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Review of Process and Extraction Effects on the Bioavailability of Anthocyanins in Grapes

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ABSTRACT
Grapes are widely consumed worldwide in various forms, including fresh and dried, or processed into products like juice, vinegar, wine, and so on. Anthocyanins, mainly found in grapes, are responsible for various health-promoting effects and contribute to their colours such as red, purple, and blue. Although grapes contain a high quantity of anthocyanins, their bioavailability is considered limited. Anthocyanins may be absorbed by the gastrointestinal wall, undergo intensive first-pass metabolism, and emerge as metabolites in systemic circulation. A significant percentage of some anthocyanins can enter the large intestine and undergo breakdown induced by digestive system microorganisms. Several factors, such as pH, temperature, light, and solvents, can affect anthocyanin bioavailability, and processing grapes into products may impact their bioavailability. Considering the high market share of grapes and grape products, it is important to understand the effects of processing on anthocyanin bioavailability. This review discusses changes in the bioavailability of anthocyanins found in grapes and grape products during food processing, the effect of extraction conditions on bioavailability, as well as the health-promoting effects of grapes and grape products.

Keywords: Metabolism, Absorption, Grape products, Anthocyanins, Process conditions

1. Introduction

Grapes are very popular and one of the most produced crops around the world. According to the Food and Agriculture Organization of the United Nations (FAO), China, Italy, Spain, France, the United States of America, and Turkey are leading grape producers (FAO 2021). Different products can be made from grapes, such as non-alcoholic fermented juices like hardaliye (a traditional Turkish beverage based on red grapes) and non-fermented beverages such as grape juice, alcoholic fermented beverages like wines, concentrated grape juice (doshab, pekmez or molasses) and grape spreads like grape jelly, jam, preserves, butter, and marmalade (Kaya & Maskan 2003; Aladeboyeje & Şanli 2021). All these grape-derived products may contain different types and quantities of anthocyanins due to various processing steps, such as fermentation, evaporation, drying, and so on.

The quantity and quality of anthocyanins in grapes and their products differ significantly from those found in other anthocyanin-rich fruits and vegetables due to factors such as the regulation of the anthocyanin biosynthetic pathway, the diversity of anthocyanin molecules, and the influence of rootstock on anthocyanin content (Alenazi et al. 2019). The main dietary sources of anthocyanins include red-coloured fruits, some vegetables and red wine. According to the FAO, the main anthocyanin-rich fruits and vegetables are produced annually in quantities of approximately 0.9 million tons for blueberries, 4 million tons for strawberries, 3.2 million tons for sour cherries and cherries, 0.7 million tons for raspberries, 4 million tons for plums and blackthorn, 1.7 million tons for eggplant, 0.5 million tons for cranberries, and 70 million tons for grapes (FAO 2021). The annual production of grapes is quite high compared to other anthocyanin-rich foods. Additionally, grapes have potential distinctive uses in food applications, setting them apart from other food sources.

Grapes and other grape-derived products have enormous potential for diverse biofunctional roles due to containing bioactive compounds, mainly anthocyanins, phenolic acids, flavonoids, stilbenes, and lipids. The health benefits of grapes have been proven in several research studies, and they can reduce the risk of cardiovascular disease, cancer, and obesity, and have anti-inflammatory, antimicrobial, and antioxidative properties (Lee et al. 2017; Henriques et al. 2020; Sabra et al. 2021). The health benefits of anthocyanins are strongly influenced by their bioavailability.

The term "bioavailability" is defined by the Food and Drug Administration (FDA) as "*the rate and extent to which the active ingredient or moiety is absorbed and becomes available at the site of action*" (FDA 2001). Although anthocyanins constitute a significant class of phenolic compounds in grapes, their bioavailability is extremely low (Han et al. 2019). The low bioavailability of anthocyanins is mainly connected to poor absorption in the gastrointestinal tract. Moreover, this limitation may be related to low water solubility due to anthocyanins being present in foods as polymers or as glycosylated forms that bond covalently to food matrices (Polia et al. 2022). Most anthocyanins consumed through foods are conjugated, digested by the colon bacteria, and eliminated in urine and feces, resulting in low bioavailability (De Rosso et al. 2012; Fang 2015). Additionally, anthocyanin degradation occurs due to several environmental conditions, including temperature, light, solvents, changes in chemical structure, and pH fluctuations during processing. The purpose of this review is to inform readers about how the extraction method and its byproducts affect anthocyanin bioavailability in grapes and their products. The health benefits of anthocyanins in grapes are also reviewed.

2. Anthocyanins found in grapes and health benefits

Anthocyanins are a major class of flavonoids and possess diverse biological functions. Anthocyanins are water-soluble pigments that give blue, purple, and red colours to grape cultivars, and are primarily present in grape skin. Hundreds of anthocyanins have been identified until now. Delphinidin-, cyanidin-, petunidin-, peonidin- and malvidin-3-*O*-glucoside are the predominant anthocyanins found in *Vitis vinifera* grapes (Han et al. 2019). In contrast, other grape species, such as *V. labrusca*, contain both anthocyanin 3-monoglucoside and 3,5-diglucosides when compared to *V. vinifera* (de Castilhos et al. 2015). The anthocyanin content of grapes varies with grape variety, and is influenced by several environmental factors such as soil type, climate, and management practices (Han et al. 2019).

Anthocyanins have various bioactive properties beneficial to human health, including the prevention of cardiovascular diseases and possess antioxidant, anti-cancer, anti-inflammatory, antidiabetic, and anti-obesity activities, as well as neuroprotective effects and improving eye and brain health. Not only grape and grape products but also grape by-products, which are left over mainly from wine and juice industries, contain beneficial phenolic chemicals, including anthocyanins. In cholesterol-fed rabbits, the administration of grape seed proanthocyanidin extract at a concentration of 0.1% resulted in a 30%–50% reduction in atherosclerosis. This effect was primarily attributed to the inhibition of low-density lipoprotein (LDL) oxidation, and a 25% reduction in malondialdehyde levels, an index of lipid oxidation, in the aorta (Yamakoshi et al. 1999). In Caco-2 cells pretreated with red grape skin anthocyanins, glucose transporter 2 (GLUT2) expression increased compared to controls, suggesting that persistent consumption of anthocyanins may improve their bioavailability. Additionally, the anthocyanins in red grape skin inhibited glucose uptake by interfering with it (Faria et al. 2009).

The effects of anthocyanin-rich diets on transcription factors and genes/proteins involved in antioxidant and anti-inflammatory defence may be related to the presence of native anthocyanins in the brain, heart, and other tissues. Trials with sour cherries, grapes, blueberries, and bilberries demonstrated this process (Seymour et al. 2011; 2013). More specifically, anthocyanins play a role in brain function as they can pass the blood-brain barrier. Grape anthocyanins (8 mg/kg body weight dose) were identified in the brains of rats at a concentration of 192 ng/g barely 10 min after being introduced into the stomach, according to Passamonti et al. (2005). In a recent study, the researchers found out that giving rats a commercial anthocyanin extract made from grape skins (200 mg/kg for 25 days) had a preventative effect on behavioural alterations in a rat model of streptozotocin-induced dementia in sporadic Alzheimer's disease. The extract had protective properties against reactive oxygen species (ROS) and antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase (Pacheco et al. 2018). In a subsequent study, where streptozotocin was injected into rats along with grapes, Jankowski et al. (2000) observed a significant drop in sugar levels in urine and blood serum. The authors proposed that anthocyanins reduce collagen, lipoproteins, and glycoprotein production, and elastase and adenosine deaminase activity, both of which are elevated in diabetics (Jankowski et al. 2000).

3. Bioavailability and metabolism of anthocyanins

Since anthocyanins are naturally present in many plant-based meals, a balanced diet inherently includes a significant amount of them (Hornedo-Ortega et al. 2021). In particular, consuming foods rich in anthocyanins, like grapes, was associated with reduced risk of several diseases (Mattioli et al. 2020). However, anthocyanin health benefits were linked to their metabolic byproducts. Bioavailability typically refers to the proportion of a nutrient that becomes accessible at its intended location for action after absorption from the gastrointestinal tract. It can also refer to the fraction of the nutrient that is absorbed and ready for use in physiological processes and storage (Fernández-García et al. 2009). Therefore, for anthocyanins to exert their metabolic functions, they must be bioavailable, requiring digestion and absorption as well as metabolism (Iglesias-Carres et al. 2019). Additionally, the bioavailability of anthocyanins can be influenced by their transport through the gut epithelium (Kamiloglu et al. 2015). Anthocyanins display a noteworthy level of stability and are capable of being absorbed during the gastric phase, with only a small proportion undergoing hydrolysis. After simulated stomach digestion, there was a modest decrease in anthocyanin stability in the gastrointestinal cavity. Alkaline conditions and microbiota in the intestine can catabolize anthocyanins into different compounds like phenolic acids and aldehydes, making the intestine a major site for anthocyanin metabolism and absorption (Soares et al. 2018). This tendency is more pronounced in red wine anthocyanins, and this could be related to the complexity of anthocyanin structure (Oliveira et al. 2019). Data indicates that anthocyanins are highly affected by the intestinal system, whereas they remain relatively unaffected in the stomach. Cyanidin, the precursor pigment for other anthocyanins, can be transformed into them by phase I and phase II reactions. Cyanidin can be converted into peonidin due to methylation at the C-3' position, which can be catalyzed by 3'-*O*-methyltransferase (Yuzuak & Xie 2022). Methylation reactions are classified as phase II reactions that occur in mammals and increase the bioavailability, stability and biological properties (Gulsunoglu-Konuskan & Kilic-Akyilmaz 2021). 3'-5'-*O*-methyltransferase catabolizes delphinidin into petunidin, and petunidin into malvidin as a result of methylation (Pomar et al. 2005). Hydroxylation is one of the most common reactions to increase antioxidant potential due to the number and the position of –OH groups (Gulsunoglu-Konuskan & Kilic-Akyilmaz 2021). According to reports, the hydroxylation of cyanidin to delphinidin is also possible by the action of 3'-hydroxylase (Pomar et al. 2005). Additionally, protocatechuic and vanillic acid can form from cyanidin and peonidin glycosides. However, phenolic metabolites of delphinidin, malvidin, and petunidin glycosides are not stable and can be broken into smaller molecules (Fang 2015). The conversion mechanism of major grape anthocyanins is shown in Figure 1.

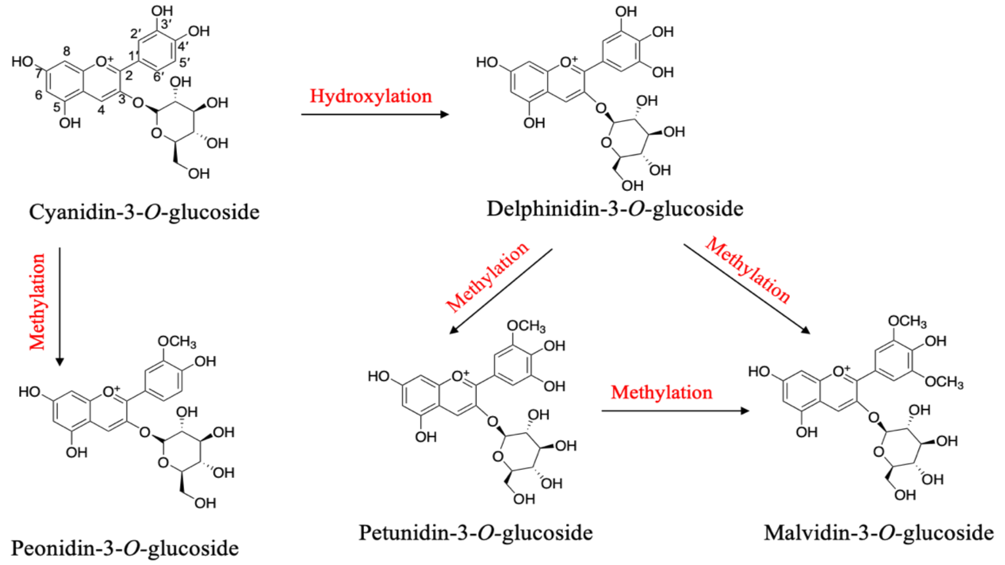

Figure 1- Schematic representation of conversion pathways for grape anthocyanins

The insoluble fraction yields a larger percentage of recovered anthocyanins (in some instances, the insoluble fraction yields all of the anthocyanins), implying that the food matrix may alter the action of digestive settings; thereby, preserving anthocyanins from degradation (Pineda-Vadillo et al. 2017). Gut bacteria may break down acylated anthocyanins more quickly than nonacylated anthocyanins. In obese mice administered a 12-week chronic dose of a Concord grape supplement rich in acylated anthocyanins, total anthocyanins in their fecal matter increased by a factor of ten compared to feces collected during the period of the late test week when antibiotics were given to suppress gut microbiota. The difference was more significant than that observed after administering nonacylated anthocyanin-rich berry supplements (Overall et al. 2017).

Compared to conventional grape-administered rats, the blood profile of rats administered organic grapes showed greater metabolite concentrations at two hours and lower metabolite concentrations at 24 hours. Consequently, the bioactivity of phenolic compounds may be influenced by their serum kinetic behaviour (Iglesias-Carres et al. 2019). Under gastrointestinal pH and temperature circumstances, the stability of anthocyanins and their breakdown products from Cabernet sauvignon red wine were investigated, and it was observed that anthocyanins can be degraded to phenolic acids under small intestine conditions when whole grapes are consumed (Yang et al. 2018). The decreased stability of anthocyanins during the intestinal phase of digestion can be attributed to factors such as the alkaline pH of the solution, leading to an increase in non-flavylium cation forms of anthocyanins. Additionally, the binding of anthocyanins to proteins and bile salts contributes to the formation of indigestible complexes, resulting in their subsequent precipitation (McDougall et al. 2005; Fleschhut et al. 2006). The pharmacokinetics and excretive profiles of phenolic metabolites were examined in 10 participants after the acute administration of a drink produced from red grape pomace. Only a few anthocyanin derivatives were tentatively discovered in trace amounts in 0–3 h urine samples from some volunteers and were consequently ignored (Castello et al. 2018). In a crossover study, 9 healthy volunteers consumed a single oral dosage of 400 mL red grape juice or red wine with dose-adjusted anthocyanin content (283.5 mg or 279.6 mg, respectively). Plasma and urine excretion both contained anthocyanin glycosides. Plasmatic antioxidant activity was also measured after the meal. Biokinetic criteria for single anthocyanins, such as AUC (the total area from zero to infinity), C_{max} (maximum plasma concentration), t_{max} (time to reach C_{max}), and the elimination rate t1/2, were computed based on plasma content. Total anthocyanin excretion in urine differed significantly, accounting for 0.18% (red wine) and 0.23% (red grape juice) of the administered dose, respectively. Additionally, when juice was consumed instead of wine, plasmatic antioxidant activity increased to higher levels. Red grape juice appeared to have better intestinal absorption of anthocyanins than red wine, suggesting a possible synergistic effect with the juice's glucose content (Bitsch et al. 2004).

Based on the findings of these studies, the concentrations of anthocyanins analyzed in both plasma and urine samples from the specific matrices and subjects were comparable, indicating a lack of significant differences. This suggests that the anthocyanins undergo minimal alterations from their entry into the bloodstream to their elimination through urine. Furthermore, anthocyanins exhibit rapid excretion within a 24-hour timeframe and possess a low absorption rate of less than 1%. Consequently, the glycosylated forms of anthocyanins were found to be more efficiently absorbed, leading to increased bioavailability.

4. Effects of processing methods on bioavailability of anthocyanin in grapes

The bioavailability of anthocyanins is influenced by key factors such as food matrix, food processing, enzymes involved in metabolism, and transport (Eker et al. 2019). Anthocyanins are highly reactive molecules and thus sensitive to degradation by exposure to oxygen, light, temperature, pH, and enzymes, affecting their chemistry, stability, and colour (Enaru et al. 2021). Degradation of anthocyanins can occur during food processing, extraction, and storage. Therefore, processing methods like heating, mechanical procedures, and domestic processes may contribute to anthocyanin degradation, potentially altering their antioxidant properties and bioavailability (Patras et al. 2010). Studies investigating the effect of thermal or non-thermal processing conditions on the bioavailability of anthocyanins found in grape and grape products are given in Table 1.

Table 1- Effect of processing on bioavailability of anthocyanins in grapes and grape products

Product type	*Grape species*	*Anthocyanin intake*	*Experiment*	*Major findings*	*Reference*
Grape juice	*V. labrusca*	17.5±22.5 µmol mv-3-glu/100 mL	Human trials	Glycemia ↓ Blood levels of uric acid ↓ Plasma lipid peroxidation ↓ Plasma antioxidant capacity ↑	(Copetti et al. 2018)
Red grape juice	ND	283.5 mg/400 mL	Human trials	C_{max} (ng/mL)=100.1 T_{max} (h)=0.5 $AUC_{0\text{-}180}$ (ng/mL.h)=168.4	(Bitsch et al. 2004)
Grape juice	*V. labrusca* Concord	238±6 µmol/350 mL	*In vitro*	C_{max} (ng/mL)=1.0–2.0 nmol/L T_{max} (h)=1.3–33 Recovery in ileal effluent=47±9 µmol Urinary excretion=612 nmol (0.26% of total anthocyanins)	(Stalmach et al. 2012)
Red wine	*V. vinifera* L. cv Syrah	126.27±14.73 mg mv-3-glu/L	*In vitro*	26% of anthocyanins released in mouth Anthocyanin content in stomach ↓ 36% pn-3-glu, 40% pt-3-aglu released in intestine	(Lingua et al. 2018)
Red wine	*V. labrusca*	66.7±10.2 µmol mv-3-glu/100 mL	Human trials	Glycemia ↓ Blood levels of uric acid ↑ Plasma lipid peroxidation ↓ Plasma antioxidant capacity↑	(Copetti et al. 2018)
Port red wine	ND	48.94±0.08 mg/100 mL	Human trials	C_{max} (mg/mL)=5.9±0.73 T_{max} (min)=90±17 $AUC_{0\text{-}120}$ (mg/mL.min)=500.64±62.52	(Fernandes et al. 2017)
Red wine	*V. vinifera* Blaufrankisch (Lemberger)	279.6mg/400 mL	Human trials	C_{max} (ng/mL)=42.9 T_{max} (h)=1.5 $AUC_{0\text{-}180}$ (ng/mL.h)=100.8	(Bitsch et al. 2004)
Grape extract	*V. vinifera*	3.85 g cyn-3-gal/kg	*In vivo*	Urinary anthocyanin concentration=33.2 nmol/L In plasma highest peaks= pt-3,5-diglu, mv-3,5-diglu	(He et al. 2006)
Red grape	*V. vinifera* L. cv Syrah	334.63±22.53 mg mv-3-glu/kg fw	*In vitro*	22% of anthocyanins released in mouth 45% of anthocyanins released in stomach 44% del-3-glu, 26% pn-3-glu, 53% pn-3-aglu released in intestine	(Lingua et al. 2018)

AUC: area under the curve of total anthocyanins, C_{max}: maximum plasma concentration, cyn-3-gal: cyanidin-3-galactoside, del-3-glu: delphinidin-3-glucoside, mv-3,5-diglu: malvinidin-3,5-diglucoside, mv-3-glu: malvinidin-3-glucoside, pn-3-aglu: peonidin-3-acetylglucoside, pn-3-glu: peonidin-3-glucoside, pt-3-aglu: petunidin-3-acetylglucoside, pt-3,5-diglu: petunidin-3,5-diglucoside, T_{max}: time to reach maximum C_{max}.

4.1. Maceration, pressing and fermentation

Maceration is the stage in which anthocyanins in grape skins are transferred to must and wine. Maceration and fermentation are not separate processes (Morata et al. 2021). Anthocyanins are transferred through diffusion from seeds and grape skins to wine during fermentation. The highest amount of anthocyanins is extracted during the first few days of maceration, after which no more extraction is usually observed, despite 30–40% of anthocyanins remaining in the crushed skins. Studies indicate anthocyanin extraction occurs in the first seven days of maceration from grape skins (Romero-Cascales et al. 2005). However, the highest anthocyanin extraction was accomplished during the first six days of fermentative maceration by freezing with dry-ice techniques (Busse-Valverde et al. 2011).

Fermentation and aging of the wine result in the chemical transformation of anthocyanins, providing pigments that can be more stable than the original anthocyanins (Ruta & Farcasanu 2019). In addition, a large number of differential metabolites were identified during the fermentation of wine and two in the post-fermentation process (Ai et al. 2021). Therefore, high amounts of anthocyanins are extracted from fermented grapes using moderate temperatures and short-time extraction (Vergara-Salinas et al. 2013). Anthocyanin concentrations in red wine range from 90 to 400 mg/L, depending on the age of the wine (Waterhouse 2002). The grapes of the *V. vinifera* variety include common wine grapes such as Cabernet sauvignon and Merlot, containing mainly anthocyanidin monoglycosides, especially malvidin-3-*O*-glucoside. Anthocyanidin diglycosides are primarily found in wine grape cultivars other than *V. vinifera*. *V. vinifera* hybrid cultivars contain monoglycosides and diglycosides of anthocyanidin (De Rosso et al. 2012). These anthocyanins contribute to the organoleptic and chemical properties of grapes and wines because of their interaction with other phenolic compounds as well as with proteins and polysaccharides.

The intestinal absorption of anthocyanins from red grape juice was improved compared to red wine, a phenomenon that can be explained by the synergistic effect of glucose content in the juice (Bitsch et al. 2004). Bub et al. (2001) reported that the C_{max} of malvidin-3-*O*-glucoside after drinking red wine or dealcoholized red wine was not substantially different (288±127 nmol.h/L and 214±124 nmol.h/L, respectively) and was approximately two times lower than that recorded after drinking red grape juice (662±210 nmol.h/L). However, C_{max} was reached after 20 minutes for red wine, while it took 90 and 180 minutes to reach this value for dealcoholized red wine and red grape juice, respectively. This difference could be attributed to the possibility that the alcohol content may increase the absorption of malvidin-3-*O*-glucoside (Bub et al. 2001). The bioavailability of flavanol-anthocyanin dimer (+)-catechin-malvidin-3-glucoside, an anthocyanin derivative found in grape skins and red wine, was investigated with an intestinal model barrier by using Caco-2 cells to examine transepithelial transportation. The research showed that the Caco-2 cell barrier model can be successfully crossed by anthocyanins and flavanols, as well as dimeric complexes that contain both anthocyanins and flavanols (Fernandes et al. 2012).

4.2. Drying

Raisins are grape products dried using the heat of the sun, solar drying or oven drying (Jeszka-Skowron & Czarczyńska-Goślińska 2020). The drying of grapes, whether pretreated with olive oil or not to produce raisins, resulted in quantitative and qualitative changes in the anthocyanin composition. However, reaching a firm conclusion regarding the variability in anthocyanin bioavailability, specifically identifying the responsible metabolizing enzymes or bacteria, is challenging (Eker et al. 2019). It was confirmed that raisins dried after treatment with olive oil preserve more anthocyanins and proanthocyanidins than raisins not treated with olive oil (Olivati et al. 2019). This could be related to the behaviour of olive oil as an edible film on the surface of raisins, protecting them from environmental conditions.

4.3. Evaporation

The purpose of evaporating grape juice is to sterilize it, extend its shelf-life and make sweet and flavoured products that can be healthier than sugar. “Pekmez” (molasses) is the traditional name of boiled juice prepared from different berry fruits such as grape and mulberry in Turkey (Bozkurt et al. 1999). Similar to pekmez, a special evaporated grape juice named “doshab” in Iran is produced by boiling and concentrating grape juice by adding “doshab soil” (Aliakbarlu et al. 2014). Another concentrated grape product is “saba” produced in Italy to make balsamic vinegar. Tagliazucchi et al. (2013) studied the effects of cooking temperature (85±2 °C) at different times on the polyphenols and anthocyanin in two different grapes. The degradation of anthocyanin was reported as more than 92% for each monomer, and the highest decrease in anthocyanin concentration occurred within the first 30 min of heating, especially for the nonacylated ones. Even though they might be less stable, nonacylated anthocyanins are more bioavailable *in vivo* than acylated anthocyanins in human investigations (Fernandes et al. 2015).

A new method in the winemaking industry is the evaporation of intermediate wine that is rich in alcohol (Ozturk & Anli 2014). This step is a pre-fermentation heat treatment technique commonly criticized for unacceptable sensory properties. Eker et al. (2019) reported that thermal treatment, such as evaporation, causes an increase in the bioaccessibility and bioavailability of anthocyanins while decreasing their concentrations. In spite of the observation of the decrease in the amount of anthocyanins, thermal treatments crack cell membranes, releasing cytoplasmic substances and increasing the absorption of anthocyanins (Barba et al. 2016; Celli & Brooks 2017). This indirectly provides higher bioaccessibility of these bioactive compounds before absorption by human digestion (Leong & Oey 2012). Overall, based on the literature reviewed, different factors contribute to the variability in anthocyanin bioavailability, including processing methods.

4.4. Fruit juice production

Grape juice is known to be a rich source of anthocyanins. In grapes, anthocyanins are primarily located in the skin and also in the flesh depending on the variety (Benmeziane et al. 2016; He et al. 2010). Delphinidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, peonidin-3,5-*O*-diglucoside, malvidin, malvidin-3-*O*-glucoside, and malvidin-3,5-*O*-diglucoside are reported to be the major anthocyanins in different varieties of grape juice (Oh et al. 2008). Non-fermented red grape juice is usually characterized by malvidin-3-*O*-glucoside as a major anthocyanin (Cahyana et al. 2019).

Anthocyanins are known to be sensitive to heat and physicochemical factors. Therefore, processes used to make grape juice, such as thermal and enzymatic treatments, grapefruit juice processing, contact time between juice and grape solid constituents (skins and seeds), pressing, and food matrix, may lead to a degradation of anthocyanins and alter their antioxidant properties (Ioannou et al. 2012; Weber & Larsen 2017). Sulphur dioxide and tartaric acid supplementation also impact the quantity and type of phenolic compounds in grape juice (Cosme et al. 2018).

In addition to processing factors that influence the bioavailability of anthocyanins, evidence suggests that anthocyanin intake could be improved by consuming smoothies and grape juices (Kuntz et al. 2015). This implies that dietary matrices, such as red wine and red grape juice, affect the bioavailability of anthocyanins. After grape juice production, anthocyanins detach and become free from interactions with other biomolecules such as proteins, bio membranes, and DNA constituents (Fernandes et al. 2019). It is reported that the structural differences and physicochemical properties of anthocyanins greatly interfere with their absorption and digestion (Crozier et al. 2009).

Bub et al. (2001) found that several variables contribute to anthocyanin bioavailability, including fecal excretion, intestinal degradation at neutral pH, gut microbiota metabolism, fast accumulation in distinct tissues, or ring fission metabolism after consuming red wine, dealcoholized red wine, and red grape juice. Furthermore, low plasma and urine quantities suggest that anthocyanins are absorbed slowly and metabolized quickly (Murkovic et al. 2001). Furthermore, grape juice's high sugar content may interfere with the intestinal absorption of these chemicals, lowering anthocyanin bioavailability (Frank et al. 2003). In another research conducted by Stalmach et al. (2012), healthy individuals who consumed Concord grape juice were investigated. Urinary excretion of anthocyanins ranged from 5.7 nmol for peonidin-3-*O*-glucoside to 368 nmol petunidin-*O*-glucuronide during a 24-hour period, and anthocyanin excretion amounted to 612 nmol, which is equivalent to 0.26% intake. Similar to other studies, researchers also claimed that grape juice anthocyanins were stable under acidic conditions in the presence of pepsin, with an average recovery of 88% for the 25 anthocyanins, lowering to 24% following simulated digestion with pancreatin and bile extract. Malvidin, peonidin, and cyanidin glycosides, with recoveries of 57%, 48% and 37% respectively, were more stable than petunidin (14%) and delphinidin (5.5%) glycosides (the only two anthocyanins detected in plasma). According to reports, the conversion of anthocyanins into reversible pseudo foundations, quinoidal foundations, and chalcones, as well as hydrolysis to various phenolic acids, for instance 3,4-dihydroxybenzoic acid (also known as protocatechuic acid), are contributing factors to anthocyanin loss under neutral pH and alkaline conditions (Stalmach et al. 2012).

5. Effects of extraction methods on bioavailability of anthocyanins in grape

Anthocyanins can be extracted from plant-based materials using various extraction techniques and solvents. Anthocyanins are dominantly found in the form of flavylium cations, giving a red colour in acidic solutions (pH 1–3). The flavylium cations convert to carbinol and chalcone, and chemical degradation occurs at pH above 4 (Fang, 2014). Under highly acidic conditions, flavylium cations remain stable. Consequently, extraction solvents used for anthocyanin extraction contain mineral and organic acids (Revilla et al. 1998). The use of an acid solvent may produce anthocyanidins, which have high bioavailability compared to their glycosylated form but are less stable in comparison (Eker et al. 2019).

Various extraction methods were suggested to obtain anthocyanin-rich extracts, typically based on organic solvents with different polarities such as methanol, ethanol, acetone, water, or mixtures in acidic or neutral conditions. In a study by Lapornik et al. (2005), red, black, and grape marc byproducts were examined for solvent-based extraction of polyphenol and anthocyanin. They applied water as a solvent along with 70% ethanol and 70% methanol. The effects of plant substance, solvent, and extraction duration (1, 12, and 24 hours) on extraction productivity and various anthocyanins were examined. Alcohol extraction was found to be superior, showing higher polyphenol and anthocyanin content compared to classic maceration. Ethanol and methanol extracts of red and black currants had twice as much anthocyanin and polyphenols, while grape marc alcohol extracts consisted of a higher amount of phenolic compounds, seven times more than water extracts. Extraction time was not effective on the water extracts of red and black currants, while it was effective for grape marc water extracts, indicating an increase in phenolic compound extraction with higher extraction time. Methanol was slightly better than ethanol, but the difference was not great. However, ethanol is considered more appropriate in the food industry. Black currant marc had the highest antioxidant activity, whereas red currant marc had the lowest.

Traditional extraction methods are considered obsolete due to their use of large amounts of organic solvents, low efficiency, time-consuming nature, and degradation of phenolic compounds (Drosou et al. 2015; Liu et al. 2018). The utilization of innovative non-thermal technologies has proven to be an effective approach for extracting anthocyanins from grapes, leading to enhanced yield, faster processing, and maintaining antioxidant capacity (Morata et al. 2021). Several extraction methods have been widely employed for extracting anthocyanins, including supercritical fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction, pulsed electric fields, high-pressure liquid extraction, high-voltage electrical discharge, and enzyme-assisted extraction (Tena & Asuero 2022). Extraction of anthocyanins using a pulsed electric field increased the antioxidant activity by four-fold, three-fold with high hydrostatic pressure and two-fold with ultrasonics compared to conventional extraction (Corrales et al. 2009). Subcritical water at 100 to 110 °C enables the extraction of anthocyanins from dried red grape skin (Ju & Howard 2005). The extraction of anthocyanins from grapes using a natural deep eutectic solvent (NaDES) (chloride, choline and citric acid) recovered 54% of anthocyanins from red grape pomace (Iannone et al. 2021).

Elmi Kashtiban & Esmaiili (2019) compared solvent extraction for phenolic compounds from black grape skins with subcritical water extraction. Subcritical water extraction is an innovative method performed at lower temperatures and higher pressures compared to solvent extraction. They concluded that subcritical water extraction was a more efficient method. They also used ultrasound as pretreatment before subcritical water extraction and it was also found to be effective. The optimum conditions for subcritical water extraction of grape phenolic compounds were temperature of 150 °C, pressure of 40 bar, and a time of 30 minutes without using any organic solvents. They observed that temperature was the most important parameter for subcritical water extraction.

Bonfigli et al. (2017) compared conventional and ultrasound extraction of grape pomace anthocyanins using a mixture of 50% ethanol-water. In both methods anthocyanin extraction straightly increased, as ultrasound-assisted extraction increased the mass transfer rate in the first stage of extraction (washing stage). In conventional solvent extraction, 80% of anthocyanins were obtained in the first 600 seconds, whereas in the ultrasound-assisted extraction method, 90% of anthocyanins were extracted in the same time. Ultrasound waves create cavities in cell walls, modifying the structure of materials and their physical and chemical characteristics. As a result, this simplified the release of bioactive compounds and increased mass transfer, while the extraction diminished with time passing. Elmi Kashtiban & Esmaiili (2019) achieved similar results, realizing that ultrasound pretreatment before subcritical water extraction methods increased the extraction yield of polyphenolic compounds. Bonfigli et al. (2017) also observed that as the temperature increased, the extraction yield also went up.

Cvjetko Bubalo et al. (2016) conducted a study on the extraction of anthocyanins in red grape skin using deep eutectic solvents (DES), combined with microwave-assisted extraction and ultrasound-assisted extraction. They tested five different choline chloride-based DES (ChCl-based containing glycerol (ChGyl), oxalic acid (ChOa), sorbose (ChSor), malic acid (ChMa), and proline and malic acid (ChProMa)) as possible extraction solvents using conventional solvent extraction with a shaker at 65 ºC as the optimal temperature. Their conclusion was that ChOa with 25% water was the best solvent. They observed that the extraction yield increased more with ultrasound-assisted extraction compared to microwave-assisted extraction and conventional solvent extraction, respectively. These findings align with similar conclusions reached by other studies, such as Esclapez et al. (2011) and Mandal et al. (2007). Bi et al. (2013) evaluated temperature as the main parameter in the extraction of anthocyanins with DES. They used alcoholic and ChCl-based DES and realized that ChCl/1,4-butanediol was the best DES for the extraction of bioactive compounds.

Panić et al. (2019) studied the extraction of anthocyanins from grape pomace using NaDES. Based on the literature, the structure and stability of anthocyanins depend on the pH value, remaining stable within the range of pH 2–7 (Cheynier et al. 2012; Cvjetko Bubalo et al. 2016; Bosiljkov et al. 2017). Previous studies showed that organic acid-based NaDES can effectively separate anthocyanins. Panić et al. (2019) used 8 different NaDES compositions: choline chloride/citric acid, choline chloride/malic acid, choline chloride/proline/malic acid, proline/malic acid, betaine/malic acid, betaine/citric acid, malic acid/glucose/glycerol, malic acid/glucose. The NaDES solutions were prepared by adding 25% (v/v) water to reduce viscosity and enhance mass transfer between liquid and solid phases (Cvjetko Bubalo et al. 2016). Low-frequency ultrasound and multimode microwave were employed individually and concurrently, and it was concluded that the optimal extraction conditions were achieved with concurrent ultrasound/microwave-assisted extraction using 30% water (v/v).

Corrales et al. (2009) investigated the extraction of anthocyanins from red grape skins using high hydrostatic pressure. They studied four parameters (pressure, time, temperature, and % ethanol) to determine the optimal conditions. Pressure exhibited a selective effect on anthocyanin extraction; for instance, at a pressure of 200 MPa, the highest levels of total anthocyanin monoglycosides were acquired, while the optimal pressure for the extraction of acylglycosides was 600 MPa. They realized that time was not a significant factor in anthocyanin extraction. However, the extraction yield was higher when higher ethanol concentrations were applied, as the highest anthocyanin recovery was obtained when pure ethanol was used. Spigno et al. (2007) obtained similar results for anthocyanin extraction using pure ethanol. Corrales et al. (2009) found that the best extraction temperature was 50–70 ℃.

Monrad et al. (2010) investigated subcritical solvent extraction of anthocyanins from freeze-dried ground Sunbelt red grape pomace using an accelerated solvent extractor (ASE). They applied the following variables: pressure was constant (6.8 MPa), temperature (40, 60, 80, 100, 120, and 140 °C), and four combined water-ethanol solvents (10, 30, 50, and 70% ethanol). Conventional solvent extraction (methanol/water/formic acid; 60:37:7, v/v/v) was compared with subcritical solvent extraction. Temperature was identified as having a significant effect on total anthocyanins using ASE, while the solvent and temperature interaction effect was insignificant. Optimum temperatures were determined to be 80, 100, and 120 °C. The solvent mixture was also found to have a significant effect with higher ethanol concentrations (50–70%), resulting in the extraction of the greatest amount of anthocyanins from grape pomace. This coincides with several investigations that obtained similar results by applying 50–95% ethanol-water solvents for polyphenol extraction from grapes. The researchers realized that a higher ethanol concentration >70% led to an insignificant resumption of anthocyanins (Lapornik et al. 2005; Luque-Rodríguez et al. 2007; Makris et al. 2008). Optimal extraction conditions (70% ethanol, 103.7 °C) were identified using regression and response surface methods. The study revealed the extraction of diverse anthocyanins, such as petunidin-3-*O*-monoglucoside, petunidin-3-*O*-monoglucoside, petunidin-3-(6-*O*-*p*-coumaroyl)-5-*O*-diglucoside, cyanidin-3-*O*-(6-*O*-*p*-coumaroyl)-monoglucoside, and petunidin-3-*O*-(6-*O*-*p*-coumaroyl)-monoglucoside, with the conventional method. The researchers concluded that employing both methods together yielded more anthocyanins. They detected 12 peaks of anthocyanins conditionally, with malvidin-3,5-*O*-diglucoside and peonidin-3-(6-*O*-coumaroyl)-5-*O*-diglucoside being the highest peaks. Pomar et al. (2005) detected 3-monoglucosides (acylated with coumaric, acetic, and caffeic acid) derivatives from 50 red table grape cultivars, while Monrad et al. (2010) also found several acylated diglycosides in Sunbelt grape pomace.

6. Conclusions

Anthocyanin metabolism after oral intake follows a unique pattern compared to other flavonoids. While anthocyanins can be absorbed in the stomach and intestine, they are among the least bioavailable bioactive compounds within the spectrum of flavonoids. This limited bioavailability is attributed to the highly reactive nature of anthocyanins, which exhibit different structural forms depending on the pH of the aqueous solution. The preservation of anthocyanins and their antioxidant activity from environmental conditions and during processing steps is crucial for developing new processes, such as encapsulation, which enable the stabilization of anthocyanins. To fully comprehend the bioavailability of anthocyanins in grape and grape products, additional *in vivo* research is required.

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