ABC AHP NCNPR
Botanical Adulterants Program
BOTANICAL ADULTERANTS BULLETIN

on Adulteration of Grape Seed Extract

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Goal: The goal of this bulletin is to provide timely information and/or updates on issues of adulteration of grape seed extract (GSE†) to the international herbal products industry and extended natural products community in general. It is intended to present the available data on the occurrence of adulteration, the market situation, and consequences for the consumer and the industry.

1 General Information

1.1 Common name: Grape[2]

1.2 Other common names:

English: European grape, wine grape[2]

Chinese: *Pu tao* (葡萄)

French: Raisin

German: Traube, Weintraube

Italian: Uva

Spanish: Uva

1.3 Accepted Latin binomial: *Vitis vinifera*[2]

1.4 Synonyms: *Cissus vinifera*[3,4]

1.5 Botanical family: Vitaceae

Grape *Vitis vinifera*
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1.6 Plant part and extract production method: The seeds of grapes, obtained as a by-product from the juice or wine industry, are used fresh, or more commonly dried, to produce a liquid extract using a solvent (e.g., water, or mixtures of water with ethanol or acetone), which is filtered, and may be subjected to further processing before it is typically spray-dried to obtain a dry extract containing high levels of naturally occurring grape seed phenolic compounds.

1.7 General use(s): GSE is known as a dietary ingredient and a number of commercial materials have received self-affirmed "generally recognized as safe" (GRAS) status as a food additive; it contains phenolic compounds with antioxidant properties for use in dietary supplements, nutritionally enhanced beverages, and functional foods. The most significant application for GSE is as an ingredient in dietary supplements (known as "food supplements" in some countries outside the United States).

2 Market

2.1 Importance in the trade: Due to the widespread history and acceptance of grapes and wine, GSE has received acceptance almost globally as an ingredient for human consumption. It is one of the more widely used botanical extracts, due to increasing scientific findings supporting health benefits. However, it remains a specialty item relative to global commodities. In the United States, GSE has ranked among the top 20 best-selling dietary supplements in the Food, Drug and Mass Market channel (excluding sales at Walmart) from 2008-2011, but not in 2012, with sales between US $1.4 million and $2.8 million.[5-9] Sales in the Mainstream Multi-Outlet channel (the new name for the Food, Drug and Mass Market channel) were down to US $1.1 million and $0.9 million in 2013 and 2014, respectively, ranking GSE at 67th in 2014. Sales in the Natural channel (excluding sales at Whole Foods Market, a major natural products retailer in the US) were US $1.5 and $1.3 million in 2013 and 2014, respectively, with GSE ranking 59th in 2014 (T. Smith [American Botanical Council] e-mail to S. Gafner, September 3, 2015). (See Table 1)

2.2 Market dynamics: GSE was at the height of its popularity in the early 2000s, with global sales of US $60 million in 2000.[10] According to data from *Nutrition Business Journal*, sales in 2011 were approximately $25 million.[11] The largest US producer of GSE is Polyphenolics, a division of Constellation Brands. Other key players in the market (in the United States and internationally) include Indena, Naturex, and Nexira. A number of Chinese manufacturers, e.g., Skyherb and JF Natural, are also active in the US GSE market. The primary application for both Europe and United States is dietary/food supplements.

2.3 Supply sources: GSE is supplied by all major grape-producing countries and regions; the main producers are the United States, Canada, Europe, South America, South Africa, and Australia.

2.4 Raw material forms: GSE is almost exclusively supplied to dietary supplement manufacturers in the form of a dry extract. The extract contains phenolic compound concentrations ranging from ca. 50-90% of the extract and sometimes there is further characterization of the phenolic compounds. The main phenolic compounds are flavan-3-ol monomers and polymers and their gallic acid esters. The polymers are known as proanthocyanidins (PACs); the term *oligomeric proanthocyanidin* (OPC) is not well defined in the sense that the number of monomer units in an oligomer varies among authors, but most often it is limited to a maximum of 10 units. Grape seeds contain predominantly B-type PACs, which are flavan-3-ol polymers where the units are linked by a single bond (Figure 1). The extract has a characteristically bitter and astringent taste. Various companies manufacture their own GSE from purchased grape seeds. Intentional adulteration of GSE can occur at the extraction facility in order to artificially increase the concentration in total phenols and to increase the volume by using other PAC-rich substances (see Section 3 below). Contract manufacturers for the dietary supplement and food industries purchase bulk dry GSE extract and produce dietary supplements and/or beverages.

3 Adulteration

3.1 Known adulterants: Peanut (*Arachis hypogaea*, Fabaceae) skin extract, pine (*Pinus* spp., Pinaceae) bark,

Table 1: Sales data for grape seed extract dietary supplements in the United States from 2012-2014.

Channel	2011		2012		2013		2014	
	Rank	Sales [US$]	Rank	Sales [US$]	Rank	Sales [US$]	Rank	Sales [US$]
Natural[a]	n/a	1,553,155	52	1,481,374	55	1,518,647	59	1,327,495
Mainstream Multi-Outlet[b,c]	17	1,261,907	37	3,468,122	64	1,075,951	67	900,560

[a]According to SPINS (SPINS does not track Whole Foods Market sales, which is a major natural products retailer in the US)
[b]According to SPINS/IRI (the Mainstream Multi-Outlet channel was formerly known as food, drug and mass market channel [FDM], exclusive of possible sales at Walmart, a major retailer in the US and beyond).
n/a: not available
[c]Data for 2012 are according to Symphony/IRI and include Walmart, club stores (Sam's, Costco), military and dollar stores
Source: T. Smith (American Botanical Council) e-mail communications, September 2, 2015 and September 3, 2015, and reference 16.

green tea (*Camellia sinensis*, Theaceae) extract, and PAC (proanthocyanidin)-rich (e.g., propelargonidin-containing) extracts from non-grape seed sources.[1,13]

Propelargonidins, a particular type of PAC, are found in the following plants and plant materials: raspberry (*Rubus idaeus* subsp. *idaeus* or *R. idaeus* subsp. *strigosus*, Rosaceae), strawberry (*Fragaria vesca* or *F. virginiana*, Rosaceae), common beans (*Phaseolus vulgaris*, Fabaceae), almond (*Prunus dulcis*, Rosaceae), cinnamon (*Cinnamomum verum*, Lauraceae), buckwheat (*Fagopyrum esculentum*, Polygonaceae), mountain ash (*Sorbus aucuparia*, Rosaceae) berries, hops (*Humulus lupulus*, Cannabaceae), and green tea.[14-18] The fact that the species above contain propelargonidins does not mean that they have been used as adulterant of GSE.

3.2 Sources of information confirming adulteration: There are at least four reports (one publication and three conference presentations) on GSE adulteration to date. Villani et al. analyzed the PACs in authentic GSEs, pine (*Pinus* spp., Pinaceae) bark, and peanut (*Arachis hypogaea*, Fabaceae) skin extracts, and in 21 commercial GSE products that were obtained from a variety of sources, including dietary supplement retailers, supermarkets, and online vendors.[1] Overall, in six of the commercial samples, GSE was considered to be substituted with peanut skin extract, while an additional three samples showed evidence of admixture of an ingredient containing A-type PACs, inconsistent with the chemical profile of GSE. Based on the evaluation of the HPLC-LC/MS profile, the adulterant appears to be peanut skin extract. Cases of adulteration of commercial GSEs with peanut skin extracts were also presented by Sudberg et al. (2014)[19] The results were similar to those of the Villani study.[1] In addition, evidence for GSE adulteration was presented in lectures at two conferences. One lecture reported on the detection of PACs (e.g., propelargonidins) from non-grape seed sources in products labeled as GSE,[13] while the other exposed a case of GSE spiking with gallic acid and epicatechin.[20]

3.3 Accidental or intentional adulteration: The motivation behind purposeful adulteration in commercial products is financial gain and to increase the concentration in PACs (aka economically motivated adulteration). Peanut skin extract, which is a high-volume byproduct of the peanut industry, is less expensive and typically available at a much greater volume than GSE. In the United States, a typical peanut mill may produce up to 17 tons of peanut skins per week, and the material sold for as little as US $0.02/kg in 2009.[21] For example, in China, in 2015, the price for peanut skin extract is at US $10-13/kg, pine bark extract at US $20-22/kg, and GSE at US $30-35/kg, although proprietary GSEs may be sold for up to US $110/kg (X. Jin, overseas sales manager at the dietary supplement manufacturer Skyherb, e-mail to S. Gafner, August 31, 2015). Thus, a bulk distributor of GSE or another manufacturer along the value chain can take advantage of the chemical similarity between GSE and peanut skin extract since the spectrophotometric assays typically used in industry are not specific enough to discriminate between grape seed PACs and PACs from other plant extracts. Due to reliance on non-specific proximate assays across the value-chain, adulteration can go undetected downstream in the commodity chain, such as those involved in distribution, packaging, wholesale, and retail sales.

3.4 Frequency of occurrence: There is limited data available on the extent of the adulteration from the available studies. Villani et al., analyzed 21 commercial GSE products that were obtained from dietary supplement retailers and supermarkets in the United States, and from online vendors, and concluded that nine products (43%) had evidence of adulteration with peanut skins.[1] In the study by Sudberg et al., out of the five commercial GSEs analyzed by high-performance thin layer chromatography (HPTLC, Figure 2), four extracts (80%) showed bands that are characteristic of peanut skin extract.[19] Using the same HPTLC approach, testing of 254 commercial GSE samples performed by Alkemist Labs, a contract analytical testing laboratory, between August 2014 and January 2016 found the presence of peanut skin extract in 67 (26%) samples (H. Johnson e-mail to S. Gafner, January 22, 2016). This suggests that GSE adulteration in the market is not uncommon.

3.5 Possible safety/therapeutic issues: The adulteration of GSE with peanut skin extracts has the potential to be damaging to consumers and the dietary supplement industry. Peanuts are a common allergen worldwide. Because of this, the US Food Allergen Labeling and Consumer Protection Act requires that all packaged food products sold in the United States that contain peanuts as an ingredient must list the word "peanut" on the label. Any peanut-containing or peanut extract-containing product that is not labeled accordingly creates a situation in which the consumer is not only deceived by buying a product that is not what it is purported to be, but due to the allergenic potential of peanuts in general (even if the allergenicity of processed peanut skins is lower than that for peanuts them-

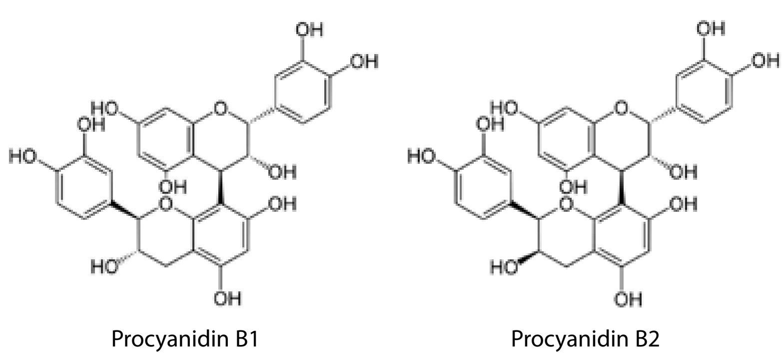

Figure 1: Chemical structures of the predominant proanthocyanidins in GSE[19]

selves),[22,23] it also represents a potential safety risk. In the United States alone, the prevalence of people sensitive to peanuts or tree nuts was estimated to be 1.4% in 2008.[24] The self-determined prevalence of peanut allergies worldwide ranges from 0% in 18-month-old children from Iceland to 15% for a group of 15–17-year-olds from France.[25] Considering that peanut skin extract contains compounds similar to those in GSE, it is not known if efficacy is compromised.

3.6 Analytical methods to detect adulteration: There are only a few published methods for the detection of GSE adulteration. High-performance thin-layer chromatography (HPTLC) was successfully used to detect adulteration with peanut skin extract.[1,19]

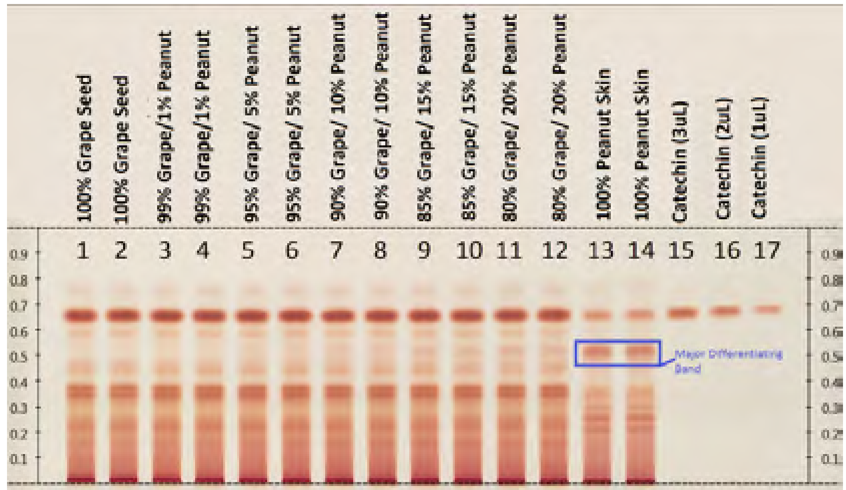

Figure 2: HPTLC analysis of authentic GSE (lanes 1-2), mixtures of GSE and peanut skin extract (lanes 3-12), authentic peanut skin extract (lanes 13-14), and the standard compound catechin (lane 20). Concentrations for extracts were ca. 100 mg/mL, and 0.5 mg/mL for catechin. Image courtesy Alkemist Labs; Costa Mesa, CA.[26]

Villani et al., also used high-performance liquid chromatography with ultraviolet and mass spectrometric detection (HPLC-UV/MS) to obtain a chemical fingerprint of grape seed, peanut skin, and pine extracts.[1] While both analytical approaches allowed the distinction between grape seed and peanut skin extracts, GSE and pine bark extract were found to have a remarkably similar qualitative profile of PAC monomers and dimers. However, GSEs were generally found to contain larger amounts of PACs than pine bark extracts. The chromatograms were submitted to cluster analysis, and while GSEs were easily distinguished from peanut skin extracts, the lower quality GSEs (i.e., those extracts containing lower concentrations of PACs) clustered with the pine bark extracts.[1] HPLC and HPLC-MS analyses were used by Kelm et al. to differentiate authentic and commercially obtained GSEs. Atypical peaks observed in HPLC profiles were further evaluated by HPLC-MS/MS, allowing the investigators to characterize structures that are un-characteristic of the PACs found in grape seeds; therefore, this approach is effective for detecting adulteration.[13]

For other methods, such as testing for peanut allergens, or genetic methods to detect peanut DNA, there are no published data available that have verified their fitness for the purpose of detecting GSE adulteration with peanut skin extracts.

3.7 Perspectives: Adulteration of GSE has been exposed only recently, but seems to be widespread. According to GSE producers, many GSE products sold on the Chinese market are adulterated (X. Jin e-mail to S. Gafner, October 2, 2015). Demand for GSE is expected to increase with more health benefits supported by human clinical studies, increasing the demand in the extract, increasing the risk of adulteration, and potentially eroding consumer confidence, safety, and efficacy if adulterants are used.

4 Conclusions

Adulteration of GSE in commercial products appears to be a significant problem. Villani et al., determined that out of the 21 commercial products, six samples contained no detectable quantities of GSE, and were composed primarily of peanut skin extract as determined by comparison to authentic peanut skin.[1] Adulteration with peanut skin extract represents a significant safety concern due to the possibility of reactions to peanut allergens. In addition, peanut skins are much less expensive than GSE and sale of adulterated lower-cost material has a significant economic impact. Companies producing authentic GSE cannot compete with adulterated products and lose sales due to consumers making a price-oriented purchasing decision. More importantly, those manufacturers that unknowingly buy adulterated products and perform analytical tests that are easily fooled are being defrauded and at risk of regulatory actions due to their GSE products being non-compliant with current Good Manufacturing Practice (cGMP) requirements.

One of the primary reasons that adulteration goes undetected is due to manufacturers relying on unspecific spectrophotometric methods for quality control of their materials. While spectrophotometric assays can provide reliable results for the contents in total phenolics, HPTLC and HPLC-UV/MS are more appropriate for the purpose of GSE identification.

*The acronym GSE should not be confused with acronym GFSE, referring to Grapefruit Seed Extract, which is an entirely different material. In some original publications on GFSE adulteration, the authors use “GSE” to refer to grapefruit seed extract.

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REVISION SUMMARY

Version # , Author,	Date Revised	Section Revised	List of Changes
Version 1, S. Kupina, S. Gafner new	n/a	n/a	none