The Truth About OPCs

By Debasis Bagchi, Ph.D.

95% OPCs … 20-50 times more potent than vitamins C and E … all grape seed extracts are the same … These are some of the myths surrounding an increasingly popular, yet poorly understood dietary supplement. Following is information intended to dispel the myths and to disclose the truth about OPCs, one of the most important new dietary supplements of the decade.

OPC is the acronym for "oligomeric proanthocyanidins," a class of polyphenolic bioflavonoids found in fruits and vegetables, which are highly concentrated in the seeds of grapes and the bark of maritime pine trees. OPCs are powerful antioxidants shown to be far more potent than the common antioxidants vitamins C, E and beta-carotene. In fact, their usefulness in fighting free radicals and helping people lead a longer, healthier life is becoming well established in the scientific literature.

The problem is, there is a wide degree of variability between the composition and biological value of the various grape seed and pine bark extracts available. There is also a lack of product standards and analytical methods available to develop and verify uniform product potency claims. Consequently, consumers, retailers and even manufacturers are often at a loss in evaluating the quality and potency of the various OPC products or raw materials available to them.

Grape Seed Extract Standards Committee

Over the past two years, members of the nutritional products industry have been meeting to discuss the various labeling and analytical challenges facing manufacturers of grape seed products. The goals of the Grape Seed Extract Standards Committee include: 1) developing test methods to verify that the product is what it says it is (i.e. a grape seed extract), 2) defining quantifiable nutrient claims, such as milligrams or percent OPC and/or polyphenolics, and 3) developing testing methods to accurately and reliably validate quantifiable nutrient claims. The recommendations of the Grape Seed Committee are to be forwarded to the National Nutritional Foods Association (NNFA) Compliance Committee for adoption as the industry standard.

Unfortunately, the Committee's task is not as simple as it seems. Currently, *there is no accurate or reliable method for quantifying OPC labeling claims*. In addition, there is disagreement in defining exactly what OPCs are.

A Very Complex Product

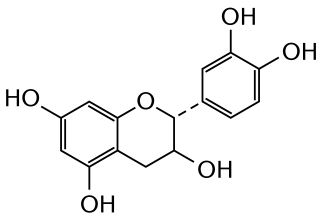
Grape seed and pine bark extracts consist of literally hundreds of known, and perhaps thousands of unknown, naturally occurring substances. In addition to OPCs, quercetin, ferulic acid, caffeic acid, coumaric acid and myricetin are some of the biologically active compounds that have been identified in grape seed and pine bark extracts. The amount and types of compounds present in a particular grape seed or pine bark extract can vary and is greatly influenced by the extraction process, as well as the source and variety of seeds or bark used.

The predominant compounds in grape seed and pine bark extracts are the OPCs, which are made up of proanthocyanidin sub-units called "monomers." The term "oligomeric" simply means more than one. Thus, oligomeric proanthocyanidins consist of two or more monomers

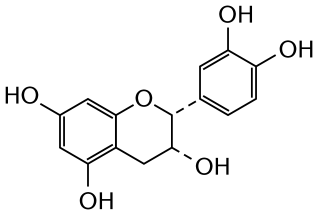
AV1-991007-000309

chemically linked together. OPCs containing two monomers are called dimers. Three monomers are called trimers. Four are called tetramers. Etcetera.

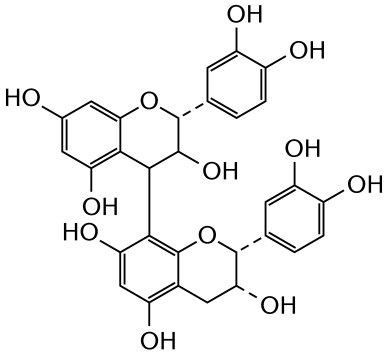
To illustrate the complexity of compounds that exist in grape seed and pine bark extracts, consider this: There are basically two proanthocyanidin monomers called “catechin” and “epicatechin.” Each of these bind at either the alpha or beta position on their molecular structures to form dimers, trimers, etc. In addition, catechin and epicatechin can also form numerous esters from gallic acid, called catechin or epicatechin “gallate,” as well as various sugar and protein molecules called “glycosides” and “peptides,” respectively. In fact, the total number of possible dimers, including gallic acid and glucose esters alone, are 162. And each time you add an additional monomer, the total number of possible combinations increases geometrically; trimers jump to over 550, tetramers over 1,200, etc. The result is, unlike a single nutrient compound such as vitamin C (ascorbic acid) or vitamin E (d-alpha tocopherol), OPCs are much more complex and difficult to identify and quantify.



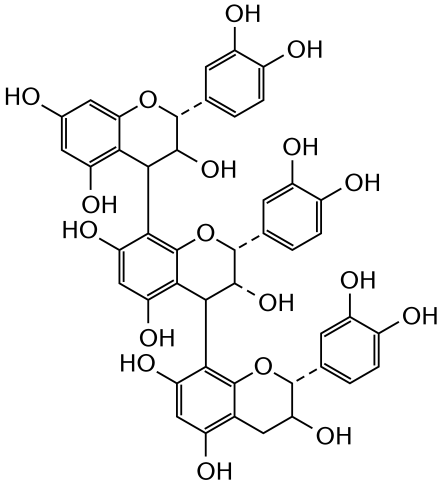
Catechin



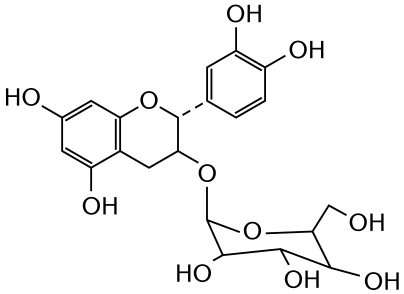
Epicatechin



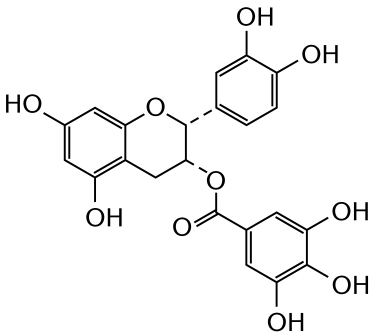
Catechin-Epicatechin Dimer



Catechin-Epicatechin-Catechin Trimer



Catechin Glycoside



Epicatechin Gallate

Challenges of Defining OPCs

The complexity of OPCs, as well as the diverging interests of many of the companies who sell grape seed and pine bark extracts, underscores the difficulty the Grape Seed Committee has in defining OPCs. Quite literally, OPCs consist of two or more proanthocyanidin monomers. However, this definition would include long chains of monomers called polymers and tannins. Some parties believe that polymers and tannins should not be considered OPCs. Others feel differently. In addition, there is general disagreement in defining what a polymer is. Five or more monomers? Seven or more? Ten or more?

Although there is evidence to suggest that smaller OPCs, such as dimers, trimers and tetramers, are more soluble and bioavailable than larger OPCs, there is also evidence to suggest that less soluble polymers and tannins are beneficial as well. Recently, Hagerman, et al., have shown that polymeric proanthocyanidins and tannins are 15 to 30 times more effective at quenching free radicals than are simple polyphenols.[1] Since polymers and tannins are not well absorbed, the researchers suggest that they could exert their antioxidant activity within the digestive tract to protect lipids, proteins and carbohydrates from oxidative damage during digestion, and spare absorbable antioxidants such as vitamins E, C and beta-carotene.

As of March 13, 1999, the consensus of the Committee was to define OPCs as all proanthocyanidins containing two or more monomers, including polymers and tannins.

What About Monomers?

Grape seed and pine bark extracts also contain monomers, some as high as 30%, others as low as one percent, depending upon the extraction process. However, grape seed and pine bark extracts are poor sources of monomers in that extracts derived from green tea provide a superior and less expensive source of monomers. Whereas green tea extracts are poor sources of OPCs, they contain significantly higher levels of the most beneficial monomers, including epigallocatechin gallate (EGCG), than do grape seed or pine bark extracts. OPCs also confer properties that monomers don't, such as collagen formation. While a combination of a high quality grape seed or pine bark extract plus a high EGCG green tea extract would provide the best of both worlds, the goal of a grape seed or pine bark extract should be to deliver the greatest amount of OPCs and the least amount of monomers.

The 95% OPC Myth

Various attempts have been made to measure the total OPC content of grape seed and pine bark extracts. The most ill-fated attempt, called the "Procyanidolic Index," has resulted in tremendous confusion in the marketplace and the misbranding of the majority of OPC products on the market today.

The Procyanidolic Index (also called the Bates-Smith Assay) involves a testing method that measures the change in color when the product is mixed with certain chemicals; the more change in color, the more OPC is present. However, the Procyanidolic Index is a *relative* value that can measure well over 100. Unfortunately, a Procyanidolic Index of 95, which appeared early on the specification sheet of a supplier's grape seed extract, was erroneously taken to mean 95% OPC and labeled that way on the finished product. In order to be competitive, many other manufacturers followed suit, erroneously labeling their products 95% OPC.

The simple fact of the matter is, there is absolutely no data available to support this claim and all current methods of analysis suggest that the actual OPC content of these products is much lower than 95%.

The Porter Assay

Another problem with the Procyanidolic Index is reproducibility. Many analytical laboratories have reported difficulty in obtaining consistent results on identical samples repeatedly tested or tested at another laboratory. This problem was largely overcome by an improved colorimetric test called the Porter Assay, which is the most common OPC assay currently in use.

The Porter Assay is a *qualitative* test that approximates the amount of OPCs present. It does not tell you the types or amounts of OPCs present. In fact, a high Porter value does not necessarily equate to a better product. Porter measures only OPC's—not monomers. It also gives higher results when there are more long chain OPCs present. Thus, a very high Porter value, say above 350, may mean a product containing mostly polymers or tannins.

Generally, a Porter value of between 250 and 350 is considered optimum. However, a Porter value of 300 could just as well indicate a large quantity of smaller OPCs, such as dimers, trimers and tetramers, as it could a mix of monomers and polymers. What does this mean? That's a good question, because currently there is simply not enough data available to draw any conclusions regarding the bio-efficacy of individual proanthocyanidin constituents.

High Performance Liquid Chromatography

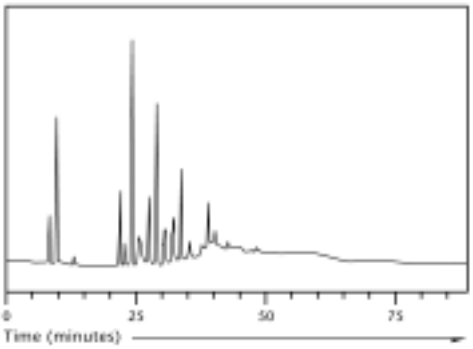
High Performance Liquid Chromatography (HPLC) is a method of analysis often used to determine a product's chemical composition. But it too has failed to provide an accurate means for determining the types and amounts of OPCs present in a given product.

For starters, HPLC is dependent upon "reference standards"—highly purified samples of the compounds you are testing for. Reference standards are run in tandem with product samples so that the HPLC "peaks" of these known compounds can be compared to the peaks of the product samples. By matching the reference standard peaks to the product sample peaks you can identify and quantify individual compounds in the product sample. This procedure works fine when you are trying to identify one or two compounds. But when you have potentially hundreds of compounds to identify and quantify, such as the myriad of constituents in a grape seed or pine bark extract, it is virtually impossible to do. What's more, there is simply a lack of OPC reference standards available to begin with.

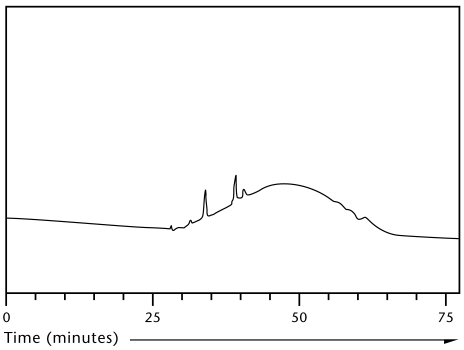
Peaks Versus Humps

Another limitation of HPLC is that it favors more highly refined OPC extracts, i.e. extracts manufactured using chemical solvents that strip away many of the naturally occurring constituents present in grape seeds. As a result, the HPLC chromatographs of these extracts show up as many distinct peaks and valleys, while the HPLC graphs of extracts that do not use chemical solvents appear as less distinct hump-like curves (see charts).

Why is this? Nobody knows for sure, but it may be due to a more complex matrix of naturally occurring compounds, such as OPC glycosides or peptides, present in extracts made without chemical solvents. These compounds may interfere with conventional HPLC analysis, resulting in a characteristic "hump."



HPLC Chromatograph of grape seed extract made with chemical solvents



HPLC Chromatograph of grape seed extract made without chemical solvents

Mass Spectrometry

Mass Spectrometry is a highly sophisticated method of analysis that actually breaks down molecules into small fragments that can be utilized to determine the structure of the molecule. Used in combination with HPLC, scientists have been able to decipher many of the OPC constituents of grape seed and pine bark extracts. However, Mass Spectrometry is a very sophisticated and expensive method of analysis, and is not practical for routine testing such as quality control analysis.

Total Polyphenolics

In the absence of an accurate and reliable means of identifying and quantifying OPCs, some manufacturers are beginning to report levels of total polyphenolics on labels and in advertising claims. While total polyphenolics represent a broader category of constituents than just OPCs, the majority of polyphenolics in grape seed and pine bark extracts are OPCs and the methods of analysis for identifying and quantifying total polyphenolics are better established. However, methods of analysis for determining polyphenolics are not with out their short-comings as well.

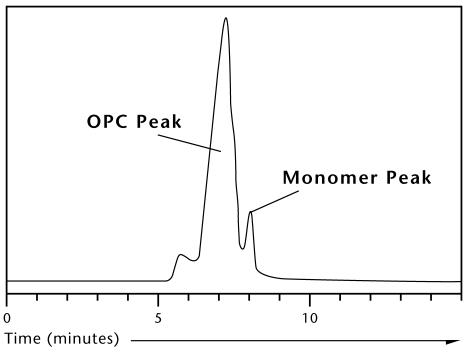
The main tests for measuring polyphenolics are the Folin-Denis and Folin-Ciocalteu methods. The Folin-Ciocalteu method, also called the GAE or Gallic Acid Equivalence method, which is the newer and more commonly used method, uses *gallic acid* as an arbitrary reference standard. Gallic acid is a phenolic compound containing two hydroxyl (OH) groups that react with a chemical reagent to produce a blue color, the intensity of which is measured by a color meter.

The hydroxyl groups of the polyphenolic compounds in grape seed extracts also react with the same chemical reagent to produce a blue color which, when compared to the colorimetric index of a known quantity of gallic acid, can be used to determine the amount of polyphenols present in a grape seed extract. The problem is, the polyphenolic constituents in grape seed extracts contain varying numbers of hydroxyl groups (i.e. one, two, three or more hydroxyl groups per molecule), which produce different results relative to gallic acid. In other words, two grape seed extracts with the same amount of polyphenols but different compositions can produce two different results using the GAE method.

Another problem with the GAE method is that it does not distinguish between monomers and oligomers. Thus, a product containing high levels of less desirable monomers can produce the same results as a product containing few monomers, but more desirable oligomers.

Gel Permeation Chromatography

The newest method of analysis being investigated by the Grape Seed Committee is Gel Permeation Chromatography or GPC. Like the GAE method, GPC measures polyphenols and is not specific for OPCs. The advantages of GPC are its accuracy and reliability, as well as its ability to distinguish monomers from oligomers (see chart). However, GPC also requires a reference standard in order to obtain *absolute* quantitative results, which are necessary for product labeling claims. Different reference standards yield different results, and researchers are working to determine what reference standard will work best. So while a method of analysis for determining quantifiable label claims has yet to be finalized, it appears that one may be close at hand.



GPC Chromatograph of grape seed extract

OPCs Versus OPPs

With no accurate and reliable means of identifying and quantifying OPCs in sight, the consensus if the Grape Seed Committee is moving towards a quantifiable label claim for polyphenolics. In order to differentiate grape seed and pine bark extracts from other simple polyphenolic products such as green tea extracts, which consist mostly of polyphenolic monomers, Grape Seed Committee members have proposed that the oligomeric polyphenolics, or OPPs, be used in the labeling of grape seed and pine bark extracts. OPPs are quantifiable and can be distinguished from monomers in the GPC method of analysis. In addition, OPPs in grape seed and pine bark extracts consist primarily of OPCs, the principle active ingredient in these products which the market has come to know.

20-50 Times More Potent Than Vitamins C and E?

Another myth that has been erroneously perpetuated about OPCs is that their antioxidant activity is 20 times more potent than vitamin C and 50 times more potent than vitamin E. In truth, the research used to support the vitamin E claim was done in a test tube study on a *single* isolated OPC compound derived from sources other than grape seed or pine bark extracts.[2] The vitamin C study was also done in a test tube using a pine bark extract (grape seed extract was not tested in this study).[3] In addition, the results of the vitamin C study were cited in a manufacturer's product patent and not reported in a peer-reviewed scientific journal.

Test tube studies, although indicative of a product's potential effectiveness, do not necessarily reflect the product's effectiveness in a living system. In fact, no study has ever shown that a grape seed or pine bark extract is 20-50 times more potent than vitamins C and E in a living system. However, studies in animals[4] and human cell cultures[5] have show that the

antioxidant activity of a commercially sold grape seed extract (ActiVin™) is significantly greater than vitamins C, E and beta-carotene. But to claim that these products are 20-50 times more potent is an exaggeration of the truth.

But Does It Work?

While considerable time and effort have been spent on the composition and analysis of OPC products, the real question is, do they work? Current methods of analysis such as Porter, GAE, HPLC and GPC are potentially useful for monitoring production batch consistency, determining product composition and quantifying ingredient label claims, but none of these analyses actually tell you if the product works. They don't tell you if it's bioavailable, biologically active, efficacious or even safe.

Research shows that many of the components of grape seed and pine bark extracts, such as OPCs, are biologically active and efficacious. However, grape seed and pine bark extracts contain more than just OPCs. They contain hundreds of known, as well as unknown, naturally occurring substances, many of which may possess biological benefits yet to be discovered.

In addition, due to manufacturing and other variances, there is a wide degree of variability between the composition and biological value of the various grape seed and pine bark extracts available. For example, extracts produced using chemical solvent are quite different from water and ethanol extracts. With respect to grape seed extracts, the variety of grape seeds used as well as seed separation, drying and storage can have a significant impact on product quality and composition.

So how do you know which ones work? The ones *proven* to work in well-controlled scientific studies published in peer-reviewed journals. Research on a given grape seed extract can be obtained by contacting the source manufacturer. But don't just ask for any research. Ask for research done *specifically* on the grape seed extract you are inquiring about. Many grape seed extract manufacturers are currently using the research done on other products to support the efficacy of their own. Since grape seed extracts vary widely in their chemical composition, there are no guarantees that one product works the same as another.❑

Debasis Bagchi is an adjunct professor at Creighton University, Omaha, Nebraska, a member of the Grant Review and Selection Committee of the National Institutes of Health and director of research for InterHealth Nutraceuticals. Dr. Bagchi is author of six books and over 100 published studies in peer-reviewed scientific journals.

REFERENCES

1. Hagerman A, et al., High Molecular-Weight Plant Polyphenolics (Tannins) as Biological Antioxidants, *J. Agric. Food Chem*., 46:1887-92, 1998.
2. Uchida S, et al., Condensed Tannins Scavenge Active Oxygen Free Radicals, *Med. Sci. Res*., 15:831-832, 1987.
3. U.S. Patent 4,698,360.
4. Bagchi D, et al., Oxygen Free Radical Scavenging Abilities of Vitamins C and E, and a Grape Seed Proanthocyanidin Extract *In Vitro*, *Res. Comm. Mol. Path. and Pharm.,* 95(2):179-189, 1997.
5. Bagchi M, et al., Smokeless Tobacco, Oxidative Stress, Apoptosis and Antioxidants in Human Oral Keratinocytes, *Free Radical Biology & Medicine* 26:992-1000, 1999.