Ng & See, 2019). Thus, the increase in the antioxidant capacity and TPC of the starch pastes, in this experiment contributes knowledge about the bioavailability and bioaccessibility of grape pomace during digestion. Further comprehensive

investigations including optimal addition levels of grape pomace, paste rheological changes, bioactive compounds loss, consumer acceptance, are necessary as they are essential for customer and manufacturer who favour the use of functional plant foods as food ingredient and additives for health-promoting purposes.

Chapter 7

The effect of grape pomace extracts on rice starch paste

7.1 Introduction

In Chapter 6, the effect of grape pomace powder on the properties of rice starch paste was investigated with promising results in terms of the value added ingredients being used to fortify food products. This chapter continues to test the effects of grape pomace powder bioactive compounds by extracting the phenolic compounds and analysing the effects of the extracted phenolic compounds on rice starch paste, in order to broaden the range of application for this valuable source of bioactive compounds.

Using plant extracts to enhance food products has been studied for a long time. Generally, most of the studies have shown promising results with the improvement of desirable attributes of food products. Researchers have focused on the recovery of leftover food products obtained post-processing. Many residues have been trialled. For instance, the use of apple pomace in food has been shown to result in an increase of higher antioxidant properties, total dietary fibre and minerals in the final food products (Mir *et al*., 2017). At the same time, berry pomace has been used to improve the sensorial characteristics of cakes while also reducing the glycaemic index of cakes (Quiles *et al*., 2018), and okara has been used to improve the quality of noodles (Pan, Liu, & Shiau, 2018).

Grape pomace is one of the largest amounts of food processing waste discarded annually, but it contains 60 – 70% of the grapes bioactive compounds, which needs to be taken into account to fully exploit its potential (Fontana, Antoniolli, & Bottini, 2016). A number of studies have focused on the bioactive profiles of grape pomace, and the possibility using these compounds in food products; however, there has been little attention paid to the changes of grape bioactive

compounds and antioxidant capacity during the digestion process. It is fundamental to evaluate the bioavailability and bioaccessibility of these compounds during and after digestion so that their potential for human health can be assessed (Saura-Calixto, Serrano, & Goñi, 2007). Chapter 6 discussed the effects of grape pomace powder the pasting properties as well as the changes in physicochemical properties of the paste after going through *in vitro* digestion process. This chapter aims to continue the study in the changes of paste properties with the replacement of freeze-dried phenolic extracts derived from the corresponding grape pomace rather than the whole pomace which not only contained the phenolic compounds but other co-passengers.

7.2 Materials and methods

7.2.1 Grape pomace preparation

As described in section 3.1.1

7.2.2 Paste preparation

As described in section 3.3.2

7.2.3 Phenolic extract preparation

As described in section 3.2.3

7.2.4 Total phenolic content determination

As described in section 3.4.3

7.2.5 Antioxidant capacity determination

As described in section 3.4.7.2 and 3.4.7.3

7.2.6 *In vitro* starch digestion

As described in section 3.5.10

7.3 Results and discussion

7.3.1 Pasting properties of paste fortified with grape pomace extracts

The pasting profiles of the five pastes fortified with grape pomace phenolic extracts are presented in Table 7.1. In general, the addition of phenolic extracts significantly affected ($p < 0.05$) the pasting parameters. A decrease in peak viscosity was observed for all pastes with grape pomace phenolic extracts compared to the properties of the control paste.

Table 7.1. Pasting properties of paste fortified with grape pomace extracts

	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Pasting temperature (°C)
RS	2553.67 ± 35.80^{a}	742.33 ± 41.04^{a}	3012.00 ± 20.30^{a}	1200.67 ± 7.64^{a}	73.75 ± 0.48^{d}
SA5	2465.00 ± 29.72^{b}	511.67 ± 27.30^{bc}	2968.33 ± 38.68^{ab}	1015.00 ± 6.08^{b}	74.88 ± 0.60^{c}
SA10	2417.00 ± 27.87^{b}	496.00 ± 26.96^{c}	2907.00 ± 26.21^{b}	986.00 ± 16.64^{b}	75.20 ± 0.60^{bc}
PG5	2444.67 ± 15.95^{b}	662.00 ± 21.00^{ab}	2777.33 ± 10.97^{c}	994.67 ± 47.00^{b}	75.00 ± 0.20^{bc}
PG10	2366.67 ± 26.27^{c}	419.00 ± 21.38^{c}	2763.00 ± 24.06^{c}	815.33 ± 24.83^{c}	75.37 ± 0.21^{bc}
GE5	2491.33 ± 22.81^{b}	551.00 ± 39.15^{b}	2865.00 ± 20.07^{b}	924.67 ± 40.67^{bc}	74.63 ± 0.45^{cd}
GE10	2368.00 ± 19.97^{c}	495.33 ± 31.77^{c}	2726.67 ± 34.53^{c}	854.00 ± 42.04^{c}	75.30 ± 0.30^{bc}
ME5	2553.67 ± 27.15^{a}	581.67 ± 49.22^{b}	2915.00 ± 13.75^{b}	943.00 ± 46.16^{bc}	75.77 ± 0.21^{b}
ME10	2294.33 ± 33.56^{d}	451.67 ± 62.40^{c}	2766.33 ± 20.53^{c}	923.67 ± 50.29^{bc}	76.10 ± 0.10^{b}
PN5	2469.67 ± 14.84^{ab}	538.33 ± 61.65^{bc}	2808.33 ± 31.77^{c}	877.00 ± 73.02^{c}	76.17 ± 0.15^{ab}
PN10	2210.33 ± 56.50^{d}	474.67 ± 43.82^{bc}	2557.33 ± 41.79^{d}	821.67 ± 62.98^{c}	77.22 ± 0.28^{a}

RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %
GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %
PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Results are expressed as mean value ± standard deviation, n = 3;
Different superscripts in the same column indicate a significant difference ($p < 0.05$).

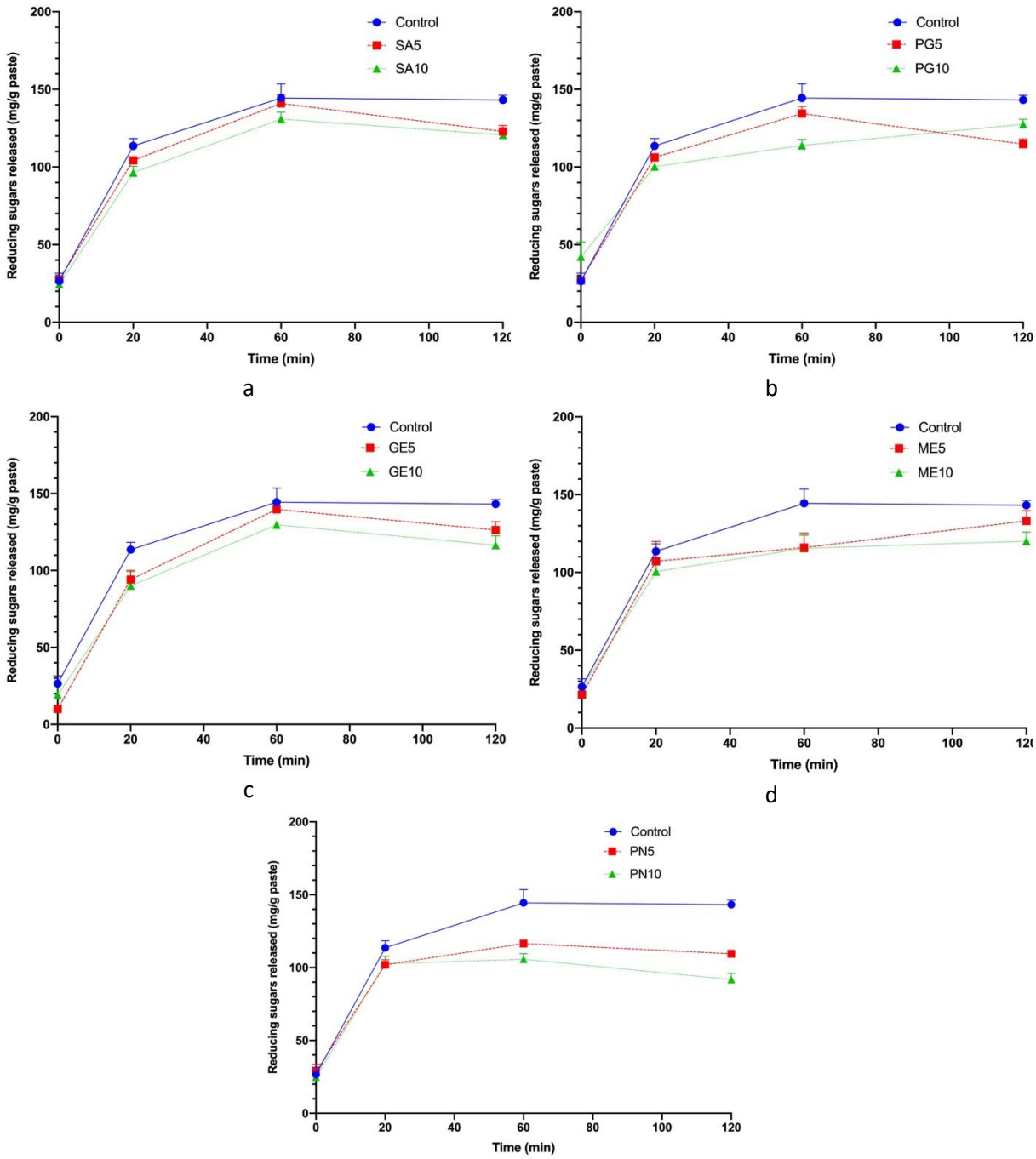
The lowest viscosities were recorded for the pastes enriched with grape pomace phenolic extracts PN10 and ME10 (2210.33 and 2294.33 cP, respectively). Similarly, the final viscosity of the samples decreased with the substitution of grape pomace phenolic extracts. However, the pasting temperatures generally increased with increasing grape pomace extract fortifications. The highest pasting temperatures were recorded for samples substituted by 5% and 10% of PN pomace extract (76.17 °C and 77.22 °C). These results are similar to the pasting profiles of pastes fortified with the whole grape pomace powder in Chapter 6. It can also be seen that samples

with 10% substitution of phenolic extracts from grape pomace had a lower pasting temperature than the ones with 10% addition of grape pomace powder by itself (Chapter 5).

The changes in pasting values are reported to depend on various factors. Zhu et. al., (2008) studied the effects of 25 single phenolic compounds on wheat starch pastes by using an RVA, and illustrated a variation in the effect of each phenolic compound on the paste. Most of the 25 phenolics increased the breakdown values of wheat paste, there were inconsistencies amongst different phenolic compounds, and that these differences were related to the chemical structure of the phenolic compounds; when trans-cinnamic acid content increased, the peak viscosity increased, whereas when catechin content was increased, the peak viscosity was decreased. Thus the type of material added to the rice starch affects the pasting properties, and the individual phenolic compounds change the degree of starch gelatinisation in different ways such as increasing or decreasing the gelatinisation or enthalpy (Sun & Miao, 2020).

7.3.2 *In vitro* starch digestibility

The amount of sugar released during an *in vitro* digestion process of pastes enriched with grape pomace phenolic extracts are shown in Figure 7.1. Barros, Awika, & Rooney, (2012), extracted polyphenols from sorghum to fortify corn starch, and these polyphenols were observed to cause a significant reduction in the digestibility of starch. The decrease was explained by the interaction between the proanthocyanidins of sorghum and amylose and amylopectin of corn starch through hydrophobic force. When comparing the results of this chapter to those in Chapter 6, the grape pomace phenolic extracts showed similar



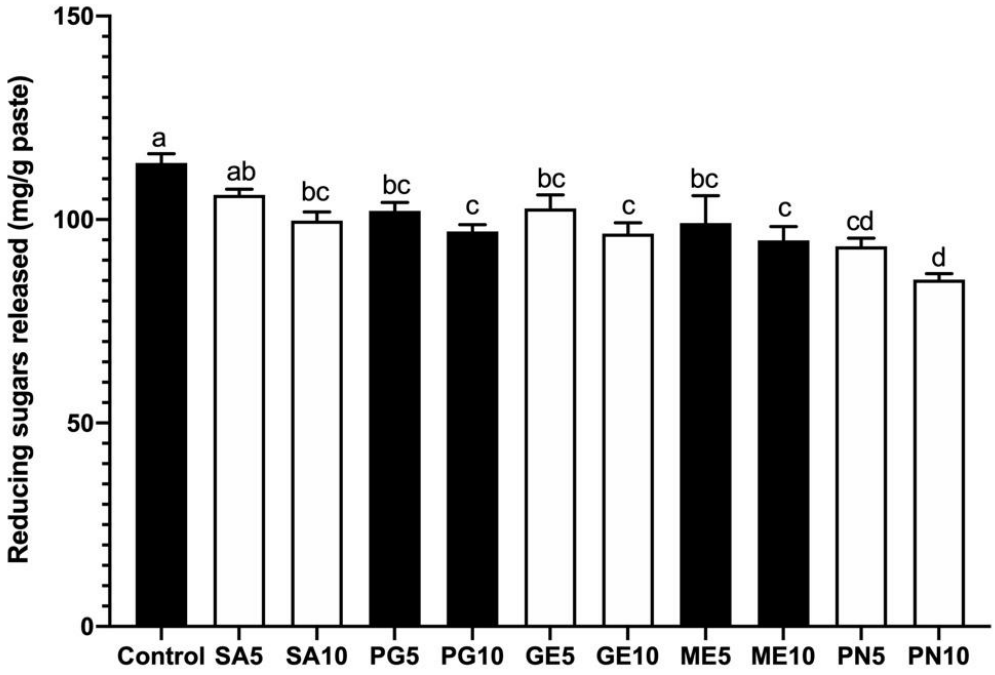
a) Sauvignon Blanc, b) Pinot Gris, c) Gewürztraminer, d) Merlot, e) Pinot Noir

RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3)

Figure 7.1. Amount of reducing sugars released (mg/g product) of pastes fortified with grape pomace extracts during *in vitro* digestion

effects while requiring smaller amounts than the grape pomace powder. This finding is important as it is likely to have less of a detrimental effect on the sensorial characteristics of final food products.

The standardised area under the curve values of pastes enriched with grape pomace extracts are illustrated in Figure 7.2. Apart from SA5, all samples showed a significant reduction ($p < 0.05$) of AUC in comparison with the control sample, and as the level of grape pomace extract increased the AUC decreased. The PN samples showed the greatest impact in the reduction of AUC; the PN5 and PN10 samples showed the lowest AUC values. Hence, red grape pomace extracts performed better as inhibitory agents of reducing sugar release. As well as having a higher amount of polyphenols than white grape pomace the red grape pomace, also contains anthocyanins, which have been reported to decrease reducing sugar release during *in vitro* digestion. Camelo-Méndez et. al., (2016) enriched maize starch with blue maize anthocyanins (mainly cyanidin-3-6''-malonylglucoside) and observed significant decreases in the glucose released with increased levels of anthocyanins. Since red grape pomaces are a good source of anthocyanins, as discussed in Chapters 5 and 6, they should be more effective at attenuating the reducing sugars release than white grape pomace extracts. In contrast, some studies stated that polyphenols have no impact on starch digestion mainly due to the low polyphenol content compared to starch (Sun *et al*., 2016; Sun *et al*., 2018). Hence, the determination of the optimum proportion of phenolic compounds in the food is important.



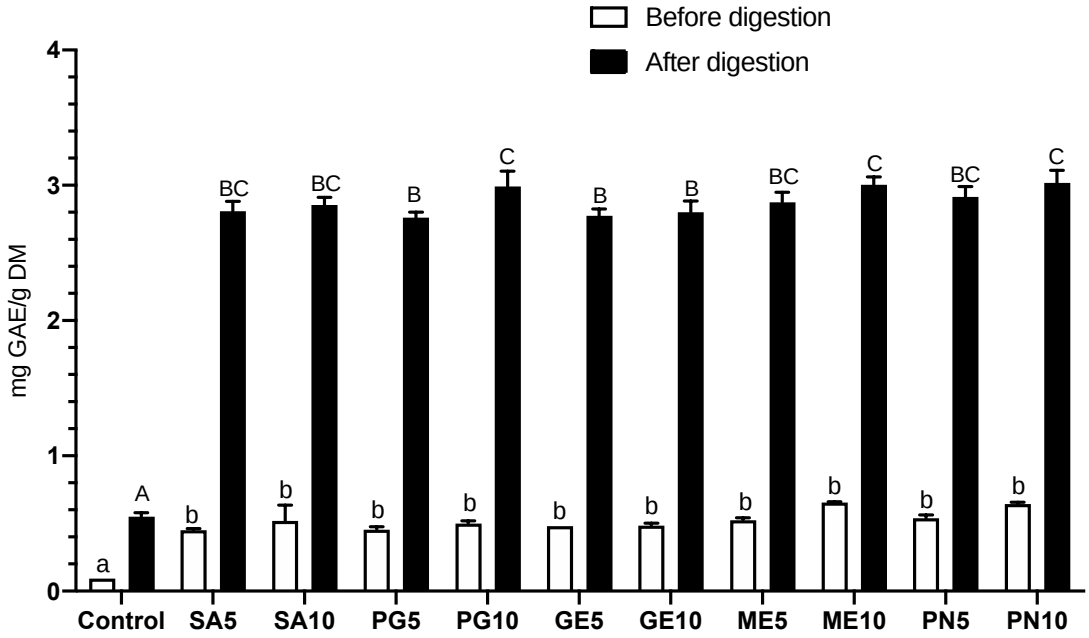
RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3).
Results with different superscripts are significantly different ($p < 0.05$)

Figure 7.2. Reducing sugar of paste fortified with grape pomace phenolic extracts after 120 minutes

7.3.3 Total phenolic content of paste

Figure 7.3 illustrates the TPC of pastes enriched with grape pomace extracts before and after *in vitro* digestion. Samples fortified with the phenolic compounds extracted from grape pomace showed a similar trend to the samples which had grape pomace powder added to them. Before digestion, the highest TPC values were observed in the samples ME10 and PN10, with the content of 0.65 and 0.64 mg GAE/g DM. The lowest TPC values were attributed to SA5 (0.45 mg GAE/g DM), although this was much higher than the control (0.09 mg GAE/g DM). However, there were no significant differences ($p < 0.05$) between all pastes enriched with grape pomace extracts. After digestion, the TPC values of all the samples rose dramatically, including the TPC values for the control sample (0.58 mg GAE/g DM). However, the increases in TPC values in pastes enriched with grape pomace extracts were much greater than that in the control sample; PN10, ME10 and PG 10 had the highest TPC values, while the lowest values were recorded for PG5 and GE5. Compared to the results of rice pastes enriched with grape pomace powder, in

the previous chapter, the TPC of pastes enriched with grape pomace extracts were at least two times higher. This fact might be due to the type of added materials, as the polyphenols were bound in the grape pomace powder, while the phenolic extracts were able to be completely dissolved in the food matrices.



RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3).
Results with different superscripts are significantly different ($p < 0.05$)

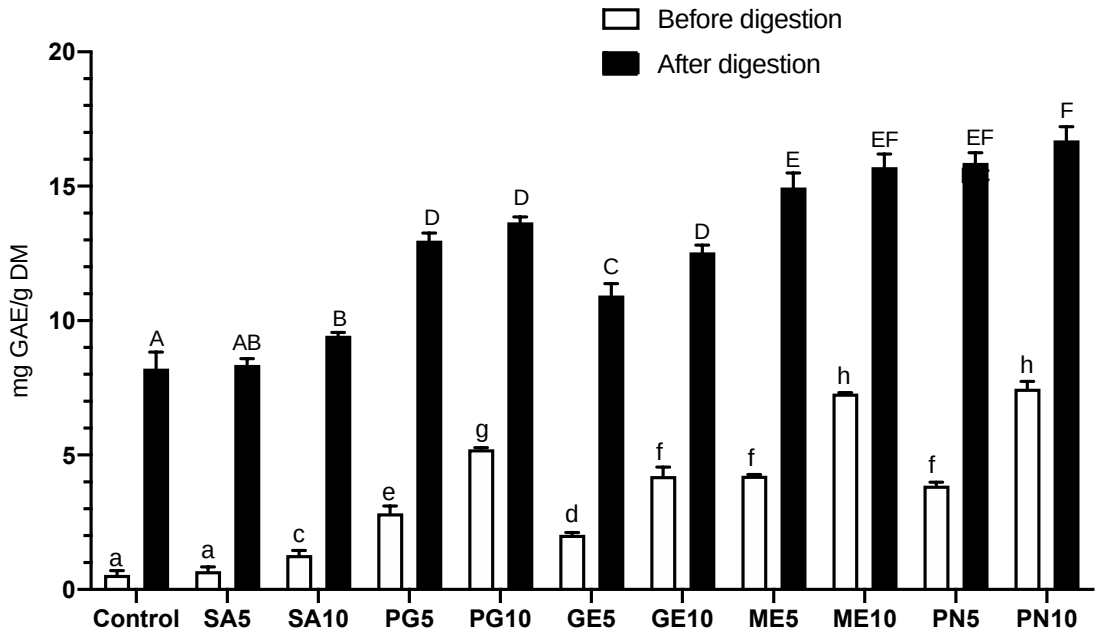
Figure 7.3. TPC of paste before and after digestion

7.3.4 Antioxidant capacity

7.3.4.1 Antioxidant capacity determined by ABTS

The radical scavenging capacity of pastes before and after digestion, as determined by ABTS assay, are presented in Figure 7.4. The radical scavenging capacity of samples increased along with the increase of grape pomace extract, and samples after digestion had higher values than before digestion. As observed in the previous chapters, red grape pomace extracts had a significantly higher scavenging capacity than white grape pomace extracts both before and after digestion. The pastes with grape pomace extract substitution had significantly higher

antioxidant capacity values compared to the control samples, and their values increased as the level of grape pomace extract increased. These observations are consistent with the results in Chapter 6 when higher inclusion of grape pomace powder gave greater antioxidant capacity.



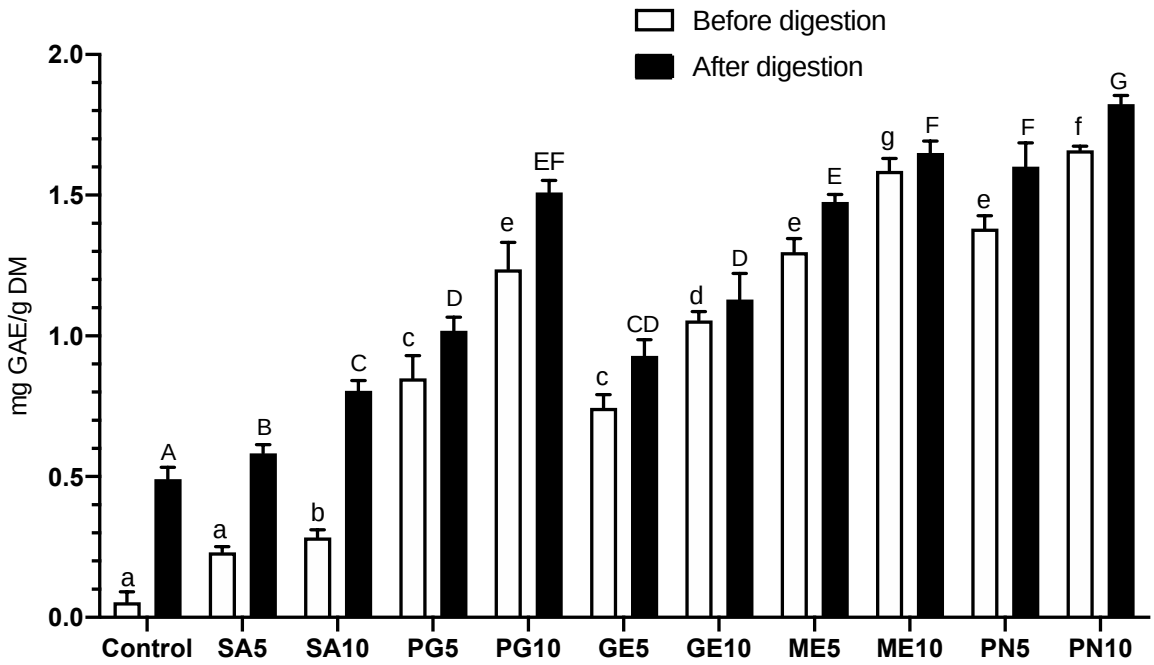
RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3).
Results with different superscripts are significantly different ($p < 0.05$)

Figure 7.4. Antioxidant capacity of paste before and after digestion determined by ABTS

7.3.4.2 Antioxidant capacity determined by FRAP

Similar to ABTS, the FRAP assay (Figure 7.5) showed the same trend in the antioxidant capacity of pastes with grape pomace extract enrichment. The scavenging capacity increased with the increasing level of extracts. Red grape pomace phenolic extracts showed high antioxidant capacity activity (both ME and PN accounted for the highest capacity before and after digestion). In the group of white pomaces, PG exhibited the highest FRAP value, and SA had the lowest radical scavenging capacity. The changes in antioxidant capacity of fruit juices before and after *in vitro* digestion have been investigated in a study by Ryan & Prescott, (2010) which showed variations based on the type of fruit used. For instance, fresh samples had reduced

antioxidant capacity levels after digestion, which was explained by the transformation of compounds to different structural form under highly alkaline conditions. On the other hand, long life samples showed an increase in antioxidant capacity activity due to the changes in the stable form of the phenolic compounds, created by heat treatment, during the digestion process.



RS: rice starch; SA5: Sauvignon Blanc 5 %; SA10: Sauvignon 10 %; PG5: Pinot Gris 5 %; PG10: Pinot Gris 10 %; GE5: Gewürztraminer 5 %; GE10: Gewürztraminer 10 %; ME5: Merlot 5 %; ME10: Merlot 10 %; PN5: Pinot Noir 5 %; PN10: Pinot Noir 10 %
Data are expressed as mean ± standard deviation (n = 3).
Results with different superscripts are significantly different ($p < 0.05$)

Figure 7.5. Antioxidant capacity of paste before and after digestion determined by FRAP

7.3.5 α – amylase inhibitory activities

Diabetes, a modern health problem has been identified as a global issue by the World Health Organisation (WHO) which estimates that the number of people with diabetes will be approximately 700 million by 2030 (Xia, Sun, Li, Zhang, & Zhao, 2017). Type 2 diabetes is related to insulin resistance, which is linked to the consumption of a high glycaemic diet (Kazeem, Adamson, & Ogunwande, 2013). In the daily diet, the rate and extent of starch digestion is crucial in determining the postprandial blood sugar and insulin response. Hence, it is important

to investigate solutions to attenuate glucose release after the intake of starchy foods in order to control diabetes (Sun *et al*., 2018). During starch digestion, α-amylase is an enzyme that is involved in two steps, binding with the starch, and the hydrolysis process which degrades starch into simple sugars (Colonna, Leloup, & Buleon, 1992). α – amylase in saliva is responsible for catalysing the digestion of dietary starch to maltooligosaccharides, which will be degraded further to glucose during digestion. These glucose units are then absorbed into the blood, causing a glycaemic response (Prodanov, Seigner, & Marchis-Mouren, 1984). The inhibitory effects of the five grape pomace phenolic extracts on α-amylase are illustrated in Figure 7.6. Compared to the control, which was the oligosaccharide acarbose which is used as a chemical inhibition of α-amylase, all of the phenolic extracts except for SA, showed a similar ability to inhibit α-amylase. However, these values were lower than those of acarbose, the positive control, at all concentrations. Among the pomaces, the extracts from PN were the most effective inhibitors with its inhibition rate being 64.3% at a concentration of 2 mg/mL. Surprisingly, GE was the second most effective phenolic extract and its rate came close to PN, 63.29%. SA was the least effective inhibitor with the percentage of inhibition being only 54.29%.

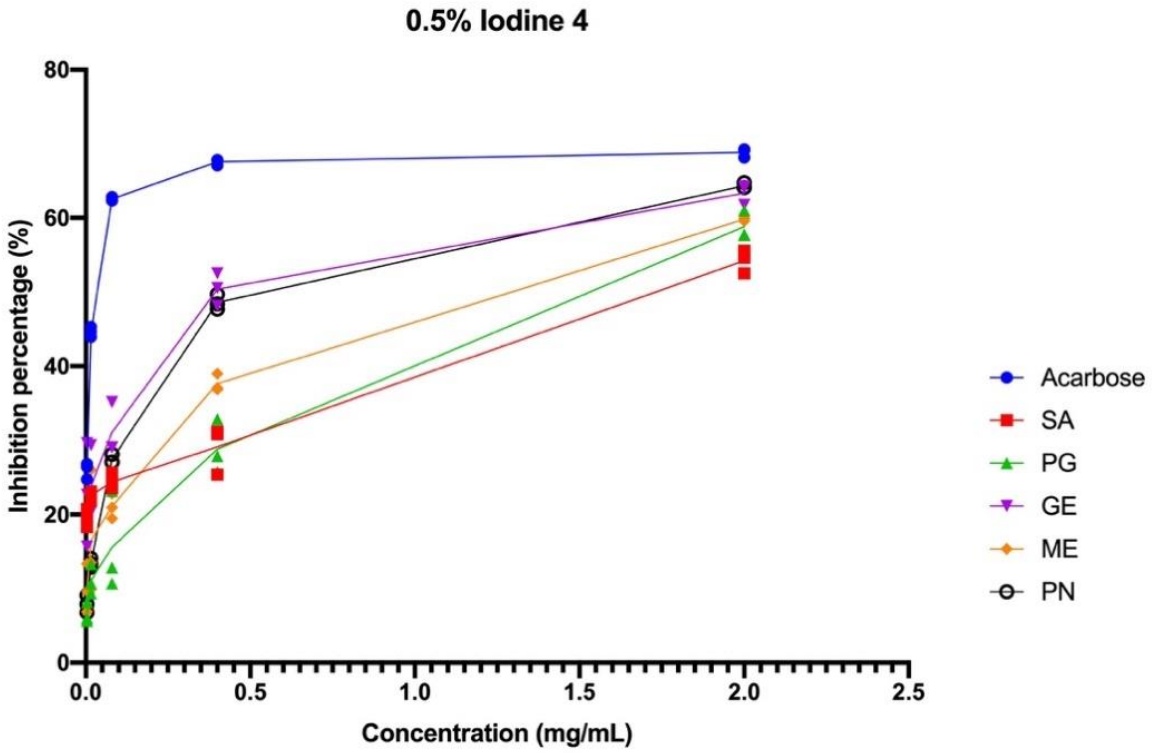


Figure 7.6. α-amylase inhibitory effects of grape pomace extracts

Polyphenols have been shown to inhibit α – amylase from catalysing the digestion of starch by interacting and binding with the enzymes, the inhibitory effect is dependent on the molecular structures of polyphenols (Piparo *et al*., 2008). This finding was confirmed in the study of Miao *et al*., (2013), who found that four different theaflavins extracted from tea were able to inhibit α – amylase. The results showed their inhibitory effects followed the decreasing orders: theaflavin-3,3'-di-O-gallate > acarbose > theaflavin-3'-O-gallate > theaflavin-3-O-gallate > theaflavin, in which the IC_{50} index of the highest one was more than ten times higher than the lowest one. The differences were explained by the molecular weight of carbohydrate moiety and backbone structure of theaflavin, in which the ligand which has more functional groups is likely to have more interactions with the enzyme and produce more docking sites to inhibit the salivary α – amylase.

Plant phenolic compounds have been proven to inhibit the activities of α-amylase by binding with the active sites of the enzyme causing competitive inhibition and/or binding with the

enzyme-substrate complex resulting in mixed-type inhibition as is the case for tea (Sun, Warren, Netzel, & Gidley, 2016), *Angelica sinensis* (dang gui), *Auricularia auricular-judae* (wood ear mushroom), *Centella asiatica* (Gotu Kola), *Clitoria ternatea* (clitoria peas flower), *Curcuma longa* (turmeric), *Cymbopogon citratus* (lemon grass), *Dioscorea polystachya* (Chinese yam), *Ginkgo biloba* (gingko seed), *Glycyrrhiza glabra* (liquorice root), *Lycium barbarum* (goji berry), *Morus alba* (white mulberry leaves and fruits), *and Musa acuminata Colla*. (banana flower) (Ng & See, 2019). Grape monomeric, and oligomeric flavan-3-ol compounds, have been found to actively inhibit α-amylase activities (Xia *et al*., 2017).

7.4 Conclusion

The addition of grape pomace extracts resulted in some significant effects on the physicochemical properties of the rice starch pastes. It reduced the peak viscosity, breakdown, final viscosity and setback and increased the pasting temperature of the pastes compared to the control paste. In comparison with grape pomace powder, the pastes with grape pomace extract had far lower pasting temperatures and setback viscosities. The pastes with grape pomace extracts showed similar attenuation effects on starch digestion as the pastes with grape pomace powder. This decrease in reducing sugar release is in agreement with other research as enrichment with plant extracts not only decrease the amount of starch in the food but decreases its digestibility. The other significant effect is that pastes with phenolic extract from grape pomace had an increased antioxidant capacity. The similarities of results in this chapter and Chapter 6 confirms the fact that enrichment with either grape pomace extracts, or grape pomace powder, have similar effects on the pastes. Both of them are able to reduce the glucose released content and enhance the nutritional quality of final products. However, when consider the huge volume of grape pomace powder required, compared to the small volumes of phenolic extracts, the latter might be more efficient in maintaining the original flavour and

sensorial appearances of food matrices. Further studies are required to evaluate the sensorial characteristics and consumer acceptance of products. Findings in these studies show the potential application of grape pomace as a value added ingredient.

Chapter 8

General Discussion and Conclusion

8.1 Summary

This study demonstrated that grape pomace is an abundant source of bioactive compounds with a high content of total phenolic, dietary fibre, and antioxidant compounds. Grape pomace powder, and the phenolic compounds extracted from grape pomace, could be incorporated into cereal-based food matrices in order to enhance the physicochemical and nutritional properties, and modify the sensory properties of modern functional food. Enrichment of grape pomace powder resulted in positive effects on cookies and pastes regarding proximate composition, physical and texture properties. The results achieved from experiments also supported the idea that grape pomace powder fortify to food matrices like cookies and pastes significantly influence on reducing starch digestibility, increasing TPC and antioxidant capacity, inhibiting α – amylase during digestion.

8.2 Discussion and Conclusion

The first aim of this thesis was to identify the phytochemical profile of grape pomaces from different grape varieties cultivated in New Zealand. Data presented in Chapter 4 showed the main compositions of those grape pomaces with their specific concentration. Based on the results achieved, it was concluded that New Zealand grape pomaces possess similar contents of bioactive compounds and other components in comparison with grape pomace in other regions in the world. This observation confirms that New Zealand grape pomace is a very rich source of bioactive compounds. The quantitative and qualitative distribution of phenolic compounds reflected in the results show significant differences between grape varieties. It is clear that red grape pomace contains a wider diversity of bioactive components than white grape pomace does, which is in agreement with number of previous studies. Besides, this study

has confirmed the advantages of UAE over conventional extracts in terms of yields and extraction time. Appropriate solvent, solvent concentration and extraction time have been examined and stated. UAE combined with methanol 60% helped to reduce the extraction time to 40 min while the corresponding time of conventional extracts was 8 – 10 h. In addition, the content of phenolic compounds and antioxidants was higher in UAE rather than the rival. Hence this study indicates the opportunities to replace conventional extraction by other advanced methods like UAE.

By inclusion at different types of grape pomace (powder and phenolic extract) into cookies and pastes, the second aim of this thesis about the feasibility of using this by – product as value – added ingredients was confirmed. Inclusion of grape pomace into food matrices resulted in promising results in terms of various aspects. For instance, in cookies, enrichment of grape pomace powder with different levels (5, 10 and 15%) showed a significant increase of some physicochemical characteristics, especially at the samples with the highest addition level.

The addition of grape pomace powder significantly enhanced the TPC and antioxidant activities of cookies as proved by the increasing values of TPC and antioxidant capacity as the levels of fortification also increased. The result was not in accordance with the phenolic profiles of raw pomaces where cookies enriched by ME powder had the highest TPC, and the phenolic content of GE at all added levels were higher than PN. In this case, the stability of each phenolic enriched cookie during the preparation and cooking process, as well as the environment they interact with, might be the key factors for the differences. In the study of Wang & Zhou, (2004), green tea catechins were relatively stable during breadmaking, but in the study about biscuits enriched with grape pomace powder of Mildner-Szkudlarz *et al*., (2013), the catechins could only be detected from 20% addition and above due to decomposition. In comparison with bread, the compositions of cookies consisted of higher content of lipids and sugar, plus an

abundance of alkaline – inducing ingredients like baking powder, sodium bicarbonate and does not have yeast during mixing. Hence, the variations of phenolic content in different cookies might be related to the combined effect of alkaline pH, interactions of specific phenolics to certain components of cookies dough, their oxidation and degradation during baking and mixing. More studies are required to thoroughly understand this loss.

Together with TPC, the presence of grape pomace powder with higher phenolic compounds in compositions of cookies was correlated to better scavenging activities. An analysis using DPPH tests, the antioxidant capacity rose by at least two times to three times depending upon the added level of grape pomace powder in comparison with added level of grape pomace powder. In FRAP test the differences were even much higher, with the highest increase was observed at PN15 sample. This result indicates that the TPC and antioxidant capacity of grape pomace had been kept and transferred to cookies after multiple processes, helping to improve the physicochemical quality of cookies. Pasqualone *et al*., (2014), also found that adding grape marc extracts helped the cookies sample to inhibit 48% of DPPH radical, and a similar report was observed by Karnopp *et al*., (2015). More recently, Hui *et al*., (2020) illustrated that the phenolic compounds from berry fruit can also be used to fortify cereal products and improve the antioxidant properties of these foods.

In Chapter 4, 5 and 6, the influence of grape pomace on starch digestibility and predicted glycaemic response of different food products were investigated. While Chapter 4 and 5 tested the impact of grape pomace powder on cookies and paste, Chapter 6 employed the grape pomace extracts to examine on paste. All the investigations revealed promising results. The content of sugar released during a simulated digestion process of all samples enriched with grape pomace powder were significantly lower than the content of control samples. This is might be due to the addition of grape pomace powder generates a “coat” surrounding starch

molecule and prevent the surface of the starch granules from access of α-amylase to starch. Thus, the rate of enzyme to approach starch and release glucose was reduced. The most efficient inhibitors were attributed to the samples with high addition level (15 %), and pomaces derived from red grapes were stronger than white grape pomaces in delaying the degradation of starch and hence the release of sugar. This may be related to the abundance of phenolic compounds in the red grape pomace, which play a crucial role in isolation of starch granules from affecting by α-amylase. A number of studies have confirmed the abilities of plant phenolic compounds in inhibition of digestion enzyme (McDougall *et al*.,2005; McDougall, Kulkarni, & Stewart, 2008; Vallée *et al*., 2017). Chapter 6 and 7 compared the impacts of grape pomace to the glycaemic index of rice starch in two different types: the raw powders and the extracts. As the results in these two chapters reflect close indexes, the influences of raw powder and the extracts were the same.

The results mentioned above answered the objective of this thesis related to the feasibility of using grape pomace as value – added ingredients into cereal-based products. It is inevitable that the appearance of grape pomaces helped to significantly improve the nutritional qualities of final products regarding TPC, antioxidant capacity and glycaemic index.

On the other hand, the inclusion of grape pomaces to foodstuffs led to some unexpected results, especially with higher enrichment. The colour of cookies was significantly changed ($p < 0.05$) by the inclusion of grape pomace powder at all levels. Cookies with the addition of grape pomace powder could be easily differentiate by lower lightness (L^*), higher red colour (a^*) and lighter yellow colour (b^*) than the control samples. Along with the increase of added grape pomace powder, cookies got darker, redder and bluer. Among grape varieties, red grape pomace (including ME and PN) showed the darkest colour (lowest L^* and b^*). It has been explained that the polyphenol content and sugar content of are responsible for the change in

colour of cookies (Mildner-Szkudlarz *et al*., 2013). Specifically, the polyphenols in grape pomace are substrates for polyphenoloxidases, the enzymes that participate in a number of hydroxylation and polymerisation in the presence of oxygen to form brown pigments. The darkness of the cookies fortified with grape pomace powder may also have resulted from Maillard and caramelisation reactions. It should be taken into account that the inclusion of grape pomace powder decreased the expansion of final products. The reduction was ascribed to the dilution of gluten when rose the levels of grape pomace powder and the high-water absorption capacity of grape pomace powder. It is of importance to examine the shrinkage as it affects the final shape of products.

Although the change of moisture content for all samples was not significant, the increase along with an added level of grape pomace was undeniable, the hardness of cookies decreased along with the increase of added levels. A similar observation has been reported by Hossain *et al*., (2017), in which wheat flour showed a decrease in moisture content, while barley flour resulted in an increase. As the water holding capacity of flour plays a crucial role in the moisture content of final baked products (Torbica, Hadnadev, & Hadnadev, 2012), the replacement of flour with grape pomace powder resulted in the change in moisture content. Increased levels of pomace addition resulted in a greater interaction between pomace and wheat flour, which caused more entrapment of water molecules through electrostatic forces, and then developed a network which is more homogeneous with less free water. Subsequently, these increases could be related to the decrease in the hardness of final cookies products in this study. A similar trend was observed in the study of Mais & Brennan, (2008), in which the increase of added sweet potato flour into wheat flour resulted in the decrease in fracture of final cookies. It was explained by the influence of added compositions that changed the pasting properties (peak and final viscosities), hence the reduction in the fracture force. More research needed to be

done to confirm this fact as it may affect the consumer acceptance of products. Since the inclusion of raw grape pomace powder may cause a significant change in physical parameters of food matrices, such as pasting properties and especially the sensorial parameters, which were illustrated by the huge different in colour and aroma of products, it could affect the original taste of products, and further is the consumer acceptance. Further investigations are required to address this problem.

8.3 Recommendation for future work

This study demonstrated that grape pomace cultivated and harvested in New Zealand are good sources of bioactive compounds, especially phenolic compounds and dietary fibre. The concentrations of those components are i.e. comparable to these in grape pomace studied in other regions around the world. However, since the phytochemical profiles of grape pomaces deeply depend on various factors such as climate conditions or winemaking processes, it is worth to implement more investigation in different harvesting seasons to have more specific profiles of compositions of grape pomaces in New Zealand. The result also confirmed that inclusion of grape pomace helps to improve the quality of food matrices. Significant effects of grape pomace powder were observed in the reduction of glucose in the *in vitro* digestion analysis. Grape pomace powder also enhance the TPC and scavenging activity of cookies and paste, meanwhile increase the ability of inhibition of α-amylase activity. For the successful inclusion of grape pomace powder, it is necessary to conduct a sensory study to understand consumers' acceptance of cookies enriched with grape pomace powder. In addition, *in vivo* analysis is required to thoroughly understand the effects of grape pomace powder as well as grape pomace extracts in improving the bioaccessibility and their benefit to human health.

Appendix A

Analysed results for extraction

Total phenolic content and grape pomaces extracted by different methods

Conventional extraction					
	SA	**PG**	**GE**	**ME**	**PN**
	11.68 ± 0.26[a]	31.28 ± 0.28[c]	26.49 ± 0.09[b]	32.19 ± 0.41[c]	48.53 ± 0.81[d]
Ultrasound assisted extraction using 60% ethanol					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	15.70 ± 0.87[a]	44.77 ± 2.67[a]	35.61 ± 1.37[a]	49.31 ± 0.65[a]	62.50 ± 2.75[a]
10’	15.90 ± 0.96[a]	46.40 ± 1.06[a]	36.54 ± 0.72[a]	56.29 ± 0.86[b]	63.31 ± 0.57[ab]
15’	16.19 ± 0.88[a]	47.72 ± 2.32[ab]	37.90 ± 1.31[a]	57.18 ± 0.82[b]	65.35 ± 3.59[ab]
20’	16.52 ± 2.60[a]	48.58 ± 0.88[ab]	38.56 ± 1.06[ab]	56.05 ± 1.40[b]	66.32 ± 2.04[ab]
30’	16.38 ± 0.26[a]	48.71 ± 0.97[ab]	40.75 ± 1.48[bc]	61.53 ± 3.07[c]	67.35 ± 0.34[ab]
40’	16.65 ± 0.65[a]	51.51 ± 1.59[b]	42.29 ± 1.32[c]	62.05 ± 0.19[c]	68.52 ± 0.68[b]
Ultrasound assisted extraction using water					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	6.37 ± 0.33[a]	23.30 ± 0.45[a]	15.19 ± 0.54[a]	19.31 ± 1.07[a]	31.32 ± 0.38[b]
10’	6.77 ± 0.17[ab]	23.25 ± 0.62[a]	15.26 ± 0.27[a]	21.47 ± 0.17[b]	29.08 ± 0.28[a]
15’	6.73 ± 0.25[ab]	24.28 ± 0.11[ab]	15.88 ± 0.36[a]	22.83 ± 0.27[bc]	32.73 ± 0.62[c]
20’	6.96 ± 0.19[ab]	24.96 ± 0.73[b]	15.38 ± 0.27[a]	21.78 ± 0.33[b]	33.42 ± 0.51[c]
30’	7.49 ± 0.33[bc]	24.96 ± 0.39[b]	16.93 ± 0.41[b]	22.50 ± 0.34[bc]	37.09 ± 0.63[d]
40’	7.94 ± 0.39[c]	25.60 ± 0.31[b]	17.18 ± 0.21[b]	24.05 ± 0.79[c]	36.26 ± 0.22[d]

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir
All values are expressed as milligram gallic acid equivalent per gram dried matter (mg GAE/g DM)
Data are expressed as mean ± SD (n=3)
Results with different superscripts in same column for each method are significantly different ($p < 0.05$)

Anthocyanin content of grape pomaces extracted by different methods

Conventional extraction					
	SA	**PG**	**GE**	**ME**	**PN**
	nd	nd	nd	2.33 ± 0.09^{b}	1.02 ± 0.16^{a}
Ultrasound assisted extraction using 60% ethanol					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5'	0.006 ± 0.027^{a}	0.034 ± 0.012^{a}	0.083 ± 0.004^{ab}	3.852 ± 0.012^{a}	1.446 ± 0.056^{a}
10'	0.028 ± 0.003^{a}	0.045 ± 0.011^{a}	0.089 ± 0.004^{b}	4.371 ± 0.011^{ab}	1.449 ± 0.025^{a}
15'	0.024 ± 0.009^{a}	0.063 ± 0.009^{a}	0.084 ± 0.015^{ab}	4.311 ± 0.009^{ab}	1.448 ± 0.078^{a}
20'	0.034 ± 0.016^{a}	0.068 ± 0.005^{a}	0.063 ± 0.011^{a}	4.471 ± 0.005^{ab}	1.476 ± 0.045^{a}
30'	0.026 ± 0.008^{a}	0.067 ± 0.006^{a}	0.087 ± 0.008^{ab}	4.654 ± 0.006^{ab}	1.451 ± 0.049^{a}
40'	0.030 ± 0.003^{a}	0.072 ± 0.032^{a}	0.092 ± 0.008^{b}	4.431 ± 0.032^{b}	1.490 ± 0.067^{a}
Ultrasound assisted extraction using water					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5'	nd	0.010 ± 0.004^{a}	nd	1.911 ± 0.043^{a}	0.488 ± 0.020^{a}
10'	nd	0.004 ± 0.001^{a}	nd	2.229 ± 0.026^{b}	0.539 ± 0.010^{ab}
15'	nd	0.009 ± 0.004^{a}	0.016 ± 0.010^{a}	2.329 ± 0.123^{b}	0.556 ± 0.035^{bc}
20'	0.003 ± 0.004^{a}	0.011 ± 0.001^{a}	0.013 ± 0.006^{a}	2.382 ± 0.040^{bc}	0.598 ± 0.018^{cd}
30'	0.006 ± 0.003^{a}	0.011 ± 0.005^{a}	nd	2.542 ± 0.017^{c}	0.646 ± 0.009^{d}
40'	0.018 ± 0.005^{b}	0.008 ± 0.004^{a}	0.008 ± 0.003^{a}	2.520 ± 0.078^{c}	0.648 ± 0.016^{d}

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir

All values are expressed as milligram Malvidin-3-o-glucoside equivalent per gram dried weight (mg mvd-3-o-glu/g DM)

Data are expressed as mean ± SD (n=3); nd: not detected

Results with different superscripts in same column for each method are significantly different ($p < 0.05$)

Tannin content of grape pomaces extracted by different methods

Ultrasound assisted extraction using 60% ethanol					
	SA	**PG**	**GE**	**ME**	**PN**
	13.21 ± 0.42^{a}	32.96 ± 0.57^{b}	32.47 ± 0.44^{b}	34.57 ± 0.64^{b}	54.51 ± 1.23^{c}
Ultrasound assisted extraction using 60% ethanol					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5'	17.13 ± 1.59^{a}	41.75 ± 1.36^{a}	43.62 ± 2.73^{a}	50.97 ± 2.94^{a}	62.84 ± 4.17^{a}
10'	18.30 ± 1.57^{a}	46.35 ± 1.27^{ab}	45.81 ± 3.62^{ab}	57.82 ± 3.21^{b}	66.04 ± 0.28^{ab}
15'	18.41 ± 0.14^{a}	49.26 ± 2.60^{ab}	46.79 ± 1.38^{ab}	60.91 ± 1.96^{bc}	70.92 ± 4.36^{ab}
20'	18.45 ± 0.99^{a}	49.80 ± 4.30^{b}	50.23 ± 3.16^{ab}	61.21 ± 3.80^{bc}	71.88 ± 2.29^{bc}
30'	19.57 ± 1.56^{a}	51.27 ± 2.35^{b}	51.17 ± 2.76^{ab}	62.14 ± 0.68^{bc}	74.82 ± 2.94^{c}
40'	20.85 ± 1.68^{a}	52.85 ± 3.93^{b}	52.87 ± 4.76^{b}	67.36 ± 3.51^{c}	76.64 ± 2.25^{c}
Ultrasound assisted extraction using water					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5'	4.14 ± 0.10^{a}	15.91 ± 1.18^{a}	9.85 ± 0.81^{a}	12.32 ± 1.05^{a}	15.15 ± 2.60^{a}
10'	4.26 ± 0.15^{ab}	16.94 ± 0.50^{a}	10.92 ± 0.35^{ab}	13.12 ± 2.98^{ab}	16.38 ± 0.96^{ab}
15'	4.63 ± 0.44^{ab}	17.41 ± 2.74^{a}	11.45 ± 0.80^{ab}	13.78 ± 2.17^{ab}	18.31 ± 2.76^{ab}
20'	4.69 ± 0.08^{ab}	17.04 ± 0.95^{a}	11.02 ± 0.57^{ab}	14.45 ± 1.24^{ab}	19.18 ± 3.89^{ab}
30'	4.54 ± 0.11^{ab}	17.31 ± 0.40^{a}	11.78 ± 1.51^{ab}	16.35 ± 1.71^{ab}	22.54 ± 1.10^{ab}
40'	4.94 ± 0.14^{b}	18.08 ± 1.06^{a}	12.62 ± 0.60^{b}	18.11 ± 2.43^{b}	22.74 ± 3.59^{ab}

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir
All values are expressed as milligram Epicatechin equivalent per gram dried weight (mg EE/g DM)
Data are expressed as mean ± SD (n=3);
Results with different superscripts in same column for each method are significantly different ($p < 0.05$)

Antioxidant capacity of grape pomaces extracted by ultrasound assisted extraction determined by ABTS assays

Ultrasound assisted extraction using 60% ethanol					
	SA	**PG**	**GE**	**ME**	**PN**
	61.73 ± 2.48^{a}	197.45 ± 2.27^{c}	153.05 ± 0.23^{b}	181.05 ± 2.44^{c}	302.91 ± 2.81^{d}
Ultrasound assisted extraction using 60% ethanol					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	137.63 ± 7.78^{a}	491.93 ± 10.02^{a}	354.15 ± 14.46^{a}	487.11 ± 5.88^{a}	758.67 ± 9.69^{a}
10’	159.30 ± 4.24^{b}	509.33 ± 12.81^{ab}	378.22 ± 13.10^{ab}	509.70 ± 15.77^{b}	759.41 ± 11.18^{b}
15’	160.59 ± 1.79^{bc}	518.96 ± 6.51^{ab}	383.04 ± 10.96^{ab}	523.78 ± 9.88^{b}	795.70 ± 8.98^{c}
20’	171.33 ± 6.31^{bc}	525.26 ± 11.18^{bc}	385.26 ± 15.77^{b}	536.0 ± 14.44^{bc}	804.59 ± 10.96^{c}
30’	171.15 ± 7.58^{bc}	538.59 ± 12.49^{bc}	396.37 ± 7.23^{b}	560.81 ± 1.28^{c}	852.74 ± 5.59^{d}
40’	183.37 ± 2.25^{c}	553.41 ± 9.25^{c}	487.48 ± 12.24^{c}	605.63 ± 7.14^{d}	862.37 ± 3.39^{d}
Ultrasound assisted extraction using water					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	51.18 ± 2.75^{a}	213.56 ± 7.33^{a}	127.16 ± 1.39^{a}	188.36 ± 3.02^{a}	293.52 ± 6.35^{a}
10’	53.58 ± 5.33^{a}	216.76 ± 3.02^{b}	145.56 ± 7.20^{b}	196.76 ± 1.39^{ab}	299.92 ± 6.04^{ab}
15’	56.18 ± 0.92^{ab}	229.16 ± 4.21^{bc}	146.36 ± 4.85^{b}	211.16 ± 1.39^{b}	315.92 ± 9.70^{b}
20’	60.78 ± 8.31^{ab}	236.76 ± 5.23^{c}	148.76 ± 1.83^{bc}	211.96 ± 4.85^{b}	352.72 ± 1.39^{c}
30’	61.98 ± 3.12^{ab}	239.96 ± 2.50^{c}	149.96 ± 2.50^{bc}	241.56 ± 2.078^{c}	371.92 ± 9.10^{c}
40’	65.98 ± 1.51^{b}	260.36 ± 7.33^{d}	161.16 ± 7.86^{c}	254.36 ± 12.60^{c}	419.92 ± 11.34^{d}

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir

All values are expressed as micromole Trolox equivalent per gram dried matter (µmol TE/g DM)

Data are expressed as mean ± SD (n=3)

Results with different superscripts in same column for each method are significantly different ($p < 0.05$)

Antioxidant capacity of grape pomaces extracted by ultrasound assisted extraction determined by FRAP assays

Ultrasound assisted extraction using 60% ethanol					
	SA	**PG**	**GE**	**ME**	**PN**
	25.54 ± 0.40^{a}	53.81 ± 0.80^{c}	31.63 ± 0.58^{b}	61.38 ± 1.35^{d}	89.01 ± 0.56^{e}
Ultrasound assisted extraction using 60% ethanol					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	38.31 ± 4.42^{a}	121.61 ± 5.41^{a}	126.91 ± 1.54^{a}	143.53 ± 9.52^{a}	167.53 ± 4.58^{a}
10’	40.71 ± 1.67^{ab}	144.91 ± 2.72^{b}	133.41 ± 4.96^{ab}	174.53 ± 8.11^{b}	211.53 ± 6.24^{b}
15’	40.21 ± 3.45^{ab}	153.31 ± 6.71^{bc}	137.31 ± 6.26^{ab}	215.78 ± 7.16^{c}	238.28 ± 8.64^{c}
20’	43.61 ± 5.28^{ab}	160.21 ± 9.66^{bc}	139.11 ± 9.70^{ab}	227.28 ± 9.38^{c}	254.53 ± 9.26^{cd}
30’	43.81 ± 3.06^{ab}	162.21 ± 9.12^{bc}	145.01 ± 6.92^{bc}	233.03 ± 7.23^{c}	265.53 ± 5.62^{d}
40’	50.01 ± 5.10^{b}	172.11 ± 8.55^{c}	151.51 ± 2.27^{bc}	261.28 ± 5.53^{d}	302.78 ± 3.97^{e}
Ultrasound assisted extraction using water					
Time	**SA**	**PG**	**GE**	**ME**	**PN**
5’	17.51 ± 1.42^{a}	79.01 ± 1.25^{a}	56.31 ± 3.16^{a}	75.53 ± 3.75^{a}	130.03 ± 3.12^{a}
10’	19.61 ± 0.17^{ab}	79.51 ± 3.70^{ab}	57.11 ± 3.48^{a}	87.28 ± 1.15^{b}	131.03 ± 3.97^{b}
15’	20.01 ± 3.34^{ab}	84.41 ± 0.76^{ab}	58.21 ± 0.62^{a}	90.78 ± 1.15^{bc}	133.03 ± 1.73^{b}
20’	20.51 ± 1.14^{ab}	86.31 ± 1.82^{b}	59.61 ± 2.40^{a}	94.03 ± 1.15^{c}	145.53 ± 4.88^{c}
30’	21.01 ± 0.46^{ab}	88.61 ± 1.35^{b}	59.81 ± 1.51^{a}	101.28 ± 5.10^{cd}	153.28 ± 4.13^{cd}
40’	22.71 ± 1.37^{b}	94.91 ± 1.51^{c}	60.91 ± 1.42^{a}	104.03 ± 0.75^{d}	163.03 ± 3.70^{d}

SA: Sauvignon Blanc; PG: Pinot Gris; GE: Gewürztraminer; ME: Merlot; PN: Pinot Noir

All values are expressed as micromole Trolox equivalent per gram dried matter (µmol TE/g DM)

Data are expressed as mean ± SD (n=3)

Results with different superscripts in same column for each method are significantly different ($p < 0.05$)

Appendix B

Chemical and Buffers used in experiment

B.1 Total phenolic determination

0.2 N Folin-Ciocalteu reagent

Folin-Ciocalteu reagent (2 N) was purchased from Sigma Aldrich, St Louis USA. Folin-Ciocalteu reagent (2 N) (20 mL) was placed in a 100 mL volumetric flask and made to volume with RO water.

7.5 % sodium carbonate (Na_2CO_3)

Sodium carbonate (7.5 g) was dissolved in 100 mL RO water.

Gallic acid solution (200 µg)

Gallic acid (C7H6O5) (0.040 g) was dissolved in 200 mL volumetric flask with RO water.

B.2 Anthocyanin dertemination

Chemicals and buffers

Bisulphite solution

Potassium metabisulfite ($K_2S_2O_5$) (1 g) was dissolved in 5 mL of distilled water, on the day of analysis.

Potassium chloride buffer, 0.025M, pH 1

Potassium Chloride (KCl) (1.86 g) was dissolved in 980 mL distilled water and concentrated HCl was used to adjust the pH of the solution to 1. Then distilled water was added to make to 1 L.

Sodium acetate buffer 0.4M, pH 4.5

Sodium acetate $CH_3CO_2Na.3H_2O$ (54.43 g) was dissolved in 960 mL distilled water, concentrated HCl was used to adjust the pH of the solution to 4.5. Then distilled water was added to make to 1 L.

B.3 2,2-diphenyl-1-picrylhydrazyl (DPPH) determination

Trolox (6-hydroxy-2, 5, 7, 8 -tetra methylchroman-2-carboxylic acid) (200mM)

A stock solution of 2mM was prepared by dissolving Trolox 0.0250 into 50 mL volumetric flask and made up with phosphate buffer. From stock solution 1 mL was added into a 10 mL volumetric flask and filled with phosphate buffer and made 200 µM Trolox solution.

B.4 Ferric ion reducing antioxidant power (FRAP) determination

FRAP reagent

Reagent A: Acetate buffer (300 mM, pH 3.6): Add 16 mL of glacial acetic acid to 3.1 g of sodium acetate trihydrate in a glass beaker, mix well using stirrer. Make up the solution to 1 litre using distilled water. The pH of solution is checked by using pH reader.

Reagent B: TPTZ solution: add 10 mL of 40 mM Hydrochloric acid (HCl) and dissolve at 50°C

Reagent C: Ferric Chloride solution: add 10 mL of distilled water to 0.054 g of Ferric Chloride and dissolve.

To prepare FRAP reagent (aim to make 30 mL): mix reagent A: B: C according the ratio 1:1:10. For example, to make 30 mL of FRAP reagent, take 2.5 mL of reagent B and 2.5 mL of reagent C and add to 25 mL of reagent A. Mix well and incubate in a water bath at 37 °C for a minimum of 10 min.

Trolox (6-hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid) (200mM): as described in Appendix B.3

B.5 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS)

ABTS radical reagent

To prepare 7 mM ABTS solution (A): take 0.0384 g of ABTS, make up to 10 mL using distilled water in volumetric flask and dissolve.

To prepare 100 mM $K_2S_2O_8$ (B): 0.27 g of potassium persulphate were made up to 10 mL using distilled water in volumetric flask and dissolve.

On the day before performing assay, 9.5 mL of solution A were mixed with 245 µL of solution B, and then made up to 10 mL using distilled water to make reagent C. The reagent C was then covered by tin foil to allow reaction in the dark at room temperature for at least 16 h.

To prepare PBS pH 7.4 solution: one PBS tablet was dissolved in 200 mL of water, the pH of solution was checked and adjusted by adding NaOH 1 M or HCl 1 M.

On the day of analysis, dilute reagent C in PBS solution until reaching the absorbance of 0.7 ± 0.02 at 734 nm.

B.6 Dietary fibre determination

95 % ethanol

78 % ethanol solution

Acetone, reagent grade

Enzymes used for TDF assay (all imported from Megazyme International Ireland): α-Amylase, heat stable (E-BLAAM, 3,000 Ceralpha Units/mL); Protease (E-BSPRT, 50 mg/mL, 350 Tyrosine Units/mL); Amyloglucosidase (E-AMGDF, 200 pNP β-maltoside Units/mL)

Celite®, analytical grade (Megazyme cat. no. G-CELTE)

MES/TRIS buffer, 0.05 M each, pH 8.2 at 24°C.

Hydrochloric acid solution, 0.561 N

pH standards

B.7 *In vitro* gastrointestinal

For measurement of reducing sugars, 3.5-dinitrosalicylic acid (DNS) is required. It is prepared by dissolving powder of DNS in appropriate amount of 2M NaOH solution overnight with vigorous stirring and heat (no more than 70 °C). Transferred solution of potassium sodium tartrate tetrahydrate to DNS solution, kept stirring until solution is totally clear. Solution was kept in dark container and cover with tin foil.

Other chemicals, buffers and enzymes

0.1 M HCL solution:

Concentrated (35 %) hydrochloric acid (8.18 mL) MW (HCl) was mixed in 1000 mL RO water.

0.1 M NaOH solution:

Sodium hydroxide (4.0 g) was dissolve in 1000 mL RO water.

Trypsin, chymotrypsin and protease enzyme:

Pepsin (1031 U/mg), pancreatin (350 U/mg), trypsin (2000 U/mg), chymotrypsin (40 U/mg) and protease (5 U/mg) from porcine gastric mucosa, were purchased from Sigma Aldrich, St Louis USA.

4 M sodium hydroxide (NaOH) solution:

Sodium hydroxide pellets (32 g) was dissolved in 150 mL RO water. Transfer it to a 200 mL volumetric flask and made to volume up to 200 mL with RO water.

1 M HCl solution:

Concentrated hydrochloric acid (16.5 mL) was mixed with 100 mL RO water in a 200 mL volumetric flask. Made to volume up to 200 mL with RO water.

0.5 m HCl solution:

Hydrochloric acid (1 mL) was mixed in a 20 mL volumetric flask. Made to volume up to 20 mL with RO water.

Sodium bicarbonate (NaHCO3) solution:

Sodium hydrogen carbonate (42 g) was dissolved in 400 mL RO water. Transferred to a 500 mL volumetric flask and made to volume up to 500 mL with RO water.

Ethanol:

Ethanol (99.5 %) was purchased from Sigma Aldrich, St Louis USA.

Invertase and Amyloglucosidase:

Invertase (300U/mg in 50 % glycerol, stored at -20 °C) and amyloglucosidase (3260 u/mg were purchased from Megazyme Inc. Wicklow, Ireland.

Glucose standard solution (5mg/mL):

D-glucose (1g) was dissolved in 150 mL RO water. Transfer to a 200 mL volumetric flask and make volume up to 200 mL with RO water. Store as 5 mL aliquots in freezer at -20 °C

Glucose standard solution (10 mg/mL):

D-glucose (2 g) was dissolved in 150 mL RO water. Transfer to a 200 mL volumetric flask and make volume up to 200 mL with RO water. Mix well. Store as 5 mL aliquots in freezer at -20 °C

Sodium maleate buffer 0.1 M pH 6:

Maleic acid (11.6 g) was dissolved in 800 mL water then the pH was adjusted to pH 6 using 4 M NaOH. Hydrated calcium chloride, $CaCl_2H_2O$ (0.3 g) was added to the solution followed by 0.23 g of sodium azide. The volume of the solution adjusted to 1 L with RO water in a volumetric flask.

Sodium acetate buffer 0.1 M pH 5.2:

Sodium acetate (13.6 g) trihydrate was added to 900 mL water, the pH was adjusted to pH 5.2 using 0.1 M acetic acid, then 4 mL of 1 M $CaCl_2.2H_2O$ was added and made to volume up to 1 L with RO water.

Dinitrosalycilate (DNS) mixture:

Ten gram of DNS (3,5-dinitrosalicylic acid) were dissolved in 400 mL of 2 M NaOH at room temperature with vigorous stirring. Dissolved 300 g sodium potassium tartrate tetrahydrate (MW = 282.22 g/mol) in 500 mL of distilled H_2O, then these two solutions were mixed and the final volume was made up to 1 L using RO water. Absorbance was read with help of spectrophotometer (VWR, V-1200) at 530 nm. Under alkaline and heating conditions, the

reducing sugars contains free aldehyde or keto groups and they can react with DNS to produce 3-amino-5-nitrosalicylate which absorbs light at 530 nm.

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