

Composition of various parts from Vitis vinifera

The European grapevine (*Vitis vinifera*) contains a range of organic compounds. Various parts of the plant (e.g. grape seeds, grape skin, and grapevine leaf) have a different chemical composition. However, all parts of the plant contain monomeric, oligomeric, and polymeric proanthocyanidins.

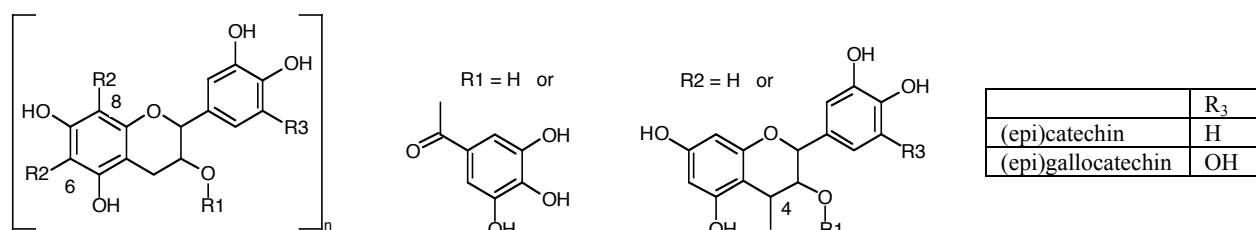


Figure 1 General structure of oligomeric proanthocyanidins.

Proanthocyanidins are oligomers of flavan-3-ol units (catechin, epicatechin, gallocatechin, and epigallocatechin), which are generally coupled through 4→6 and 4→8 links. The most common classes are the procyanidins, which are oligomers of (epi)catechin and their gallic acid esters, and prodelphinidins, which are oligomers of (epi)gallocatechin and their gallic acid esters [Porter (1989) in **Lazarus** et al (1999)]. Grape seeds only contain procyanidins, while other parts (grape skins and stems) also contain prodelphinidins. Therefore, the absence of trihydroxylated flavan-3-ol units (gallocatechin and epigallocatechin) confirms the authenticity of products derived from grape seeds [Vivas et al (2004) and Souquet et al (2000) in **Monagas** et al (2005)].

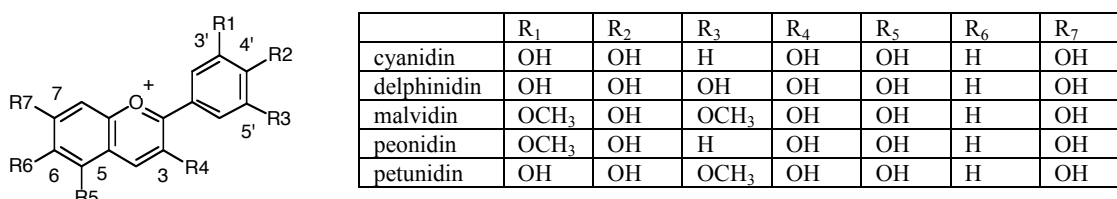


Figure 2 General structure of anthocyanidins.

Anthocyanins are the 3-O-monoglucosides and 3-O-acetylated monoglucosides of the five main anthocyanidins (delphinidin, cyanidin, petunidin, peonidin, and malvidin). Acetylation may occur at the C-6 position of the glucose molecule by esterification with acetic, p-coumaric, and caffeic acid [Mazza et al (1993) in **Monagas** et al (2006)]. The anthocyanins are water-soluble plant pigments, which are present in the grape skins and grapevine leaves of red cultivars. Glucosides are more abundant than acetylated glucosides. Malvidin-3-O-glucoside is the main pigment in the grape skins, while peonidin-3-O-glucoside, cyanidin-3-O-glucoside, and malvidin-3-O-glucoside are the main pigments in the grapevine leaves [**Monagas** et al (2006)].

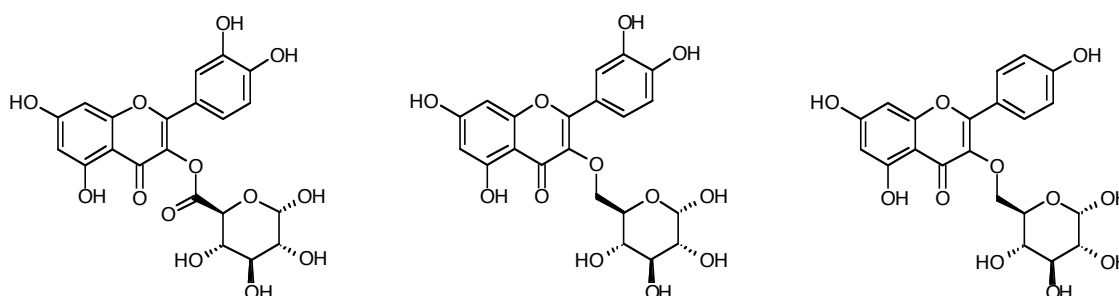


Figure 3 Quercetin-3-O-β-D-glucuronide, quercetin-3-O-β-D-glucoside, and kaempferol-3-O-β-glucoside.

Flavonols exist as the 3-O-glycosides of myricetin, quercetin, kaempferol, and isorhamnetin. Glucose, galactose, and glucuronic acid are the most common sugar units [Monagas et al (2005) in **Monagas** et al (2006)]. Flavonols are present in grape skins and grapevine leaves of both white and red cultivars. Quercetin derivatives are more abundant in grapevine leaves than kaempferol derivatives. Quercetin-3-O-glucuronide is the most important flavonol, followed by quercetin-3-O-glucoside (isoquercitrin), and kaempferol-3-O-glucoside [**Monagas** et al (2006)].

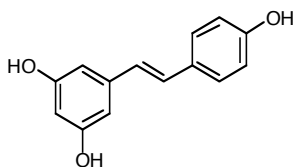


Figure 4 Resveratrol

trans-Resveratrol (3,5,4'-trihydroxystilbene) is an antimicrobial and antifungal compound that is naturally produced by grapevines upon infection. It is accumulated in grapevine leaves and grape skins in response to various fungal organisms, UV radiation, or chemicals [Jeandet et al (1995) and Langcake et al (1976) in **Orea** et al (2001)].

Grape seeds

Grape seeds contain procyanidins. They do not contain prodelphinidins or flavonoid compounds, such as anthocyanins and flavonols [Waterhouse et al (1995) in **Yamakoshi** et al (2002)]. Since 55% of the procyanidins that are extracted from grape seeds contain more than five monomer units, it is concluded that grape seeds contain a mixture of procyanidin monomers, oligomers, and polymers [Prieur et al (1994) in **Yamakoshi** et al (2002)].

Grape seed extracts are generally prepared by extraction with highly polar solvents (e.g. water or mixtures of short chain alcohols and water). Consequently, only water soluble compounds will be present. Grape seed extract is expected to contain mainly procyanidin dimers, trimers, tetramers, and their gallic acid esters. Some (epi)catechin and gallic acid and small amounts of procyanidin pentamers, hexamers, heptamers and their gallic acid esters are also expected to be present.

Grape skins

Grape skins contain procyanidins and prodelphinidins. Anthocyanins are only present in grape skins of red cultivars. They also contain flavonols and *trans*-resveratrol.

Grapevine leaves

Grapevine leaves contain procyanidins and prodelphinidins. Anthocyanins are only present in the grapevine leaves of red cultivars. They also contain flavonols and *trans*-resveratrol. Grapevine leaves also contain various other (non-phenolic) compounds like organic acids (e.g. mainly malic and oxalic acid, but also tartaric acid and traces of citric, fumaric, and succinic acid), carotinoids, and vitamin C [Beck (1997) in **Lardos** et al (2000)].

Grapevine leaf extracts are generally prepared by extraction with highly polar solvents (e.g. water or mixtures of short chain alcohols and water). Consequently, only water-soluble compounds will be present. According to the French Pharmacopoeia monograph, red vine leaf extract ('*extrait de vigne rouge*') should contain not less than 0.2% anthocyanins and not less than 4% polyphenols, which includes oligomeric proanthocyanidins and flavonols.

Support for claims regarding the use of grape seed extract
General applications

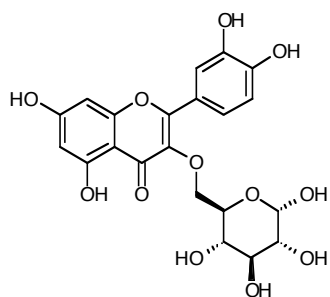


Figure 5 Quercetin-3-O- β -D-glucoside.

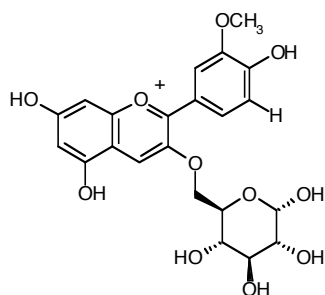


Figure 6 Peonidin 3-O- β -glucoside.

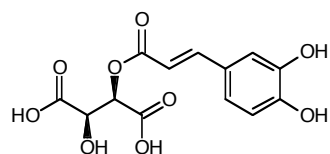


Figure 7 Caffeic acid, tartaric acid ester

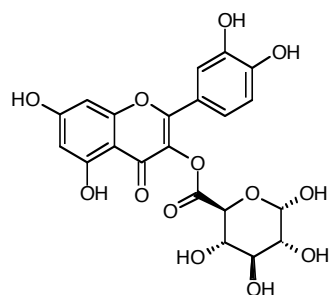


Figure 8 Quercetin-3-O- β -D-glucuronide.

According to the French Pharmacopoeia, identification of dry red vine leaf extract is carried out by thin layer chromatography (TLC), which monitors the following substances: quercetin-3-O- β -D-glucoside, peonidin 3-O- β -glucoside, tartaric acid ester of caffeic acid, and quercetin-3-O- β -D-glucuronide. Tartaric acid ester of caffeic acid unambiguously identifies the botanical source, as it is unique for the *Vitis vinifera* species [Jaworski et al (1987), Oszmianski et al (1990), and Macheix et al (1997) in Lazarus et al (1999)]. Quercetin-3-O- β -D-glucoside and quercetin-3-O- β -D-glucuronide are the major polyphenolic components of grapevine leaves [Monagas et al (2006)]. Peonidin 3-O- β -glucoside, an anthocyanin, is characteristic for the red grape skins and grapevine leaves.

General applications of grape seed extract

Antioxidative properties of grape seed proanthocyanidin extract

Free radicals have been implicated in various disease conditions in humans, including arthritis, haemorrhagic shock, atherosclerosis, advancing age, ischaemia and reperfusion injury, Alzheimer and Parkinson's disease, gastrointestinal dysfunctions, tumour promotion and carcinogenesis, and AIDS. Oligomeric proanthocyanidins, antioxidants that occur naturally in fruits, vegetables, nuts, seeds, flowers and bark, have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress. Grape seed proanthocyanidin extract is highly bioavailable and provides significantly greater protection against free radicals and free-radical induced lipid peroxidation and DNA damage than vitamins C and E, and β -carotene. It was also shown to exhibit cytotoxicity towards human, breast, lung, and gastric adenocarcinoma cells, while enhancing the growth and viability of normal human gastric mucosal cells. Furthermore, it provided significantly better protection against tobacco-induced oxidative tissue damage (lipid peroxidation and DNA fragmentation) and apoptotic cell death in human oral keratinocytes than vitamins C and E, singly or in combination. It also demonstrated excellent protection against paracetamol overdose-induced liver and kidney damage as well as myocardial ischaemia-reperfusion injury and myocardial infarction. Finally, it enhanced sun protection and improved chronic pancreatitis [**Bagchi** et al (2000)].

Reference data for the ORAC assay

As has been discussed before, it is recommended to analyse a number of food samples in order to obtain reference data for the ORAC assay. These reference data may be used to support the claim that grape seed extract capsules offer similar or better antioxidative activity than foods, which are well-known for their antioxidative properties or health benefits.

We suggest to analyse the following foods: pomegranates, cranberries, oranges, kiwis and apples (in order of decreasing expected ORAC value). Although pomegranates and cranberries may not be well-known to the general public, juices of these fruits are currently advertised for their antioxidative properties. Oranges, kiwis, and apples are interesting, since they are well-known to the general public and expected to show significantly different ORAC values. Although oranges and kiwis both contain large amounts of vitamin C, oranges are anticipated to show higher ORAC values, since they also contain polyphenolic compounds. Vitamin C and E reference material may also be analysed.

Based on the obtained results, it may be possible to include a statement on the container or in the information leaflet (e.g. 1 grape seed extract capsule is equivalent to 3 oranges). Alternatively, a graph may be included on the container or in the information leaflet, which shows the ORAC values of different foods and of grape seed extract capsules.

Conclusions regarding the use of grape seed extract

Taking into account the fact that the grape seed extract capsules will be marketed as a food supplement, the following (non-medical) claims may be allowed in the Netherlands, based on the Dutch so-called KOAG-KAG list (with English translation between brackets):

Support for claims regarding the use of grape seed extract
General applications

- 'versterkt de levenskracht' (enhances vitality)
- 'gunstige invloed op de gezondheid' (beneficial effect on health)
- 'voor behoud gezondheid' (for preservation of health)
- 'voor een gezond leven' (for a healthy life)
- 'verhoogt de weerstand van het organisme' (increases the resistance of the organism)
- 'ondersteunt het afweersysteem' (supports the immune system)
- 'aktiveert het afweersysteem' (activates the immune system)
- 'bevordert het (natuurlijk) afweersysteem' (strengthens the (natural) immune system)
- 'bevordert het (natuurlijk) afweersysteem van de lichaamscellen' (strengthens the (natural) immune system of the body cell)
- 'helpt het (natuurlijk) afweersysteem van de lichaamscellen' (supports the (natural) immune system of the body cell)
- 'ondersteunt het immuunsysteem' (supports the immune system)
- 'stimuleert het immuunsysteem' (stimulates the immune system)
- 'gunstige invloed op ouderdomsverschijnselen' (beneficial effect on symptoms of old age)
- 'beschermt tegen ouderdomsverschijnselen' (protects from symptoms of old age)
- 'versterkt zowel inwendig als uitwendig bij ouderdomsverschijnselen' (enhances both internally and externally in case of symptoms of old age)
- 'beschermt bij veroudering' (protects from aging)
- 'verhoogt de vitaliteit' (enhances vitality)
- 'houdt jong' (keeps young)
- 'houdt uw uiterlijk fris en jeugdig' (retains/keeps your appearance fresh and youthful)
- 'ter aanvulling van anti-oxydanten' (to supplement antioxidants)
- 'bij veroudering door vrije radicalen' (in case of aging by free radicals)
- 'ter bescherming tegen vrije radicalen' (for the protection against free radicals)
- 'vrije-radicalen vanger' (free radical scavenger)
- 'ter bescherming van gezonde cellen en weefsels' (for the protection of healthy cells and tissues)
- 'celbeschermend' (cell protective)
- 'voor behoud van gezonde cellen en weefsels' (for the preservation of healthy cells and tissues)
- 'ondersteunt herstel/aansterken na ziekte' (supports the recovery/recuperation after disease)
- 'versnelt herstel na ziekte' (accelerates recovery after disease)
- 'in herstelperiodes' (during periods of recovery)

The most interesting claims have been underlined.

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