ABC AHP NCNPR
Botanical Adulterants Program
BOTANICAL ADULTERANTS BULLETIN

on Adulteration of Grapefruit Seed Extract

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Goal: The goal of this bulletin is to provide timely information and/or updates on issues regarding adulteration of grapefruit *(Citrus paradisi*) seed extract to members of the international herb industry and the extended natural products community in general.

1. General Information

1.1 Common name: Grapefruit seed extract (GFSE)[1]

1.2 Other common names: There are commercial names for various GFSE products in the marketplace. Note: many products and some literature refer to grapefruit seed extract as GSE, but this acronym is commonly accepted as referring to grape (*Vitis vinifera,* Vitaceae) seed extract, an entirely different and distinct plant and product. The ABC-AHP-NCNPR Botanical Adulterants Program uses GFSE in order to distinguish the two separate materials.

French: Extrait de pépins de pamplemousse

German: Grapefruitkern Extrakt

Italian: Estratto di semi di pompelmo

Spanish: Extracto de semilla de pomelo

1.3 Accepted Latin binomial: *Citrus paradisi* [2]

1.4 Synonyms: *Citrus* x *paradisi* [1]

1.5 Botanical family: Rutaceae

1.6 Plant part and form: Extracts of ground seeds (sometimes also containing ground peel and/or pulp) made with water, or water plus additional solvents (e.g., glycerin-water mixtures).[3,4]

Saw Palmetto *Serenoa repens*
Photo ©2017 Steven Foster

On its website, a major supplier provides a summary of its manufacturing process, which entails treatment of an aqueous extract of seeds, and spent (from juicing) ground peel and pulp with ascorbic acid and ammonium chloride under heat and pressure, then heating with hydrochloric acid.[5] Another supplier of finished products provides a bit more detail on this process (or a very similar one).[6] Also noteworthy is the original patent[7] on which these preparations are purportedly based; it contains elements of the preparations described above, but is far more complex and includes the use of tetrachloroethylene, ethylene glycol phenyl or ethyl ethers, 2,3,5,6-tetrachloro-4-(methylsulfonyl) pyridine, 3,4,5-tribromosalicylanilide, 2,4,4'-trichloro-2'-hydroxydiphenyl ether (triclosan), and at least one substituted dimethyl benzyl ammonium chloride. (These last four ingredients are all known synthetic microbicides, and the latter two are microbicides that have been reported to be present in commercial GFSE products). The grapefruit portion of the reaction mixture/formula is described as the product of reacting grapefruit pulp (not seeds!) with a nontoxic polyhydric alcohol (e.g., glycerin or propylene glycol) under ultraviolet light.[7]

1.7 General use(s): To the author's (and peer reviewers') knowledge, neither grapefruit seed extract nor grapefruit seed, nor its preparations, has any documented use in traditional herbal medicine practices or associated materia medica. For example, no citrus seed, its extracts, or other preparations thereof have been advocated for use in infectious disease by either Traditional Chinese or Ayurvedic medicine.[8]

GFSE is currently marketed primarily for its professed antimicrobial and preservative properties.

2. Market

2.1 Importance in the trade

2.2 Supply sources: Grapefruit seeds are abundantly available as byproducts of the citrus juice industry.

2.3 Raw material forms: Whole or ground dried seeds; in some instances, ground peel and pulp are also included.[3,4]

3. Adulteration

3.1 Known adulterants: The secondary metabolites of grapefruit seeds are predominately limonoids and flavonoids. Naringin, a diglycoside of the common flavanol naringenin, is the dominant flavonoid in grapefruit seeds.

Limonoids are a unique subset of triterpenes in which the conventional triterpene skeleton is significantly oxidized and cleaved in one or more places. Seven limonoids, along with seven limonoid glycosides (the glycosides have one or several sugars attached to the triterpene core), have been reported from grapefruit seeds.[9-14] Limonin, the most abundant grapefruit seed limonoid, comprises ~0.5% of the dry weight of the seeds,[15] and the total limonoid content could approach 1%.[16]

It is noteworthy that none of the published analyses of commercial grapefruit seed extracts has indicated the presence of either limonoids or flavonoids in those products, except for the most recent paper in the series,[4] even though limonoid glycosides have been isolated from citrus seeds extracted with aqueous acid in the presence of pectinase.[17-19] Instead, a series of analyses of commercial GFSE products over a span of two-and-a-half decades has revealed the presence of a number of synthetic microbicides and preservatives, including triclosan, methyl and propyl parabens, benzalkonium chloride, benzethonium chloride, cetrimonium bromide, and decyl trimethylammonium chloride.

Any commercially available quaternary ammonium salt with at least one lipophilic (hydrophobic) ring or chain could conceivably be used as a microbicide in purported GFSE products. This class of compounds exerts its considerable microbicidal effect by lysing (breaking down or disintegrating) cell membranes.

Manufacturers have claimed that a proprietary extraction process, involving treatment/reactions with ascorbic and hydrochloric acids and ammonium chloride, produces compounds similar to the known antimicrobial quaternary amine compound benzalkonium chloride,[3] although no rational organic chemistry mechanism has been provided for this non-credible purported transformation. Further, the compounds in question are not just similar to benzalkonium chloride or benzethonium chloride; researchers have isolated the compounds in question from commercial prod-

Table 1: US Dollar sales data for grapefruit seed extract dietary supplements from 2012-2015

	2012		2013		2014		2015	
Channel	**Rank**	**Sales**	**Rank**	**Sales**	**Rank**	**Sales**	**Rank**	**Sales**
Natural[a]	44	1,718,106	n/a[c]	1,753,970	45	1,757,207	43	1,787,853
Mainstream Multi-Outlet[b]	82	334.778	n/a[c]	125,902	118	117,981	92	111,735

[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 from 2013-2015)
[c]n/a: not available
Source: Smith T [American Botanical Council] email communications, September 2-3 2015; K. Kawa [SPINS] email communication, July 11, 2016.

ucts and, using authentic reference standards, have shown them by high-performance liquid chromatography (HPLC) retention times and ultraviolet, nuclear magnetic resonance (NMR), and mass spectra to be identical to those authentic reference materials.[20-23]

3.2 Sources of information supporting confirmation of adulteration: A series of 13 publications over 25 years comprises the evidence for adulteration of GFSE. A variety of analytical methods were utilized in studies emanating from Japan, European countries, and the United States. The results show a consistent pattern of adulteration; moreover, the results also show that after the first two of the aforementioned publications appeared, the synthetic microbicides present began to shift. Table 1 summarizes the chronological record of the appearance of the various GFSE adulterant microbicides.

3.3 Frequency of adulteration: A sense of the frequency of adulteration can be gained by looking at the results reported in the 13 articles listed in Table 2. Triclosan was reported in nine out of 21 samples analyzed in five different studies. Parabens were found in six of 15 samples analyzed in four different studies. Benzethonium chloride was detected in 27 of 46 samples analyzed in six of the reports, while benzalkonium chloride was found in nine of 15 samples analyzed in four reports. These are very high frequencies of adulteration, even if one considers the relatively modest number of ingredient suppliers of GFSE and the history of changing adulterants. An important additional consideration is that, in cases where researchers compared antimicrobial or microbicidal activity to the presence or absence of these synthetic adulterants, such activity was observed only in those products containing one or more of the listed adulterants.[26]

3.4 Possible safety issues: Triclosan is a long-used disinfectant, labeled for external use only in the United States, found in hand soaps and cleaning products such as dish detergents. Recent studies indicating disruption of hormone regulation in animal models and possible impact on the immune system have prompted the US Food and Drug Administration (FDA) to initiate a new review of the safety and regulatory status of this compound.[32,33] In September 2016, the FDA issued a rule effectively banning the use of triclosan in hand soaps due to a lack of perceived benefits over regular hand soap, and the above-mentioned safety concerns.[34] However, it is still approved by the FDA as an OTC drug ingredient in combination with a copolymer in toothpaste to reduce gingivitis.

Benzethonium chloride is a broad-spectrum microbicide, effective against many species of bacteria, fungi, and yeasts. The FDA specifies that a safe and effective concentration in first aid treatments is 0.1-0.2%. Benzethonium chloride is not approved for use as a food additive in the United States or Europe.

Benzalkonium chloride is a skin, eye, and mucosal membrane irritant, with a 0.1% solution regarded as the highest concentration not eliciting an irritant response in skin, while eye drops use concentrations between 0.004 and 0.01%. Benzalkonium chloride is classified as a Category III antiseptic active non-prescription (over-the-counter) drug ingredient by the FDA. Ingredients are placed in Category III when "available data are insufficient to classify as safe and effective, and further testing is required." Benzalkonium chloride and benzethonium chloride were not

Table 2. Time sequence of the detection of adulterants in GFSE products

Year, First Author	Adulterants 1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]
1991 Nishina et al.[24]	√	√					
1996 Sakamoto et al.[25]	√	√					
1999 von Woedtke et al.[26]	√	√		√			
2001 Takeoka et al.[20]				√			
2001 Terreaux et al.[27]				√	√		
2004 Spitaler et al.[28]	√	√	√	√	√		
2005 Takeoka et al.[21]					√		
2006 Ganzera et al.[22]		√	√	√	√		
2007 Avula et al.[29]	√			√			
2007 Spinosi et al.[30]				√		√	√
2008 Sugimoto et al.[31]				√	√		
2008 Bekiroglu et al.[23]				√			
2016 Avula et al.[4]				√			

[a]**1** – triclosan; **2** – methyl paraben; **3** – propyl paraben; **4** – benzethonium chloride; **5** – benzalkonium chloride; **6** – cetrimonium bromide; **7** – decyltrimethyl ammonium chloride

included in the recently published (2013) FDA rule on the safety and effectiveness of consumer antiseptics and topical antimicrobial over-the-counter drug products; thus, for now they will remain Category III ingredients.[33]

3.5 Analytical methods to detect adulteration: A variety of analytical methods have been used in the analysis of GFSE products for the presence of adulterants: thin-layer chromatography (TLC);[26] high performance liquid chromatography-ultraviolet spectroscopy (HPLC-UV);[25-27] high performance liquid chromatography-mass spectrometry (HPLC-MS);[22,25,28,31] gas chromatography-mass spectrometry (GC-MS);[30] and proton nuclear magnetic resonance spectroscopy (^{1}H-NMR).[21,23] Due to the continuing change in the composition and content of microbicidal compounds in commercial products labeled as GFSE over time (see Table 2), none of the published methods has been evaluated for the detection and quantification of all the known adulterants of GFSE. The HPLC-UV method of Avula et al.[29] and the HPLC-UV-MS method of Ganzera et al.[22] have been validated, making them attractive methods to develop further for all the potential adulterants. The very recent paper by Avula et al.[4] improves on those two methods, in that the UHPLC-UV-MS method has been modified to resolve and identify not only the suspected adulterant microbicides, but also the limonoids and flavonoids expected in a true extract of grapefruit seed. Thus, one can now look at a product labeled as GFSE with one analytical method to examine whether it is made from grapefruit seeds or other citrus (lemon and orange seeds are abundantly available as byproducts of the juice industry) and/or whether it contains any adulterating synthetic microbicides.

It should be noted that cetrimonium bromide and decyl trimethylammonium chloride (detected by Spinosi et al.[30]) do not contain a UV chromophore. Therefore, a mass spectrometry-based method might be the most appropriate approach for analyzing GFSE products for any of the known or suspected adulterants.[4,22,30] Fortunately, samples of all the potential adulterants are commercially available, facilitating development and validation of analytical methods.

For additional details on the adulteration of GFSE, readers can refer to the Botanical Adulterant Program's original review on this topic in *HerbalGram* in 2012.[3]

4. Conclusions

The 13 published analyses of so-called "grapefruit seed extract" (GFSE) products, conducted and reported over the course of 25 years, revealed that a consistently high percentage of the tested materials were adulterated with synthetic microbicidal compounds. Further, during that time period, the specific microbicides that were detected changed, as illustrated in Table 1. A variety of analytical methods have been developed for the purpose of identifying and/or quantifying adulterant microbicides in GFSE and can be relatively easily adapted for use by commercial and in-house industry laboratories.

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REVISION SUMMARY

Version # , Author,	Date Revised	Section Revised	List of Changes
Version 1, J. Cardellina	n/a	n/a	none