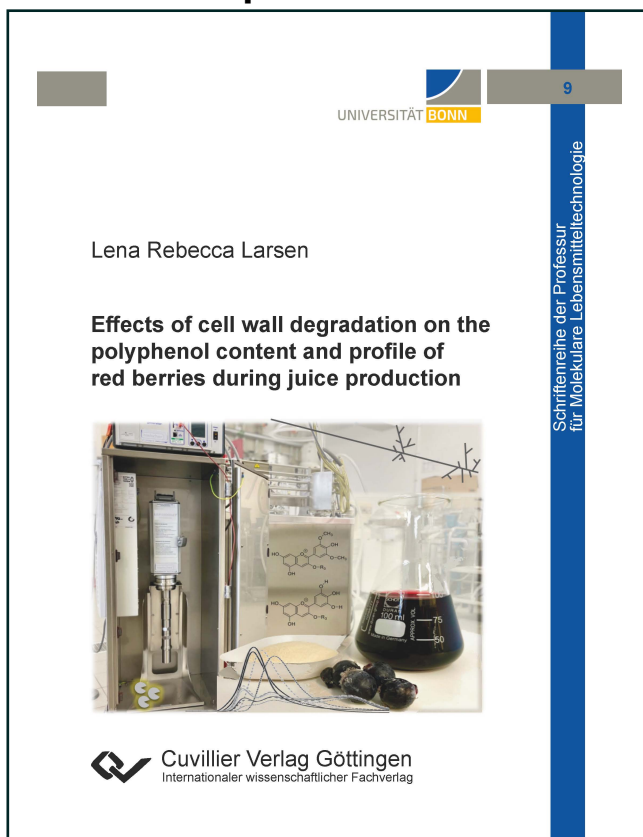




Lena Rebecca Larsen (Autor)

# Effects of cell wall degradation on the polyphenol content and profile of red berries during juice production



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Telefon: +49 (0)551 54724-0, E-Mail: [info@cuvillier.de](mailto:info@cuvillier.de), Website: <https://cuvillier.de>

# Chapter 1

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## General Introduction

### 1 Red juice as functional foods

We live in an age of significant technological changes, with tangible improvements in every part of life, such as in the food segment. Today, food products are high in quality and microbial safety, rich in aroma and color, and have long shelf lives. Lately, the nutritional value of food has shifted in the focus of the consumers' interests. Nowadays, food should not only be merely satiating, but also has additional functional features. This has inspired academia and the food industry to research for foods with beneficial nutritional values and preferably mild production technologies.

Red berries and their derived juices have gained great popularity due to the growing evidence of health-promoting potential and their appealing taste and color. Scientists are primarily holding the high contents of the phenolic flavonoids, in particular, the red to purple colored anthocyanins responsible for these beneficial qualities.

Besides red berries and their derived products, flavonoids are abundantly spread throughout foods consumed by humans such as fruits, vegetables, nuts, seeds, herbs, spices, and whole grains. Similarly, manufactured products originating from plant sources contain high amounts of polyphenols, e.g., a glass of juice, a cup of coffee, or tee includes about 100 mg polyphenols (Scalbert et al. 2005; Spencer et al. 2008). Flavonoids are synthesized by all vascular plants, as they are responsible for essential functions like natural attractants, repellents, or protection against UV light since they often possess colored and aromatic properties (Pandey and Rizvi 2009). With approximately 10,000 different compounds identified in plants so far, flavonoids represent the most prevalent

phenolic groups, while the list is constantly growing (Birt and Jeffery 2013; Cheynier et al. 2013).

In the last decade, a growing number of epidemiological studies and associated meta-analyses concluded that a polyphenol-enriched long-term diet goes along with protection against the development of cancer, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases. The potential health benefits seem to be countless; in fact, many positive effects are still under investigation and need to be proven in clinical studies (Pandey and Rizvi 2009). Until now, anti-cancerogenic, anti-inflammatory, and immunomodulating properties have been postulated (Arts and Hollman 2005). The flavonoid intake from berries, in particular, demonstrated positive effects against diabetes and slowing of neurodegenerative diseases (Devore et al. 2012).

The health-related effects of polyphenols assessed by their bioaccessibility, bioavailability, and bioactivity not only depend on their structure and quantity but also on their intermolecular interactions with other food matrix compounds. Jakobek (2015) emphasized the significant role of dietary fiber composed of plant cell wall polysaccharides by directly modulating the bioaccessibility and bioavailability of polyphenols and indirectly influencing their nutritional and potential health benefits.

The interaction between polyphenols and plant cell wall polysaccharides occurs spontaneously after the disruption of plant tissue; thus, the naturally separated compounds get into contact. This process happens either during consumption, starting by simple chewing, and continues inside the human digestive tract or occurs under the food processing conditions of several common foods such as fruit juice, purees, jams, or wine.

The complexation is assumed to stabilize polyphenols against degradation processes, which bears two advantages. From a nutritional point of view, complexes are supposed to reach the colonic tract since fibers are only metabolized by the microbiota of the large intestine. Here, they provide a beneficial prebiotic effect and an antioxidant environment towards microbiota in the gastrointestinal tract and an antimicrobial activity against pathogenic bacteria (Molan et al. 2009; Puupponen-Pimiä et al. 2005). From a food technology perspective, complexes enhance the stability of food products like juices

regarding taste and color since these are strongly determined by polyphenols. In general, polyphenols significantly contribute to the bitterness, astringency, color, and oxidative stability in food.

Thus, there is a growing awareness from manufacturers' and academia's perspectives of the importance of polyphenol-polysaccharide complexes providing bio- and techno-functional properties, which are particularly important in the red juice sector (Liu et al. 2020; Phan et al. 2017).

This dissertation reflects upon polyphenol-polysaccharide complexation during red juice production, taking a closer look at the cell wall degradation during the maceration process and the corresponding complexation of pectin and anthocyanins, the two main representatives of plants cell wall polysaccharides and polyphenols in red berries. Several novel technologies are part of recent research aiming at gentle process conditions to produce high-quality products rich in natural components and with less undesired process-induced alterations compared to common procedures. As a promising technology, ultrasonication is discussed in the present dissertation to sustainably improve fruit juice production (**Section 4**).

## **2 Red berry fruits**

Red berries providing high amounts of flavonoids are growing in popularity due to their appealing color and sweet taste accompanied by promising health-promoting properties. Berries are consumed fresh or processed into juices, concentrates, purees or jams. Juices (concentrates) and frozen berries are used as food ingredients and natural colorants.

Botanically, a berry is a simple fleshy indehiscent fruit derived from a single ovary of an individual flower. A berry usually contains more than one seed, has a pulpy endocarp and a thin skin or exocarp. Typical red berries are cranberry (*Vaccinium macrocarpon* L.), bilberry (*Vaccinium myrtillus* L.), and black currant (*Ribes nigrum* L.). Botanical berries also include tomatoes, eggplants, and guavas. In common parlance, several small, round, and fleshy fruits are also termed berries, e.g., elderberry (*Sambucus nigra* L.), chokeberry (*Aronia melanocarpa* Michx.), raspberry (*Rubus idaeus* L.), and blackberry (*Rubus* sect. *Rubus*). However, in the botanical sense, they mostly belong to stone fruits or aggregate

fruits consisting of several smaller fruits. Due to their transport sensitivity and short shelf life, these berries are also defined as soft fruits in food traits.

Berries appear in attractive orange, red, violet, purple, and blue colors like many flowers, fruits, and vegetables. This is the result of the presence of anthocyanins, which account for one of the six flavonoid subclasses of the polyphenol family. These water-soluble pigments, along with other polyphenols, are enclosed in the vacuoles mainly of the deeply colored skin, encapsulated by tonoplast and cytoplasmic lipid membranes of the plant cell wall (Padayachee et al. 2012).

The highest content in total anthocyanin concentration among berries was found in chokeberries (1480 mg/100 g fresh weight) and elderberries (1374.4 mg/100 mg fresh weight) (Wu and Prior 2005). Each berry provides a specific anthocyanin profile that may be consulted for authentication and chemotaxonomic investigations, like a fingerprint pattern. However, it needs to be considered that the anthocyanin profile may be altered depending on the plant's environmental conditions, the processing of the fruit, and the extraction method (Heffels et al. 2015; 2017). Additionally, anthocyanin type and structure show variable stability, which will be introduced in more detail below.

Next to anthocyanins, berries are a rich source of other phenolic compounds like phenolic acids and flavanols, which also bear high antioxidant activities and impart health properties. Well-known are the proanthocyanidins, also referred to as condensed tannins, consisting of polymerized derivatives of flavanols. These are responsible for the astringent taste that occurs due to the complexation of human saliva proteins.

Besides phenolic compounds, berries are a rich source of nutritive compounds like sugars, vitamins, and minerals, all entrapped in the dietary fiber of the cell wall matrix consisting of cellulose, hemicellulose, pectin, lignin, and cutin-like polymers. Soluble solids mainly include sugars with a high proportion of fructose, providing them as valuable for individuals with diabetes diseases.

The total fiber content of berries is relatively high (bilberries 4.9 g/100 g, blackberries 3.2 g/100 g, blackcurrants 3.5 g/100 g, raspberries 4.7 g/100 g) compared to other fruits like apples (2.3 g/100 g), and bananas (2.0 g/100 g) (GMF 2002). Pectin, as the main part of the soluble fibers, undergoes nearly complete fermentation by colonic microflora and

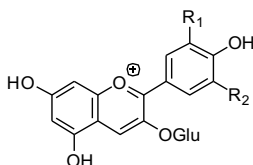
leads to the generation of short-chain fatty acids, which lower the colonic pH and prevent colon cancer (Moore et al. 1998). It also has the potential to lower blood cholesterol levels and affect glucose metabolism. Many studies have suggested potential beneficial effects on cholesterolemia and diabetes, the risk for diverticulosis, colon cancer, and coronary heart disease (Voragen et al. 2009). Insoluble fibers like cellulose are not fermented but are essential for intestinal transition activities.

In summary, red berry fruits have been classified as superfoods due to the amount of health-promoting compounds they contain, especially anthocyanins. However, during fruit processing and consumption, these compounds are degraded and affected by oxidation and complexation reactions, as discussed in **Section 3**.

## 2.1 Anthocyanins

Anthocyanins naturally occur in their glycoside form, where the aglycon, called anthocyanidin, is linked to a sugar moiety. Until now, >500 different anthocyanins and 23 different anthocyanidins have been identified. The six most common anthocyanidins are listed in **Table 1-1** in their 3-glucoside form. Their distribution in fruits and vegetables was examined as: cyanidin 30%, delphinidin 22%, pelargonidin 18%, peonidin 7.5%, malvidin 7.5%, and petunidin 5% (Andersen and Jordheim 2005).

The main structural differences are the number and position of hydroxy and methoxy groups in the B-ring, the nature and number of linked sugars, and the aliphatic and aromatic acids attached to the sugar moieties. Glycosylation increases their water solubility. The most common monosaccharides attached primarily at C3 are glucose, rhamnose, galactose, arabinose, and xylose (in the same order of frequency as mentioned). The structure and substitution variety results in broad shades of colors for anthocyanins due to variances in the absorption intensity (hyperchromic effect) and its wavelength (bathochromic/hypsochromic shifts).

**Table 1-1:** Chemical structure of anthocyanins

Anthocyanin	R <sub>1</sub>	R <sub>2</sub>
Perlagonidin-3-glucoside	H	H
Cyanidin-3-glucoside	OH	H
Delphinidin-3-glucoside	OH	OH
Peonidin-3-glucoside	OCH <sub>3</sub>	H
Petunidin-3-glucoside	OCH <sub>3</sub>	OH
Malvidin-3-glucoside	OCH <sub>3</sub>	OCH <sub>3</sub>

The structure and color of anthocyanins depend on a pH-dependent equilibrium reaction. In acidic milieu (pH <2), the red flavylium cation is predominant, occurring primarily with a chloride counterion. With increasing pH, deprotonation occurs in two steps (pK<sub>a</sub> 4 and 7) and the equilibrium shifts via the purple, neutral quinonoid base to the bluish, anionic quinonoid base. Since the quinoid species are thermodynamically unstable, hydration of the flavylium cation at C2 is more likely. Above pH 2, hydration results in the formation of the colorless hemiketal that is prone to tautomerization into isomeric chalcones at pH 6-7. Above pH 7, the chalcone irreversibly degrades by a C-ring opening reaction depending on its substituent groups (Castaneda-Ovando et al. 2009; Trouillas et al. 2016). Red berries contain an acidic environment around pH 3-4, where anthocyanins appear in an equilibrium of the charged flavylium cations, the neutral quinonoid base, and the hemiketal form.

Besides the pH, the individual anthocyanin structure determines the molecule's reactivity and susceptibility toward hydration and oxidative reactions. In general, glycosylated anthocyanins have been shown to be more stable than their aglycone. Anthocyanidins tend to form the chalcone structure eventually followed by an irreversible cleavage,

resulting in an aldehyde and phenolic acid. Hydroxy groups and methoxy groups on the B-ring also affect reactions differently. Thus, delphinidin, petunidin, and cyanidin, which contain an *ortho*-di-hydroxylated B-ring, are generally more prone to oxidation than malvidin and peonidin, which lack this pattern (Rossi et al. 2003). Also, the type of glycosylation has an influence on the stability; while pentosides have been demonstrated to be less stable than hexosides, diglucosides were more stable than monoglucosides (Rein and Heinonen 2004). Additionally, the acylation of the glycoside residues by aliphatic or aromatic acids increases the chemical stability and can prolong the half-time values toward thermal degradations (Zhao et al. 2017).

Next to their structure, several exogenous aspects determine anthocyanin stability. Due to their electron-rich aromatic structure, polyphenols are generally strong nucleophiles reacting with other molecules. They also tend to self-associate or are readily oxidized. Polymerization or degradation of the molecule can lead to a decrease in anthocyanin concentration and a reduction in the attractive color. In general, reactions are accelerated tremendously by heat and exposure to light, oxygen, or oxidizing enzymes, all of which are crucial during red juice production.

On the other hand, complex formation like co-pigmentation, including self-association and metal complexation, provide stabilizing effects by protection against hydrolysis and deglycosylation. For example, acylation of hydroxycinnamic acid promotes their stability around neutral pH values due to intramolecular co-pigmentation. This is based on a stacking phenomenon of the hydrophobic acyl moiety and the flavylium nucleus (Castaneda-Ovando et al. 2009; Clifford 2000; Giusti and Wrolstad 2003; Rein 2005; Shikov et al. 2008). A wide range of mostly colorless natural compounds bear a  $\pi$ -electron-enriched system and have been discovered as intermolecular co-pigments, like colorless phenols such as flavonoids and phenolic acids, or alkaloids, amino acids, and organic acids. Co-pigmentation was suggested to be the main mechanism stabilizing the flavylium cation chromophore and thus the color in plants (Mazza and Brouillard 1987; Patras et al. 2010; Rein 2005). However, polymerization to high-molecular-weight adducts can also lead to the formation of brown polymeric pigments, being less attractive in food processing purposes. In general, inter- and intramolecular co-pigmentation with



other moieties, polyglycosylated, and polyacylated anthocyanins, were proven to provide greater stability toward changes in pH, heat, and light.

The fruit matrix contains additional putative complexation compounds like polysaccharides and proteins, which may protect anthocyanins from degradation. Even though ample research has been conducted to examine these complexations, detailed binding mechanisms, because of their complexity, still remain under-investigated (Jakobek 2015; Liu et al. 2020; McManus et al. 1985; Phan et al. 2017). The present dissertation focuses on the polysaccharide complexations during red berry juice production.

### 2.2 Plant cell wall polysaccharides

Pectin, hemicellulose, and cellulose are the main polysaccharides of the primary plant cell wall of fruits and vegetables, with cellulose and hemicellulose providing rigid strength and pectin providing flexibility and fluidity through a gelatinous matrix. Pectin is also the predominant compound in the middle lamella encasing every cell (Chanliaud and Gidley 1999; Ochoa-Villarreal et al. 2012).

The cell wall function includes structural integrity and maintaining the internal pressure. It also has a crucial role in plant growth, cell differentiation, water transport, intercellular communication, and plant defense (Cosgrove 2005; McCann et al. 1990; Ochoa-Villarreal et al. 2012).

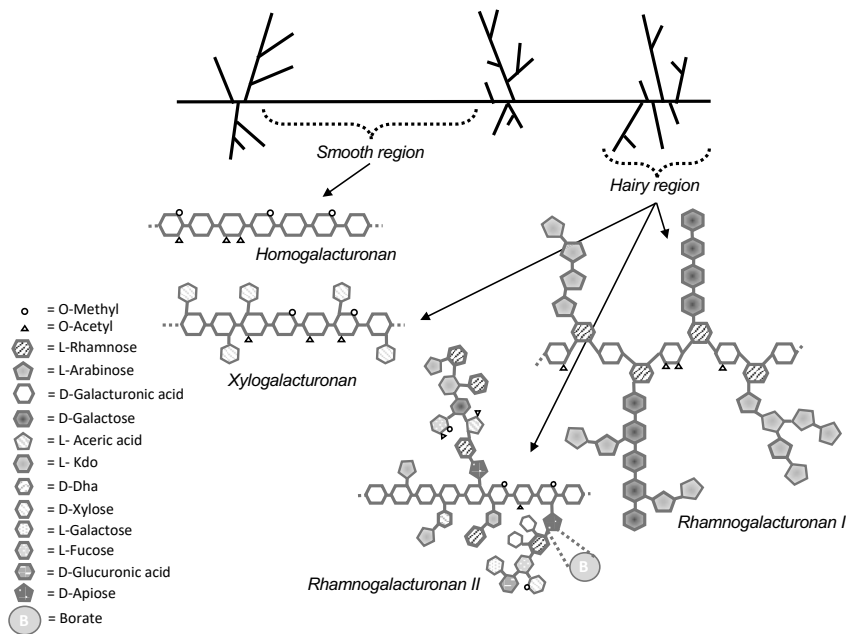
The structure of the plant cell wall network is very dynamic and complex; microfibrils of cellulose (30%) are embedded in an amorphous matrix of pectin (35%), while hemicellulose (30%) is stabilized by minor components of protein (glycoproteins, <5%) and phenolic compounds (lignin, insoluble proanthocyanidins, <5%) (data on dry weight basis from Ochoa-Villarreal et al. 2012). Due to its anionic nature, pectin is mainly involved in the regulation of ion transport. It also determines the porosity of walls and controls permeability for enzymes by forming a network independent from the hemicellulose/cellulose network (McCann et al. 1990).

Cellulose microfibrils ( $\emptyset$  approx. 3 nm) are formed by aggregated linear unbranched chains of  $\beta$ -(1,4)-D-glucose residues. These polymers are highly crystalline and insoluble, chemically stable, and resistant to most enzymatic activities.

Hemicelluloses are low molecular weight polysaccharides directly attached to lignin and cellulose. They consist of a heterogeneous group of polysaccharides with a similar structure of  $\beta$ -(1,4)-linked backbones with an equatorial configuration at C1 and C4 (Scheller and Ulvskov 2010). Xyloglucan is the most abundant hemicellulose comprising of a  $\beta$ -(1,4)-glucan backbone, where three out of four glucose residues are substituted with  $\alpha$ -xylose at C6 (Ochoa-Villarreal et al. 2012). Other substitutions of galactose, galactosyl-fucose, or arabinose were detected in olives (Vierhuis et al. 2001). Three domains of xyloglucan can be differentiated; the first is enzyme accessible occurring in free loops and cross-links, the second is connected to cellulose microfibrils by hydrogen bonds and extractable by concentrated alkali, and the third is only accessible when cellulose is degraded, due to an intense entanglement with amorphous microfibrils of cellulose (Pauly et al. 1999).

Pectic polysaccharides provide an extraordinary versatile complex, whereby its precise molecular structure has not yet been finally identified. The current status of scientific knowledge was recently reviewed by Ropartz and Ralet (2020). Pectin is composed of as many as eighteen distinct monosaccharides connected through twenty different linkages, while their arrangement is controversially discussed. A broad consensus was found for the smooth and hairy region model postulated by Schols and Voragen (1996), where the unbranched homogalacturonan (HG) domain refers to the smooth region and every branched domain of primary rhamnogalacturonan (RG) and galacturonans forming the hairy region (**Figure 1-1**). The first consists of 1,4-linked  $\alpha$ -D-galacturonic acid residues forming a linear unbranched homopolymer chain. In the primary cell wall, HG can be methyl esterified at the C6 position of the galacturonic carboxylic group and acetylated at the C2 or C3 position. Regarding the degree of methylation (DM) and the degree of acetylation (DA), polymers of HG can form gels that increase viscosity, depending on the pH or sugar content of the surrounding media. HG chains occur cross-linked by calcium bridges, where free carboxy groups of HG interact with calcium ions, structurally similar to an “egg-box,” determining porosity and fluidity of pectin gel (Grant et al. 1973).

The hairy region mostly consists of RG polysaccharides, bearing a backbone of the repeating disaccharide 1,2-linked  $\alpha$ -L-rhamnosyl 1,4-linked  $\alpha$ -D-galacturonic acid where acetylation can occur at O-2 and/or O-3. The rhamnose residues have been identified to be substituted with different side chains of neutral polysaccharides at O-4 such as arabinan, arabinogalacturonan (type I and II), and xylogalacturonan. Depending on the nature of the plant, the length of side chains varies enormously ranging from one galactose residue up to chains of 50 residues composed of arabinose/galactose. Side chains also strongly determine the pore size and flexibility of the pectin structure (Ochoa-Villarreal et al. 2012; Schols 1995).



**Figure 1-1:** Pectin smooth and hairy region model. The smooth region consists of homogalacturonan, and the hairy region includes three types of subunits: xylogalacturonan, arabinan- and type II arabinogalactan substituted rhamnogalacturonan I (RG I). Rhamnogalacturonan II (RG II) is highly branched by five side chains varying in complexity from a single arabinose unit to highly heterogeneous non-saccharides and cross-linked by borate diesters. Ketodeoxyoctonic acid/L-3-deoxy-D-manno-2-octulosonic acid (L-Kdo), L-3-desoxy-D-lyxo-2-heptulosaric acid (L-Dha). Adapted from Harholt et al. 2010 and Ropartz and Ralet 2020.