Concepts Important in Understanding the Health Benefits of Phenolics in Fruits and Vegetables: Extractable & Non-Extractable Phenolics and the Influence of Cell Wall Polysaccharides on Bioaccessibility & Bioavailability

Kelly A. Ross*[1]

Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre
4200 Highway 97, Summerland, BC, Canada, V0H 1Z0

*[1]Kelly.Ross@agr.gc.ca

Received Dec 1, 2013; Revised Feb 17, 2014; Accepted Mar 10, 2014; Published Jun 13, 2014

Abstract

The health benefits associated with a diet high in fruits and vegetables are well established and fruits and vegetables are a key source of phenolic compounds. Phenolic compounds have been shown to demonstrate antioxidant, anti-inflammatory, and anti-carcinogenic activities. However in order to provide health benefits , these compounds must be bioaccessible and bioavailable in the body after ingestion. Evidence is emerging that demonstrates the importance of plant cell wall polysaccharides as a key factor affecting the bioaccessibility and consequent bioavailability of phenolics. Also, the health benefits of phenolics does depend on their concentration in fruits and vegetables and the phenolic content in fruits and vegetables has been commonly under-reported due to the aqueous organic extraction method typically used. Determination of the extractable and non-extractable phenolics content using a more complete extraction method is necessary to obtain accurate measurements of phenolics in foods. This review discusses the following: the concepts of extractable and non-extractable phenolics in fruits and vegetables; the influence of cell wall polysaccharides on the bioaccessibility of phenolic compound including a discussion of the types of polysaccharide-phenolic interactions; an examination of the release of phenolics from polysaccharides during simulated gastrointestinal digestion; and an examination of how polysaccharides may influence the uptake/bioavailability of phenolics. The aim of the review paper is to stimulate increased research efforts in the area of polysaccharide-phenolic interactions and their consequences on human health.

Keywords

Cell Wall Polysaccharides; Extractable Phenolics; Non-extractable Phenolics; Polysaccharide-phenolic Interactions; Bioaccessibility; Bioavailability; Gatrointestinal Digestion; Colon Fermentation

Introduction

Fruits and vegetables are well known sources of vitamins, minerals and dietary fibre (cell wall polysaccharides) as well as phenolic compounds and other phytonutrients. Polyphenolics/phenolic compounds are a major group of phytochemicals in fruits, including: flavonoids (proanthocyanidins, anthocyanidins anthocyanins, flavanols, flavonols, flavones, flavanones, chalcones, and isoflavones); phenolic acids; stilbenes, lignans, hydrolyzable tannins; and other polyphenols (hydroxytyrosols, alkylresorcinols etc). (Crozier et al., 2006; Perez-Jimenez and Torres, 2011; Saura-Calixto, 2012). Studies have implicated phenolic compounds as important bioactive compounds (Herken and Guzel, 2010). Research examining the health promoting properties of fruits and vegetables has found that phenolic compounds and their metabolites have antioxidant, anti-inflammatory and/or anti-carcinogenic properties (Palafox-Carlos et al., 2011). High consumption of

fruits has been linked to the prevention of degenerative diseases such as cardiovascular diseases, diabetes, cancer, and arthritis (Adnan et al., 2011). As such, it is well established that the phenolic component of fruits and vegetables may be important components of health promoting diets (Herken and Guzel, 2010).

The health benefits of phenolics depend on their concentration in fruits and vegetables, along with their bioaccessibility and bioavailability after ingestion (Palafox-Carlos et al., 2011). The concentrations of phenolics in different fruits and vegetables and the bioavailability of phenolic compounds after their ingestion has been studied in detail (Palafox-Carlo et al., 2011; Parada and Aguilera 2007). Bioavailability is an important concept in food for health studies (Palafox-Carlos et al., 2011; Manach et al., 2005) and bioavailability is defined as the proportion of a compound that is digested, absorbed, and utilized in normal metabolism. However, for a compound to be bioavailable the compound must be bioaccessible. Bioaccessibility is defined as the amount of an ingested nutrient that is available for absorption after digestion (Hedren et al., 2002; Palafox-Carlos et al., 2011). Bioaccessibility of phenolics has not been studied to the same extent as bioavailability of phenolics. Furthermore, the health benefits associated with high dietary intake of fruit and vegetable phenolics is a well-researched subject, yet the role of the plant cell wall polysaccharides on the bioaccessibility and bioavailability of phenolic compounds is not (Paydayachee et al., 2012a, 2012b; Padayachee et al., 2013).

Also, it has been assumed that the polysaccharides of plant cell walls are resistant to digestion by human enzymes in the stomach and small intestine and are delivered to the colon/large intestine in a chemically unaltered state, where they are fermented by colonic microbiotica (Carnachan et al., 2012). Small chemical or structural changes in polysaccharides can substantially affect the bioavailability/absorption of biologically important compounds (Carnachan et al., 2012; Mishra and Monroe, 2012). It is important to discuss the information existing on fruits and vegetables that have been subjected to vitro digestion and report on the physical and chemical changes that occur in the cell wall polysaccharides and any influence on the bioavailability of biologically important compounds. Also, it should be noted here, yet beyond the scope of this review, that polysaccharides have been shown to possess bioactivity on their own right (Ross and Mazza, 2011; Ross et al., in press) and digestion may impact bioactivity (Ovodova et al., 2009; Popov et al., 2011). The concept of bioactive polysaccharides is an area of research that is gaining importance.

In addition to bioaccessibility and bioavailability, the health benefits of phenolics does depend on their concentration in fruits and vegetables, (Palafox-Carlos et al., 2011) and knowledge of the phenolic content of foods is essential for studies on diet and health (Saura-Calixto, 2012). However, the majority of studies examining the phenolic content of foods have focused on measuring phenolics that can be extracted with organic aqueous solvents. This has likely resulted in the phenolics content of fruits, vegetables, and other plant foods containing phenolic compounds to be underestimated as it has been reported that an appreciable amount of phenolics remains associated with cell wall polysaccharide residues remaining after aqueous organic extractions (do Socorro M. Ruffino, 2010; Saura-Calixto, 2012). However phenolics associated with cell wall polysaccharides may be bioaccessible and bioavailable in the human digestive system (Saura-Calixto et al., 2007) and they may have an important role in digestive health and contribute to human health benefits (Saura-Calixto, 2012). A discussion of the relevance of these phenolics is warranted. Phenolics associated with cell wall polysaccharides must be discussed in terms of their characterization using both chemical and physiologically relevant extraction regimes with the aim of understanding their contributions to human health.

This review will discuss the following: the concepts of extractable and non-extractable phenolics in fruits and vegetables; the influence of cell wall polysaccharides on the bioaccessibility of phenolic compound including a discussion of the types of polysaccharide-phenolic interactions; an examination of the release of phenolics from polysaccharides during simulated gastrointestinal digestion; and an examination of how polysaccharides may influence the uptake/ bioavailability of phenolics. The aim of the review paper is to stimulate increased research efforts in the area of polysaccharide-phenolic interactions and their consequences on human health.

Concept of Extractable and Non-Extractable Phenolics

Extractable Phenolics Obtained from Chemical Solvent Based Extractons

In plants, polyphenols/phenolic compounds are chemically described as secondary metabolites that

possess an aromatic ring that has more than one hydroxyl groups present (Crozier et al., 2006). Phenolic compounds are a heterogeneous class of secondary plant metabolites that can be divided into two main groups: flavonoid compounds, with a C6-C3-C6 structure (anthocyanins, flavan-3-ols, flavonols, flavones, and flavanones) and non-flavonoid compounds (i.e. other phenolic compounds) with C6-C1 and C6-C3 structures (Crozier et al., 2006). These other phenolic compounds include stilbenes, hyrdrolyzable tannins, and simple phenolic acids. (Denny and Buttriss, 2005). Fruits and vegetables are two key sources of phenolics in the diet. Traditional studies regarding phenolics in the diet have focused on their impact on the sensory properties of foods and beverages such as colour, bitterness, astringency, and haze formation (Saura-Calixto, 2012). However current studies have focused on the health benefits of phenolics in foods as dietary phenolics have been attributed to play a significant role in the prevention of chronic disease such as cardiovascular diseases, diabetes, cancer, and arthritis (Adnan et al., 2011). This has resulted in much research focused on determining the phenolics content of foods (Saura-Calixto et al., 2007). In many of these works, chemical solvent based extraction methods were employed and the solvents most commonly used were 100% ethanol or aqueous ethanol at various concentrations along with a variety of other acidified or non-acidified aqueous organic solvents (methanol, acetone, ethyl acetate) (Perez-Jimenez and Saura-Calixto, 2005). Therefore there is much data available on "extractable phenolics" in fruit and vegetables obtained using a chemical solvent based extraction method (Perez-Jimenez and Torres, 2011; Saura-Calixto, 2012).

The extractable phenolics obtained from chemical solvent based extraction methods and information about these extractable phenolic have been typically used to populate databases providing information characterizing the phenolic compounds in foods (Bravo et al, 1994; Saura-Calixto, 2012). Extractable phenolics are typically lower molecular weight compounds, mainly monmers and decamers (Saura-Calixto, 2012), with molecular mass less than 5000 Da (Bravo et al., 1994) and examples of extractable phenolics that have been extracted from fruits and vegetables using chemical solvent based methods include: flavonoids (extractable proanthocyanidins, anthocyanidins anthocyanins, flavanols, flavonols, flavones, flavanones, chalcones, and isoflavones); phenolic acids (hydroxybenzoic and hydroxycinnamic acids, including soluble ester conjugates); stilbenes, lignans, hydroxytyrosols, alkylresorcinols, and extractable hydrolyzable tannins (Perez-Jimenez and Torres, 2011; Saura-Calixto, 2012). Furthermore, these phenolics extracted with chemical solvent based methods have been the focus of studies assessing the in vitro bioactivity of phenolics (Perez-Jimenez and Torres, 2011; Saura-Calixto, 2012).

Non-Extractable Phenolics Remaining after Chemical Solvent Based Extractions

However, a considerable amount of phenolics remain unextracted/unaccounted for in the residues of the chemical solvent extracted materials, and these phenolics are termed "non-extractable phenolics" (Bravo et al., 1994; Perez-Jimenez and Torres, 2011; Saura-Calixto, 2012). Non-extractable phenolics include mainly tannins (non-hydrolzyable/condensed and hydrolyzable) of higher molecular weight (>5000 Da) (Bravo et al., 1994) yet also include lower molecular weight phenolics bound to cell wall polysaccharides and protein (Saura-Calixto, 2012). Different associations responsible for the binding interactions include: hydrogen bonds, hydrophobic interactions, and possible covalent bonds (Bravo et al., 1994; Perez-Jimenez and Torres, 2011). Non-extractable phenolics is a concept wider than the term "bound phenolic" which typically corresponds to insoluble hydrocinnamic and benzoic acids linked by ester bonds to cell walls. These bound phenolics are only a part of non-extractable phenolics (Saura-Calixto, 2012).

Non-extractable phenolics can be found in either a bound form or free form (Bravo et al., 1994; Perez-Jimenez and Torres, 2011). As noted, phenolics can be inherently associated with cell wall polysaccharides (do Socorro M. Ruffino et al., 2010; Pinelo et al., 2006), yet free phenolic compounds can exist in the vacuoles of plants. These phenolics are separated from other cellular components via vacuolar containment and can become associated with plant cell wall polysaccharides after cell disruption as vacuoles can rupture accidentally via injury or deliberately via processing (comminution/particle size reduction) and mastication (Padayachee et al., 2012a, 2012b; Pinelo et al., 2006).

Some researchers have championed the concept of non-extractable phenolics and have noted the importance of non-extractable phenolics and their potential bioactivity (do Socorro M Ruffino et al., 2010; Perez-Jimenez and Saura-Calixto, 2005; Perez-Jimenez

and Torres, 2011; Saura-Calixto et al., 2005; Saura-Calixto, 2012). Literature has noted that treatment of chemical solvent extracted residues with acids (HCl, H_2SO_4), alkali, or with enzymes (cellulases, proteases, digestive enzymes, bacterial enzymes), releases significant amounts of phenolic compounds from the food matrix residue, which can then be analyzed in the corresponding hydrolysates (Perez-Jimenez and Torres, 2011; Saura-Calixto, 2012).

Physiologically Relevant Extractable and Non-Extractable Phenolics

Furthermore, for quantifying phenolics and determining the potential health benefits of phenolics, the extraction of phenolics via a simulated gastrointestinal digestion regime is also of relevance (Bouayed et al., 2011; Gawlik-Dziki et al., 2012; Tagliazucchi et al, 2010). Phenolics released from the food matrix during gastrointestinal digestion can be considered physiologically extractable phenolics and are bioaccessbile and potentially bioavailable (Palafox-Carlos et al., 2011). However, some phenolic compounds are not released during gastrointestinal digestion and can be considered physiologically non-extractable phenolics yet these phenolics may still be physiologically/biologically relevant. These phenolics are typically associated with indigestible cell wall polysaccharides and proteins (to a lesser degree) and are not absorbed at the small intestine yet upon reaching the colon/large intestine, these compounds may be fermented by colonic microflora (Saura-Calixto, 2012; Padayachee et al., 2013). Colonic fermentation produces short-chain fatty acids (SCFA) (acetic, propionic, butyric) from polysaccharides along with nitrogen compounds from protein, and releases lower molecular weight phenolic compounds from the cell wall polysaccharides (Chandrasekara and Shahidi, 2012; Fogliano et al., 2011; Perez-Jimenez and Torres, 2011; Saura-Calixto, 2012). Therefore an important feature of physiologically non-extractable phenolics is bioaccessibility and potential bioavailability in the colon as the fermentation process enhances the intestinal antioxidant status. As such, phenolics associated with cell wall polysaccharides may have an important role in gastrointestinal health (Halliwell et al., 2000). The topic of bioaccessibility and bioavailability of phenolic compounds during gastrointestinal digestion and colonic fermentation will be further discussed in the section entitled: "Bioavailability and Bioaccessibility of Phenolics as Affected by Cell Wall Polysaccharides".

It should be noted here that phenolics remaining associated with cell wall polysaccharides after gastrointestinal digestion may be considered extractable and non-extractable upon treatment with chemical extraction methods. This is of relevance for characterization and quantification of phenolics in food materials. This concept along with a description of chemical and physiologically relevant extraction regimes will be discussed further in the section entitled: "Types of Extraction Regimes for Determination of Extractable and Non-Extractable Phenolics.

Types of Extraction Regimes for Determination of Extractable and Non-Extractable Phenolics

The determination of the phenolic content of foods is essential for studies on diet and health (Saura-Calixto, 2012) as the health benefits of phenolics does depend on their concentration in fruits and vegetables (Palafox-Carlos et al., 2011). Therefore it is important to describe extraction methods used for determining the phenolics content of foods. As noted, extraction regimes for obtaining extractable and non-extractable phenolics can include both chemical methods that use an organic extraction medium and simulated in vitro digestion methods that use a physiologically relevant extraction medium, both of which have been described by Perez-Jimenez and Saura-Calixto (2005) and do Socorro M Rufino et al. (2010).

Chemical Organic Solvent Based Extraction Regime for Obtaining Extractable and Non-Extractable Phenolics

This chemical method used to determine the extractable phenolics as described by Perez-Jimenez and Saura-Calixto (2005) and do Socorro M Rufino et al. (2010) involved hydrolyzing sample (0.5 g) in 20 mL of acidified aqueous methanol (methanol / water /HCl (50:50, v / v; pH 2)) at room temperature for 1 h. The extract was recovered by centrifugation and the resulting supernatant was reserved. The residue was then subjected to extraction with 20 mL of acetone / water (70:30, v / v) at room temperature for 1 h. This extract was recovered by centrifugation and the resulting supernatant was added to the supernatant obtained from acidic aqueous methanolic extraction. This combined extract contained the extractable phenolics obtained from chemical extraction and they

were quantified with the Folin-Ciocalteau procedure of Singleton et al. (1999). The residue of these extractions were divided in two parts and were subjected either to hydrolysis with H_2SO_4 in methanol to obtain hydrolysable tannins content or treatment with butanol /HCl/ $FeCl_3$ to determine condensed tannins content. The hydrolysable tannins and condensed tannins represent the non-extractable phenolics. A method for determining hydrolyzable phenolics content was given by Perez-Jimenez and Saura-Calixto (2005) and Hartzfeld et al. (2002). It was reported that the residue (200mg) was mixed with 20 mL of ethanol and 2 mL of concentrated H_2SO_4 heated at 85 °C for 20 h. The samples were then centrifuged and the supernatant was recovered. This supernatant contained the hydrolysable tannins and the Folin-Ciocalteau method of Singleton et al. (1999) was used to determine the phenolics content of the supernatent.

The condensed tannins were determined by a method reported by Perez-Jimenez and Saura-Calixto (2005) and Reed et al. (1982). It was stated that the residues were treated with HCl/buthanol (5:95, v/v) at 100 °C for 3 h, and the absorbance was read at 538.5 nm to measure the condensed tannins content.

Physiologically Relevant Extraction Regime for Obtaining Extractable and Non-Extractable Phenolics

As noted, extractable and non-extractable phenolics can also be determined from samples subjected to simulated in vitro gastrointestinal digestion. Perez-Jimenez and Saura-Calixto (2005) and do Socorro M. Ruffino (2010) provided the following methods for simulated in vitro gastrointestinal digestion. Sample (900 mg) was incubated with pepsin (0.2 mL of a 300 mg/mL solution in a buffer of 0.2 M HCl-KCl, pH 1.5, 40 °C, 1 h), pancreatin (1 mL of a 5 mg/mL solution in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h), and α-amylase (1 mL of a 120 mg/mL solution in 0.1 M tris-maleate buffer, pH 6.9, 37 °C, 16 h). Then, samples were centrifuged and supernatants were incubated with 100 μL of amyloglucosidase for 45 min at 60 °C. The extractable phenolics were present in the resulting supernatant and were quantified with the Folin-Ciocalteau procedure (Singleton et al., 1999). The residue of this extraction contained the non-extractable phenolics. As described above these were quantified by hydrolysis with H_2SO_4 in methanol to obtain hydrolysable tannins content or treatment with butanol /HCl/ $FeCl_3$ to determine condensed tannins content.

Determining the Extractable and Non-Extractable Phenolics Associated with Plant Polysaccharides

Recognizing the biological importance of phenolics associated with the polysaccharide fraction of fruits, do Socorro M. Ruffino et al. (2010) also presented a protocol for the chemical determination of 1) extractable phenolics associated with the soluble indigestible cell wall polysaccharides obtained from simulated in vitro gastrointestinal digestion of acerola fruit and cashew apple; and 2) the extractable and non-extractable phenolics associated with the insoluble indigestible cell wall polysaccharides obtained from simulated in vitro gastrointestinal digestion of acerola and cashew apple. In order to quantify the extractable phenolics present in the soluble indigestible polysaccharide cell wall residues and the extractable and non-extractable phenolics present in the insoluble indigestible polysaccharide cell wall residues remaining after simulated digestion , these materials were subjected to chemical extraction to determine the extractable and non-extractable phenolics content. Extractable phenolics may still be associated with the insoluble indigestible polysaccharide cell wall residues after simulated gastrointestinal digestion due to a possible strong association with the non-extractable phenolics (Matthews et al., 1997; Perez-Jimenz and Torres, 2011).

To obtain soluble and insoluble indigestible cell wall polysaccharides do Soccoro M. Ruffino et al. (2010) used the simulated in vitro gastrointestinal digestion procedure as described previously. After treatment with amyloglucosidase , the supernatant was transferred to dialysis membranes (12 000–14 000 molecular weight cutoff) and dialysed against water for 48 h at 25 °C to eliminate digestible compounds. The retentate in the dialysis tubing was termed the soluble cell wall polysaccharide fraction while the residue remaining after centrifugation was the insoluble cell wall polysaccharide fraction. The chemical extraction method noted previously was used to determine the extractable phenolics associated with the soluble indigestible cell wall polysaccharide fraction , and the extractable and non-extractable phenolics associated with the insoluble indigestible cell wall polysaccharide fraction.

Quantification of Extractable and Non-Extractable Phenolics as Affected by Extraction Regime

The work of do Socorro M. Ruffino et al. (2010) reported that the extractable and non-extractable

phenolics content of acerola fruits and cashew apples as determined using a chemical extraction method was given on a dry matter basis as approximately 170 mg gallic acid equivalents (GAE)/kg (acerolo-extractable phenolics), 28 mg GAE/kg (cashew apple-extractable phenolics), 4mg GAE/kg (acerola-non-extractable phenolics), and 64 mg GAE/kg (cashew apple-non-extractable phenolics). This work does show that for different fruits the non-extractable phenolics content may be more than the extractable phenolics content

With the aim of determining extractable phenolics content in fruits, Tagliazucchi et al. (2010) performed chemical extractions and simulated in vitro gastrointestinal digestions on grapes while Bouayed et al. (2011) performed chemical extractions and simulated in vitro gastrointestinal digestions on different apple varieties. It was reported that the total (extractable) phenolics content of the grapes, as determined via gastrointestinal digestion, was 87 mg catechin equivalents/100 g grapes and the total (extractable) phenolics content of grapes determined using organic aqueous extractions was 70 mg catechin equivalents/100 g grapes. It was reported that the total (extractable) phenolics content of the different apple varieties as determined via gastrointestinal digestion ranged from 230-370 mg GAE/100 g. The total (extractable) phenolics content of the different apple varieties determined using organic aqueous extractions ranged from 120-180 mg GAE/100 g. In the works of Tagliazucchi et al. (2010) and Bouayed et al. (2011) comparisons were made against chemically extractable phenolics versus extractable phenolics obtained from simulated gastrointestinal digestion, however there was no quantification of non-extractable phenolics. Perez-Jimenez and Saura-Calixto (2005) performed both chemical and simulated in vitro gastrointestinal digestion extractions on a variety of cereal products (wheat flour, bread, raw and boiled rice, wheat bran, and oat bran). Their work found that when acidified aqueous methanol was used as the extraction solvent, the extractable phenolics content ranged from 200-3400 mg GAE per kg sample. The non-extractable phenolic content of the residue remaining after chemical extraction ranged from 2500-16500 mg GAE/kg sample. These workers also performed a simulated in vitro gastrointestinal digestion and reported that the extractable phenolic content of the digestate, ranged from 3000-8600 mg GAE/kg sample while the non-extractable phenolics content of the insoluble residue ranged from 1200-10000 mg GAE/kg sample. Overall, all of these works (Perez-Jimenez and Saura-Calixto, 2005; Bouayed et al., 2011; Tagliazucchi et al., 2010) demonstrated the effect of extraction method of levels of phenolics measured.

The work of do Socorro M. Ruffino et al. (2010) reported that the extractable phenolics contents of the soluble indigestible cell wall polysaccharides from the acerola fruit and cashew apple were 9.6 and 0 mg GAE/kg, respectively while the extractable phenolics content of the insoluble indigestible cell wall polysaccharides from the acerola fruit and cashew apple were 3.6 and 3.9 mg GAE/kg, respectively. The non-extractable phenolics content of the insoluble indigestible cell wall polysaccharides from the acerola and cashew apple were reported to be 3.8 and 104.9 mg GAE/kg, respectively. Also, the work of Saura-Calixto et al. (2007) used both a chemical extraction method and a simulated gastrointestinal digestion on a variety of foods commonly consumed in a Spanish diet (cereals, vegetables, nuts, fruits, legumes, and vegetable oils). These workers focused on characterizing the extractable phenolics associated with the soluble indigestible cell wall polysaccharide portion of the foods consumed along with the extractable and non-extractable phenolics associated with the insoluble indigestible cell wall polysaccharide portion of the food consumed. The goal of their work was to determine the actual intake of total phenolic compounds in a typical Spanish diet and found that the mean dietary intake of phenolic compounds ranged from 2590-3016 mg/person/day. They found that the amount of non-extractable phenolic compounds was nearly two times the amount of extractable phenolic compounds.

All of these works show that use of simple organic aqueous extraction does result in the under-reporting of the phenolics present in food materials and these non-extractable phenolic compounds comprise a significant part of dietary phenolics. Additionally, a simulated digestion extraction regime does result in the determination of different levels of phenolics content and both extractable and non-extractable phenolics are associated with material that has been subjected to simulated digestion studies. Figure 1 shows a representation of the extractable and non-extractable phenolic compounds in food as characterized with chemical and simulated gastrointestinal digestion regimes. Further work is required to determine the content of the non-extractable phenolics of the wide variety of fruits and

vegetables that are important in the human diet as well use of a simulated gastrointestinal digestion extraction regime as this will aid in fuller understanding to the real contribution dietary consumption of phenolic compounds on human health.

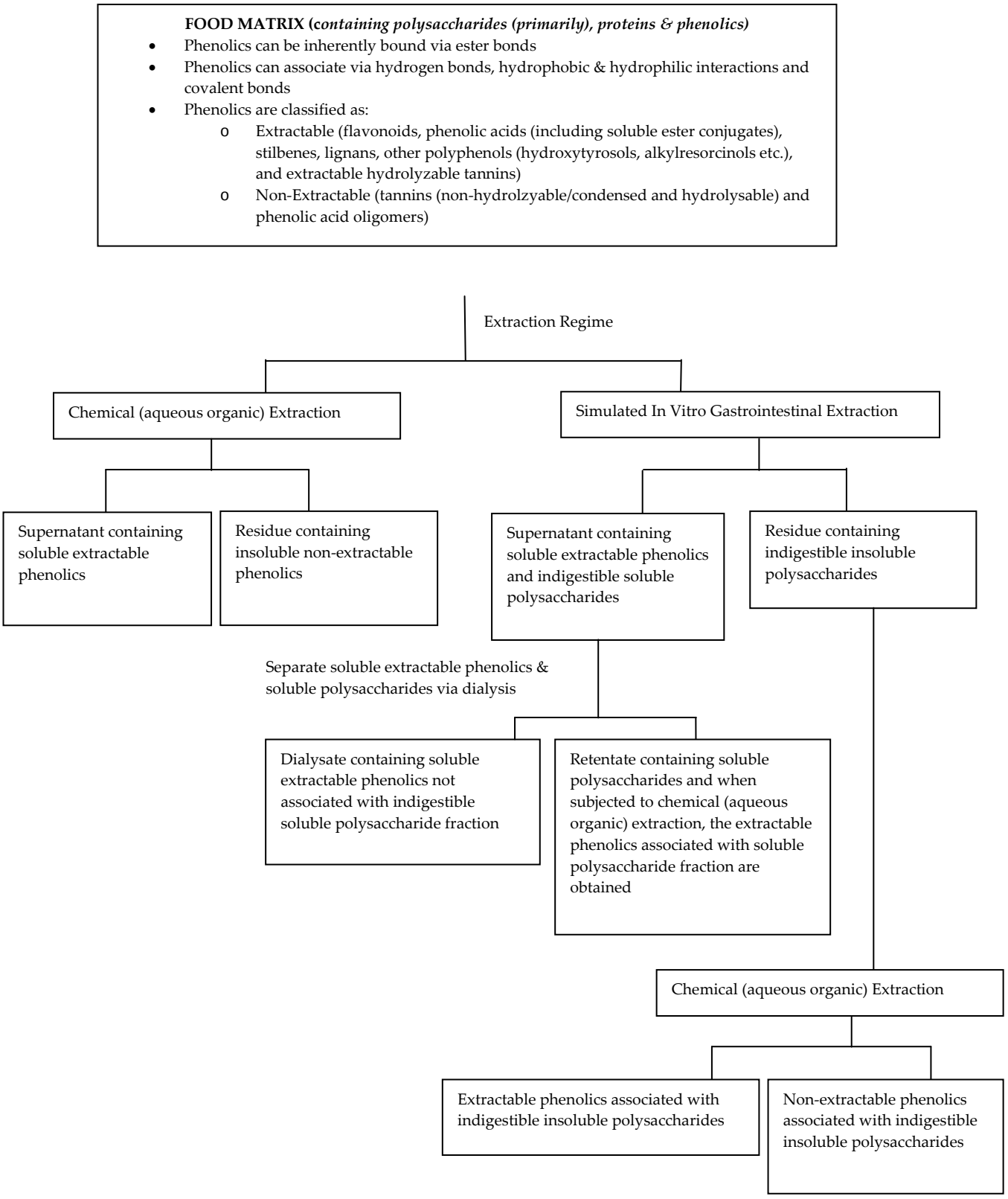

FIG 1. Representation of the Extractable and Non-Extractable Phenolic Compounds in Food as Characterized with Chemical and Simulated Gastrointestinal Digestion Regimes (Adapted from do Socorro M. Ruffino et al., 2010; Perez-Jimenez and Torres, 2011; Sauro-Calixto et al., 2007; Saura-Calixto, 2012)

Cell Wall Polysaccharide-Phenolic Interactions

Plant cell wall polysaccharide-phenolic interactions are complex and is an area that requires continued study in terms of the influence of; different families of phenolic compounds, different species of phenolic compounds, degree of polymeriziation (influences. number of hydroxyl groups and size of molecule), concentration effects (phenolic, polysaccharide), and ratio and type of polysaccharide (cellulose, possibly lignin, and pectin including varying degrees of methyl esterification) (Perez-Jimenez and Torres, 2011). Factors related to the food, such as degree of ripeness, storage conditions, and processing conditions may also influence the degree to which polysaccharide-phenolic interactions occur (Downey et al., 2003; Verries et al., 2008; Perez-Jimenez and Torres, 2011).

The impact of the association of cell wall polysaccharides and phenolic compounds on nutritional and biofunctional properties of fruits and vegetables may be significant (Le Bourvellec et al., 2009; Padayachee et al., 2012a, 2012b). Therefore increased understanding of the bioaccessibility and bioavailability of phenolic compounds during digestion requires the study of cell wall polysaccharide and phenolic interactions. The following sections provide a discussion of work that has performed characterizing interactions between cell wall polysaccharides and procyanidins, anthocyanins and phenolic acids

Interactions of Cell Wall Polysaccharides and Procyanidins

Renard et al. (2001) ; Le Bourvellec et al. (2004); Le Bourvellec & Renard, (2005); and Le Bourvellec et al. (2005) studied apple cell wall polysaccharide- phenolic (procyanidin) interactions. These workers extracted and purified procyanidins from apple material and then resubjected the procyanidins to contact with extracted cell wall polysaccharide material. They found that non-covalent bonds (i.e. hydrogen bonding and hydrophobic binding) were important in polysaccharide-phenolic interactions, whichwere influenced by the specific cell wall polysaccharides (ie. cellulose, hemicellulose, pectin, lignin) present (Padayachee et al., 2012a). Bindon et al. (2010) also indicated that the specific cell wall materials may influence polysaccharide-phenolic interactions. It was reported that cell wall material with a higher proportion of pectin possessed a structure with greater flexibility and thereby allowed for an enhanced surface contact and more phenolic interactions (Perez-Jimenez and Torres, 2011). It has been reported that cell wall material with higher proportions of lignin and cellulose is more rigid and the degree of phenolic interactions is lessened (Bindon et al., 2010; Perez-Jimenez and Torres, 2011). Le Bourvellec et al. (2004; 2005) also established that procyanidins bind to the individual polysaccharide constituents of the plant cell wall at different rates; association with pectin was favoured over cellulose. From their work studying polysaccharide-procyanidin binding in apple pomace Le Bourvellec et al. (2007) found that while an increased concentration of procyanidin enhanced the degree of interaction with plant cell wall polysaccharides, increasing the amount of cell wall material also resulted in an increase in the association of phenolics with cell wall polysaccharide. The molecular weight and degree of polymerization of procyanidins has been shown to enhance binding with plant cell wall polysaccharides (Le Bourvellec et al., 2004; Le Bourvellec and Renard, 2005; Le Bourvellec et al., 2005). The importance of the physical properties of the cell wall polyssacharide matrix has been shown to influence binding of phenolics (Le Bourvelle and Renard, 2005). It has been reported that the food processing operation of drying reduces the number of polysaccharide-phenolic associations due to a reduction of available pores (Perez-Jimenez and Torres, 2011). Le Bourvellec and Renard (2005) reported that apple cell wall polysaccharides with lower porosity levels had demonstrated a lower apparent affinity of binding with procyanidns on a per mass basis. However, if the data were presented on the basis of amount of procyanidins bound per suface area of cell wall polysaccharide, the apparent affinity of binding increased in samples with lower porosity (Le Bourvellec and Renard, 2005). Also, pore size has been reported to have affected binding interactions of cell wall polysaccharides with procyanidins (Padayachee et al., 2012a).

Interactions of Cell Wall Polysaccharide and Anthocyanins and Simple Phenolic Acid

With the aim of understanding the type of binding between polysaccharides and phenolics that may occur upon the release of vacuole contained phenolics upon particle size reduction (processing or mastication), Padayachee et al. (2012a, 2012b) examined the binding of key acylated and non-acylated anthocyanins, and phenolic acids

(chlorogenic acid, caffeic acid and ferulic acid) found in purple carrot (juice) with 1) bacterial cellulose and 2) a model cell wall polysaccharide system (bacterial cellulose-pectin composite containing either a high or low degree of esterification). It should be noted that the cellulose pectin composite with a low degree of esterification possessed a higher pectin content (38.8%) compared to the pectin content (17.1%) of the cellulose pectin composite with a high degree of esterification. Padayachee et al. (2012a) found that anthocyanins interacted with both cellulose and pectin over a two-stage process with initially ~13–18% of anthocyanins binding to cellulose or cellulose/pectin composites on a minutes-hours timescale. On a longer timescale of day-weeks, an increase of anthocyanin binding was observed. At the 12 day timepoint, 83, 41, and 34 % of the anthocyanins were bound to the cellulose-pectin composite containing a low degree of esterified pectin, cellulose-pectin composite containing a high degree of esterified pectin, and pure cellulose, respectively. Acyalation had only a minor effect on binding as the acylated form exhibited slightly (~5–10%) more binding. It was reported that the cellulose-pectin system with the highest pectin content and lowest degree of methyl esterification showed the greatest anthocyanin binding (~80–90%), which suggested existence of both ionic/charge interactions (with pectin) and self-association forming hydrogen bonds and hydrophobic interactions (with cellulose). Padayachee et al. (2012b) also reported that a two stage process was also found to describe the binding process between the phenolic acids and the cellulose material along with the model cellulose-pectin composite. Association of the phenolic acids with pure cellulose and the model cellulose-pectin composite systems was noted within 30s to 1h of contact and slightly greater levels of phenolic acid binding was observed with pure cellulose compared to the model cellulose-pectin composite system, 20% versus 10-15%, respectively. Within the 30s to 1 h timescale the model cellulose-pectin composite system with the highest degree of esterification exhibited the lowest (10%) level of binding of phenolic acids. However after prolonged exposure (day timescale), comparable levels of phenolics binding (~30%) to cellulose and the model cellulose-pectin composite system was observed. On the minutes-hours timescale, the negative charge carried by the pectin in the cellulose-pectin composites was speculated to cause ionic charge repulsion between the phenolic acids and pectins thereby resulting in the decreased rate of interactions between the phenolic acids and the cellulose pectin composites (Padayachhe et al., 2012b)

The extent of binding was influenced by phenolic acid type as caffeic acid showed the highest extent of binding followed by chlorogenic acid then ferulic acid. Although the work of Le Bourvellec et al. (2004), Le Bourvellec and Renard (2005) and Le Bourvellec et al. (2005) noted that higher molecular weight procyandins showed enhanced binding to cell wall polysaccharides, the size of the phenolic acids could not be implicated as the key factor for higher binding of caffeic acid as both caffeic and ferulic acid are of comparable molecular weights, 180 and 194 g/mol, respectively and chlorogenic acid has the highest molecular weight (354 g/mol) (Padayachee et al., 2012b)

In all, based on the findings regarding the interaction of anthocyanins and phenolics acids with the model cellulose-pectin composites , Padayachee et al (2013) proposed that there was an initial rapid deposition of both anthocyanins and phenolic acids with the model polysaccharide cell wall material driven by hydrophobic and hydrogen bond interactions with (primarily) cellulose. Additionally, ionic interactions were implicated in being involved in the binding of anthocyanins and phenolic acids with the pectin component of the cell wall composites. Pectin and the phenolic acids had a partial negative charge and anthocyanins have a positive charge at pH 4 (the pH of purple/black carrots (juice)) used in the work of Padayachhe et al. (2012a, 2012b). It was found that more anthocyanins bound with the more anionic low methoxy pectin cellulose cell wall composite than high methoxy pectin cellulose cell wall composite or cellulose material. Less interactions between the phenolic acids and the low methoxy pectin cellulose cell wall composites were observed and therefore more phenolic acids were initially able to bind to the cellulose and high methoxy composites . These interactions may have consequences in terms of bioaccessibility.

Bioavailability & Bioaccessibility of Phenolics as Affected By Cell Wall Polysaccharides

Bioavailability of Phenolic Compounds

Understanding the role of cell wall polysaccharides on the bioavailability and bioaccessibility of phenolic

compounds is essential to define the health benefits associated with the consumption of phenolic compounds (Manach et al., 2004; Manach et al., 2005), yet it is not an area of research that has received considerable study (Palafox-Carlos et al., 2011). Bioavailability at the small intestine has been a key topic studied with respect to defining the beneficial effects of phenolics (Boayad et al., 2011). The bioavailability of a dietary compound is dependent upon its release from the food matrix (bio-accessibility), its stability, and the efficiency of its absorption at the small intestinal epithelium (Shimizu, 2010; Tagliazucchi et al., 2010). Both type of phenolic compound and source of the compound have been shown to affect bioavailability at the small intestine (Manach, et al., 2005). Some phenolic compounds, such as anthocyanins, have been reported to possess low bioavailabiity which has been attributed to the high instability of these molecules in the mild alkaline condition of the small intestine (Bouayed et al., 2011). Furthermore, the hydrophilic nature and chemical structure (most phenolic compounds are present in foods as esters, glycosides or polymers that cannot be absorbed in their native form) are factors that contribute to the low absorption of phenolic compounds at the small intestine (Bouayed et al., 2011; Manach et al., 2005). However, it should be noted that regardless of their bioavailability/uptake at the small intestine, phenolic compounds have been reported to provide health benefits directly to the gastrointestinal tracts via prevention of oxidative damage (i.e. antioxidant activity) and cancer (Halliwell et al., 2000). Phenolic compounds that are not absorbed by the small intestine and therefore present in plasma at low concentrations may be present in the gastrointestinal tract at much greater concentrations after ingestion. In this respect , bioaccessibility and stability under gastrointestinal conditions are key factors in determining the potential beneficial effects of phenolic compounds on gastroinestinal epithelial cells (Tagliazucchi et al., 2010).

Bioaccessibility of Phenolic Compounds

Some researchers have studied the effect of simulated digestion on the bioaccessibility of phenolics. The work of Cilla et al. (2009) reported that the phenolic content (extractable) of grape–orange–apricot fruit juice decreased by 47% after being subjected to simulated in vitro gastrointestinal. Tagliazucchi et al. (2010) reported that only 62% of the phenolic compounds (extractable) initially measured in grapes were bioaccessible following gastrointestinal digestion. Bouayed et al. (2011) used an in vitro model that simulated gastrointestinal digestion to investigate the bioaccessibility of phenolics in different apple varieties. These workers also incorporated a dialysability aspect to their study, which served as a simplified model of the small intestinal epithelial barrier, this assessed free soluble phenolics obtained from gastrointestinal digestion of apples and provided an indication of the potential bioavailability of apple phenolics after gastrointestinal digestion. Their work showed that release of phenolics, which was indicative of bioaccessibility, was mainly achieved during the gastric phase with about 65% of phenolics (extractable) being released and a smaller further release (<10%) during intestinal digestion was noted. The dialysis aspect of the experiment showed that free soluble dialysable phenolics were approximately 20% lower than that of the phenolics content (extractable) of gastrointestinal digestate. Bouayed et al. (2010) suggested that some of the non-dialysable phenolics should be bound to macromolecular compounds and concentrations in final digesta would overestimate polyphenol bioavailability in terms of uptake at the small intestine. Nevertheless, these phenolic compounds are bioaccessible and may be of relevance as they may provide health benefits directly to the epithelial cells of gastrointestinal tract and the colon via prevention of oxidative damage (i.e. antioxidant activity) and cancer.

Influence of Simulated Digestion on Cell Wall Polysaccharides and Potential Impacts on Phenolics Bioaccessibility

The influence of the chemical and structural features of polysaccharides may influence the bioaccessibility and bioavailability of phenolics. The physico-chemical properties of plant cell wall polysaccharides in the gastrointestinal tract (including the colon) are important in modulating processes that influence health (Mishra and Monro, 2012). An important physiological effect of cell wall polysaccharides (i.e. dietary fibre) in the small intestine of the gastrointestinal tract is the reduction of the rate and the extent of release of nutrients which is accomplished by (1) physical entrapment of nutrients and (2) increased viscosity of gastric fluids that limits the peristaltic mixing process that allows for transport of enzymes to their substrates, bile salts to unmicellized fat, and soluble nutrients to the epithelial cells of the small intestine along with inhibition of

diffusion of nutrients (Palafox-Carlos et al., 2011). Mishra and Monro (2012) investigated the physicochemical properties of kiwifruit cell wall polysaccharides subjected to simulated in vitro gastrointestinal digestion. Soluble and insoluble indigestible polysaccharide fractions were isolated and the hydration properties and the capacity to retard diffusion and mixing in a simulated small intestinal segment were determined. The indigestible polysaccharides from kiwifruit had strong water retention and swelling capacities, and retarded both glucose diffusion and mixing significantly. These researchers concluded that the indigestible cell wall polysaccharides of kiwifruit showed substantial potential to influence the properties of gastrointestinal contents. From this work, it is likely that indigestible cell wall polysaccharides influence the diffusion/transfer of phenolic compounds in the gastrointestinal tract.

Further, it has typically been assumed that the polysaccharides of plant cell are unaltered by gastrointestinal digestion, however, minor chemical or structural changes in polysaccharides can substantially change properties that determine their impact on health yet which has not been well studied (Carnachan et al., 2012). Recently, Carnachan et al. (2012) studied whether chemical or structural changes occurring in kiwi fruit cell wall polysaccharides after simulated in vitro gastrointestinal digestion. Their work showed that the chemical composition and structure of the indigestible cell wall polysaccharides remained largely unchanged. However, the degree of methylesterification of galacturonic acid residues present in the pectin-rich indigestible soluble polysaccharide material decreased after gastrointestinal digestion. There was also an observed decrease in the molecular weight of the higher molecular weight fraction of the indigestible soluble polysaccharide material and an increase in the lower molecular weight fraction of the indigestible soluble polysaccharide material. It was noted by Carnachan et al. (2012) that these changes may affect the physicochemical properties and fermentability of kiwifruit polysaccharides/dietary fibre in the gastrointestinal tract including the colon/large intestine, which may affect the bioaccessibility of the phenolic compounds and degree of association with the polysaccharide material.

With respect to bioaccessibility of phenolics as influenced by polysaccharide interactions, Palafox-Carlos et al. (2011) indicated that the type of chemical interactions between phenolic compounds and cell wall polysaccharides included: non-covalent bonds between hydroxide groups from phenolic compounds and polar groups from polysaccharide molecules (hydrogen bonds, electrostatic and dipolar interactions, van der Waals attractions); and these interactions were relatively instable and their formation and disruption were affected by small changes in pH or solvent quality of the gastrointestinal tract (i.e. the type and concentration of dissolved solids in the digesta).

As noted, Padayachee et al. (2012a, 2012b) studied the binding of anthoycanins and phenolic acids on model cell wall polysaccharide composites and subsequently studied the release of bound anthocyanins and phenolic acids from model cell wall polysaccharide composites and carrot plant cell walls during simulated gastric and small intestinal digestion (Padayachee et al., 2013). Upon examining the release of these phenolics during simulated in vitro gastrointestinal digestion , these researchers found that out of the anthocyanins and phenolic acids that were initially bound to either the model plant cell wall polysaccharide system consisting of a bacterial cellulose-pectin compostie or purple/black carrot puree cell wall material, less than 2% was released during either gastric or small intestinal digestion showing that the amounts of polyphenols released under the simulated in vitro gastrointestinal conditions were low and that the majority of anthocyanins and phenolics acids bound to cell wall polysaccharides remained bound. Of the phenolics that were released, ~2% more acylated anthocyanins were released than non-acylated anthocyanins.

An effect of contact time on cell wall polysaccharides and phenolic compounds interactions was noted as there was slightly more anthocyanin released from the the model cellulose-pectin composites subjected to contact with phenolics for a short time (up to 24 h), but this progressively decreased for the longer contact time samples (Padayachee et al., 2013). The release of phenolic acids from the model cellulose material and cellulose-pectin composites was affected by the composition of the model cell wall material. Up to 30% of total phenolic acids were released after gastric digestion for the cellulose-pectin composites and only about 10% of bound phenolic acids were released from pure cellulose (Padayachee et al., 2013). Also, Padayachee et al. (2013) reported that the amount of

anthocyanins and phenolic acids released after in vitro gastric digestion (simulated stomach digestion) was greater than the amount released after sequential gastric and small intestinal digestion suggesting that anthocyanins and particularly phenolic acids released during gastric digestion could be re-adsorbed onto the cell wall material under small intestinal conditions.

In all, Padayachee et al. (2013) reported minimal release of anthocyanins and phenolic acids after simulated gastrointestinal digestion, indicating that phenolic compounds bound to plant cell wall polysaccharides would be transported to the colon where they were expected to be released by the action of cell wall degrading bacteria, which was a relevant area of future research.

Conclusions

Improving the understanding of the real contribution of phenolic compounds from fruits and vegetables consumption to human health is an important area of research. Non-extractable phenolics (i.e. phenolics not extracted with conventional organic aqueous solvents) are a major part of phenolic compounds and further studies to determine the non-extractable phenolic contents of foods are necessary to create a data base that reflects the true phenolics content of foods. Some researchers have studied the nature of interactions between cell wall polysaccharides and phenolic compounds and have indicated that the type of chemical interactions between phenolic compounds and cell wall polysaccharides includes non-covalent bonds between hydroxide groups from phenolic compounds and polar groups from polysaccharide molecules (hydrogen bonds, electrostatic and dipolar interactions, van der Waals attractions); and these interactions are relatively instable and their formation and disruption are affected by small changes in pH or solvent quality of the gastrointestinal tract. As such, polysaccharide-phenolic interactions which are complex requires continued study to provide greater insight into the true contribution of consumption of dietary phenolics human health. Additionally, determining the bioaccessibility and bioavailability of phenolic compounds present in fruits and vegetables upon simulated gastrointestinal digestion is an important area of food research. Even if phenolic compounds are released and are available during digestion, these phenolic compounds may bind to polysaccharides at some point within the gastrointestinal tract which may influence bioavailability. Moreover, it has been reported that the majority of phenolics in fruits or vegetables that bind strongly to the cell wall polysaccharides show minimal release during simulated gastric and small intestinal digestion, indicating that significant levels of phenolics may be delivered to the colon with the potential to exert a positive health benefits. An aspect of future work should include examining the effect that phenolics bound to plant cell wall polysaccharides have on colonic bacteria in terms of the metabolites produced and the mode of absorption from the colon.

REFERENCES

Adnan, L., Osman, A., and Hamid, AA. "Antioxidant Activity of Different Extracts of Red Pitaya (*Hylocereus polyrhizus*) Seed." International Journal of Food Properties 14 (2011): 1171-1181.

Bindon, KA., Smith, PA., Holt, H., and Kennedy, JA. "Interaction Between Grape-Derived Proanthocyanidins and Cell Wall Material. 2. Implications for Vinification." Journal of Agricultural and Food Chemistry 58 (2010): 10736–10746.

Bouayed, J., Hoffman, L., and Bohn, T. "Total Phenolics, Flavonoids, Anthocyanins, and Antioxidant Activity Following Simulated Gastro-Intestinal Digestion and Dialysis of Apple Varieties: Bioaccessibility and Potential Uptake." Food Chemistry 128 (2011): 14-21.

Bravo ,L., Abia, R., and Saura-Calixto, F. 1994. "Polyphenols as Dietary Fiber Associated Compounds. Comparative Study on In Vivo And In Vitro Properties." Journal of Agricultural and Food Chemistry 42 (1994): 1481–1487.

Carnachan, SM., Bootten, TJ., Mishra, S., Monro, JA., and Sims, I.M. "Effects of Simulated Digestion *In Vitro* on Cell Wall Polysaccharides from Kiwifruit (Actinidia spp.)." Food Chemistry 133 (2012): 132-139.

Chandrasekara, A. and Shahidi, F. "Bioaccessibility and Antioxidant Potential of Millet Grain Phenolics as Affected by Simulated In Vitro Digestion and Microbial Fermentation." Journal of Functional Foods 4 (2012): 226-237.

Cilla, A., González-Sarrías, A., Tomás-Barberán, FA., Espín, JC., and Barberá, R. "Availability of Polyphenols in Fruit Beverages Subjected to In Vitro Gastrointestinal Digestion and their Effects on Proliferation, Cell-Cycle and Apoptosis in Human Colon Cancer Caco-2 Cells."

Food Chemistry 114 (2009): 813–820.

Crozier, A., Jaganath, I.B., and Clifford, M.N. "Phenols, Polyphenols and Tannins: An Overview." In Plant Secondary Metabolites Occurrence, Structure and Role in the Human Diet. Edited by A. Crozier, M.N. Clifford, and H. Ashihara, 1-24. Oxford, UK: Blackwell Publishing Ltd, 2006.

Denny, A. and Buttriss, J. "Synthesis Report No 4-Plant Foods and Health: Focus on Plant Bioactives." In European Food Information Resource (EuroFIR) Consortium, 2005.

do Socorro M. Ruffino, M., Perez-Jimenez, J., Tabernero, M., Aleves, RE., and de Brito, ES. "Acerola and Cashew Apple as Sources of Antioxidants and Dietary Fibre." International Journal of Food Science and Technology 45 (2010): 2227-2233.

Downey, MO., Harvey, JS., and Robinson, SP. "Analysis of Tannins in Seeds and Skins of Shiraz Grapes Throughout Berry Development." Australian Journal of Grape Wine Research 9 (2003): 15–27.

Fogliano, V., Corollaro, ML., Vitaglione, P., Napolitano, A., Ferracane, R., Travaglia, F., Alorio, M., Costabile, A., Klinder, A. and Gibson, G. "In Vitro Bioaccessibility and Gut Transformation of Polyphenols Present in the Water-Insoluble Cocoa Fraction." Molecular Nutrition Food Research 55 (2011): S44-S55.

Gawlik-Dziki, U., Jezyna, M., Swieca, M., Dziki, D., Baraniak, B., and Czyz, J. "Effect of Bioaccessibility of Phenolic Compounds on In Vitro Anticancer Activity of Broccoli Sprouts." Food Research International 49 (2012):469-476.

Halliwell, B., Zhao, K., and Whiteman, M. (2000). "The Gastrointestinal Tract: A Major Site of Antioxidant Activity. Free Radical Research." 33 (2000): 819-830.

Hedron, E., Diaz, V."Estimation of Cartenoid Accessibiility from Carrots Determined by an *In Vitro* Digestion Method." European Journal of Clinical Nutrition 56 (2002): 425-430.

Herken, EN., and Guzel, S. "Total Antioxidant Capacity and Total Phenol Contents of Selected Commercial Fruit Juices." International Journal of Food Properties 13 (2010): 1373-1379.

Kulp, KS., Fortson, SL. Knize, MG., Felton, JS. "An In Vitro Model System to Predict the Bioaccessibility of Heterocyclic Amines from a Cooked Meat Matrix." Food and Chemical Toxicology 41 (2003): 1701–1710.

Le Bourvellec, and Renard, CMGC. "Non-Covalent Interaction between Procyanidins and Apple Cell Wall Material. Part II: Quantification and Impact of Cell Wall Drying." Biochimica et Biophysica Acta 1725 (2005): 1-9.

Le Bourvellec, C., Bouchet, B., and Renard, CMGC. "Non-Covalent Interaction between Procyanidins and Apple Cell Wall Material. Part III: Study on Model Polysaccarides." Biochimica et Biophysica Acta 1725 (2005): 10-18.

Le Bourvellec, C., Guyot, S., and Renard, CMGC. "Interactions between Apple (*Malus x domestica Borkh.*) Polyphenols and Cell Walls Modulate the Extractability of Polysaccharides." Carbohydrate Polymers 75 (2009): 251-261.

Le Bourvellec, C., Guyot, S., and Renard, CMGC. "Non-Covalent Interaction between Procyanidins and Apple Cell Wall Material. Part I. Effect of Some Environmental Parameters." Biochimica et Biophysica Acta 1672 (2004): 192-202.

Le Bourvellec, C., Le Quere, JM., and Renard, CMGC. "Impact of Noncovalent Interactions Between Apple Condensed Tannins and Cell Walls on their Transfer from Fruit to Juice: Studies in Model Suspensions and Applications." Journal of Agricultural and Food Chemistry 55 (2007): 7896-7904.

Manach, C., Scalbert, A., Morand, C., Remesy, C., Jimenez, L. "Polyphenols: Food Sources and Bioavailability." American Journal of Clinical Nutrition 79 (2004): 727–747.

Manach, C., Williamson, G., Morand, C., Scalbert, A., and Rémésy, C. "Bioavailability and Bioefficacy of Polyphenols in Humans. I. Review of 97 Bioavailability Studies." American Journal of Clinical Nutrition 81 (2005): 230s-242s.

Mathews, S., Mila, I., Scalbert, A., and Donnelly, DMX. "Extractable and Non-Extractable Proanthocyanidins in Barks." Phytochemistry 45 (1997): 405–410.

Mishra, S. and Monro, J. "Kiwifruit remnants from digestion in vitro have functional attributes of potential importance to health." Food Chemistry 135 (2012): 2188-

2194.

Ovodova, RG., Golovchenko, VV., Popov, SV., Popova, G Y., Paderin, NM., Shashkov, AS., Ovodov, YS. "Chemical Composition and Anti-Inflammatory Activity of Pectic Polysaccharide Isolated from Celery Stalks." Food Chemistry 114 (2009): 610-615.

Padayachee, A., Netzel, G., Netzel, M., Day, L., Mikkelsen, D., and Gidley, MJ. "Lack of Release of Bound Anthocyanins and Phenolic Acids from Carrot Plant Cell Walls and Model Composites During Simulated Gastric and Small Intestinal Digestion." Food and Function 4 (2013): 906-916.

Padayachee, A., Netzel, G., Netzel, M., Day, L., Zabaras, D., Mikkelsen, D., Gidley, MJ. "Binding of Polyphenols to Plant Cell Wall Analogues-Part 1: Anthocyanins." Food Chemistry 134 (2012a): 155-161.

Padayachee, A., Netzel, G., Netzel, M., Day, L., Zabaras, D., Mikkelsen, D., Gidley, MJ. (2012b). "Binding of Polyphenols to Plant Cell Wall Analogues-Part 2: Phenolic Acids." Food Chemistry 135 (2012b): 2287-2292.

Palafox-Carlos, H., Ayala-Zavala, JF., Gonzalez-Aguilar, G.A. "The Role of Dietary Fiber in the Bioaccessibility and Bioavailability of Fruit and Vegetable Antioxidants." Journal of Food Science 76 (2011): R6-R15.

Parada, J. and Aguilera, JM. "Food Microstructure Affects the Bioavailability of Several Nutrients." Journal of Food Science 72 (2007): R21-R32.

Pérez-Jiménez, J., and Saura-Calixto, F. "Literature Data May Underestimate the Actual Antioxidant Capacity of Cereals. Journal of Agricultural and Food Chemistry 53 (2005): 5036−5040.

Pérez-Jiménez, J., and Torres, JL. "Analysis of Nonextractable Phenolic Compoundsin Foods: The Current State of the Art." Agricultural and Food Chemistry 59 (2011): 12713-12724.

Pinelo, M., Arnous, A., and Meyer, AS. "Upgrading of Grape Skins: Significance of Plant Cell-Wall Structural Components and Extraction Techniques for Phenol Release." Trends in Food Science and Technology 17 (2006): 579-590.

Popov, SV., Ovodova, RG., Golovchenko, VV., Popova, GY., Viatyasev, FV., Shashkov, AS., and Ovodov, YS. "Chemical Composition and Anti-Inflammatory Activity of a Pectic Polysaccharide Isolated from Sweet Pepper Using a Simulated Gastric Medium." Food Chemistry 124 (2011): 309-315.

Renard, C M G C., Baron, A., Guyot, S., and Drilleau, JF. "Interactions Between Apple Cell Walls and Native Apple Polyphenols: Quantification and Some Consequences." 29 (2001): 115-125.

Ross, KA., Mazza, G. "Conjugated Polysaccharides as Potential Health Promoting Compounds in Berries and Cherries. In Berries: Properties, Consumption and Health Benefits, edited by C. Tuberoso, 55-81. New York: Nova Science Publishers Inc, 2012.

Ross, KA., Siow, Y., Brown, D., Isaak, C., Fukumoto, L., and Godfrey, D. "Characterization of Water Extractable Crude Polysaccharides from Cherry, Raspberry, and Ginseng Berry Fruits: Chemical Composition and Bioactivity." International Journal of Food Properties, in press.

Saura-Calixto, F. "Concept and Health Related Properties of Non-extractable Polyphenols: The Missing Dietary Polyphenols." Journal of Agricultural and Food Chemistry 60 (2012): 11195-11200.

Saura-Calixto, F., Serrano, J., Goni, I. "Intake and Bioaccessibility of Total Polyphenols in a Whole Diet." Food Chemistry 101 (2007): 492-501.

Shimizu, M. "Interaction between Food Substances and the Intestinal Epithelium." Bioscience Biotechnology and Biochemistry 74 (2010): 232-241.

Singleton, VL., and Rossi, JA. "Colorimetry of Total Phenolics with Phosphomolybdic–Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture." 16 (1965): 144-158.

Tagliazucchi D, Verzelloni E, Bertolini D, and Conte A. " In Vitro Bio-Accessibility and Antioxidant Activity of Grape Polyphenols." Food Chemistry 120 (2009): 599–606.

Verries, C., Guiraud, JL., Souquet, JM., Vialet, S., Terrier, N., and Olle, D. "Validation of an Extraction Method on Whole Pericarp of Grape Berry (*Vitis vinifera* L. Cv. Shiraz) to Study Biochemical And Molecular Aspects of Flavan-3-ol Synthesis During Berry Development." Journal of Agricultural and Food Chemistry 56 (2008): 5896–5904.

Kelly A. Ross, Ph.D. was born in Winnpeg, MB, Canada. Dr. Ross joined Agriculture and Agri-Food Canada (AAFC) as a

Research Scientist in October 2008. Dr. Ross received her Ph.D. from the Department of Food Science at Purdue University. Prior to joining AAFC, at the Department of Food Science and the Department of Agricultural and Biological Engineering at the University of Manitoba, Dr. Ross performed post-doctoral research assessing levels of bioactives in pulses, evaluating chemical components of pulses, and determining how storage and processing conditions affected chemical changes and their influence on product quality. Dr. Ross' research is currently focussed on isolating and characterizing the polysaccharides present in tree fruits, such as cherries and apples along with berries and ginseng. Ongoing work is concerned with defining the relationships between the chemical composition, and structure of the polysaccharides and bioactivities. Dr. Ross' research is striving to provide fundamental knowledge of production and processing practices that affect the nutritional attributes of foods, including impact of food structure on nutrient release (i.e. the importance of bioaccessability). Dr. Ross' most significant research contributions include: (1) isolation and characterization of bioactive compounds from crops and biomass; (2) employing principles from chemistry, engineering, and polymer science to solve food structure function problems in order to attain high quality, health promoting, and safe value-added products for new market opportunities; and developing and optimizing green technologies for bioenergy and bioproducts production.