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Food Coloring Agents and Plant Food Supplements Derived from *Vitis vinifera*: a New Source of Human Exposure to Ochratoxin A

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Abstract

Grape pomaces are increasingly being used as starting material in the industrial production of plant food supplements (PFS), food coloring and tartrates but they are at risk of ochratoxin A (OTA) contamination, a mycotoxin with nephrotoxic and carcinogenic effects. We analysed 24 commercial PFS and 13 food coloring samples derived from *Vitis vinifera*, mainly pomaces, using a HPLC-FLD method for OTA determination. OTA was found in 75% of PFS samples and 69% of food coloring samples at levels of <1.16–20.23 µg/kg and <1.16–32.00 µg/kg, respectively. The four commercial leavening agents containing tartrates were found to be negative for OTA. All eight samples collected in two distilleries that use grape pomaces and wine lees to produce tartrates and other byproducts, contained OTA at levels of <1.16–240.93 µg/kg. The high incidence of OTA contamination in PFS and food coloring agents derived from *V. vinifera* suggests maximum permitted level(s) should be established for this mycotoxin in these products.

Keywords: ochratoxin A, plant food supplements, food coloring agents, *Vitis vinifera*, grape pomaces, tartrates

INTRODUCTION

Food supplements are defined as "foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities".[1] Plant food supplements (PFS) are widely consumed because they are suggested to maintain and promote health or reduce the risk factors for diseases. A recent survey on the usage of PFS in six European countries estimated that 18.8% of screened survey respondents used at least one PFS. Across countries, 491 different botanicals were identified in the PFS products used, with *Ginkgo biloba* (ginkgo), *Oenothera biennis* (evening primrose) and *Cynara scolymus* (artichoke) being most frequently reported.[2] Within the top-40 botanicals consumed in these countries, derivatives of *Vitis vinifera* ranked 15th.[2]

Grape pomaces are used to produce pomace brandy, grape seed oil, fodder, fertilizer, biogas, grape polyphenols, food coloring, tartrates and food supplements.[3] Extracts of *V. vinifera* (grape skins, grape pomaces and to a less extent leaves), are commonly used to formulate food antioxidant supplements due to their high content of polyphenols.[4] The healthy action of polyphenols is claimed by their antioxidant properties: in this way they can inhibit LDL (low density lipoprotein) oxidation, a pathological process involved in generation of atheromatous plaques and can decrease ROS (reactive oxygen species) accumulation, which may induce cellular damage. [5-7] Grape pomaces can be used as starting material for the PFS industries and the Food and Drug Administration (FDA) approved also the use of this by-product for coloring of beverages.[5] Unfortunately, grape pomaces can be contaminated by ochratoxin A (OTA), **1** (Figure 1), a mycotoxin produced mainly by *Aspergillus carbonarius* on grape berries in the field. Moreover, 95% of the OTA present in the grape accumulates in fermented grape pomaces during the winemaking process.[8-9] However, the levels of OTA in grape and derived products can be quite different depending on geographical area,

conditions of cultivation, grape variety, weather and year vintage.[9-13] OTA is a potent nephrotoxic compound and a possible human carcinogen, therefore a tolerably weekly intake (TWI) of 120 ng/kg bw, equivalent to a tolerably daily intake (TDI) of 17.14 ng/kg bw, was established.[14] The more recent scientific information on the toxicity of OTA were assessed by the European Food Safety Authority in 2010 who concluded that an update of the opinion on OTA was not necessary.[15] In this study we analysed for OTA a number of commercially available samples of PFS, food coloring agents and leavening agents containing tartrates derived from *V. vinifera* and evaluated the impact of this mycotoxin on the safety of these products. The occurrence of OTA in samples collected during the processing of grape pomaces and wine lees in two distilleries was also performed.

MATERIALS AND METHODS

Reagents and Materials. Solid standard of ochratoxin A (OTA) was purchased from Sigma-Aldrich (Milan, Italy). The stock solution (1 mg/mL) was prepared by weighing 1 mg of the toxin that was dissolved in 1 mL of toluene/acetic acid (99:1, v/v). To assess the exact concentration of the OTA stock solution, an aliquot was evaporated to dryness, redissolved in methanol at concentration of about 10 μg/ml and spectrophotometrically tested (ε=6,330 cm^2/mmol, at λ=332 nm). Standard solutions of OTA for HPLC calibration or spiking purposes were prepared by dissolving adequate amounts of the stock solution, previously evaporated to dryness under nitrogen stream, in the HPLC mobile phase. Acetonitrile, methanol, water (HPLC grade), and glacial acetic acid were purchased from Mallinckrodt Baker (Milan, Italy).

Sodium chloride (ACS grade), polyethylene glycol (PEG 8000), and sodium hydrogen carbonate ($NaHCO_3$, ACS grade) were purchased from Sigma-Aldrich. OchraTest immunoaffinity columns were purchased from Vicam (Watertown, MA). Filter paper and GF/A glass microfiber filters were obtained from Whatman (Maidstone, U.K.).

Commercial samples. Commercially available PFS, food coloring agents and leavening agents containing tartrates, all derived from *V. vinifera*, were purchase from retail stores in Bari province (Italy) or via internet for a total of 41 samples. The dose form of PFS was tablet (7 samples), powder (3 samples), solution (2 samples), ampoule (2 samples), comfit (1 sample), syrup (1 sample), or capsule (8 samples). Food coloring agents were as powder (6 samples) or liquid (7 samples) whereas the leavening agents containing tartrates (4) were as powder.

Samples from distilleries. Eight additional samples were collected in two processing industries (distilleries) located in Puglia region (South Italy) that use wine lees (distillery 1), grape pomaces (distillery 2) and wine as starting materials for the production of ethyl alcohol, tartrates, dried grape seeds and dried grape skins. In particular, grape pomaces or lees are mixed with wine or water and distilled to obtain ethyl alcohol. Dealcoholated pomaces or lees are washed with warm water to obtain a liquid borlanda. Washed pomaces are dried and separated into grape skins and grape seeds. Borlanda is used to precipitate and collect tartrates. Exhausted borlanda and exhausted lees are mixed and submitted to digestion in a purification plant constituted of an anaerobic fermenter to produce biogas followed by an aerobic fermenter containing activated sludge. Samples of 0.5-1 kg of starting materials (lees, grape pomace) and final materials (dried grape seed, dried grape skins, tartrates and mud) were collected and sent to our laboratory. These samples were collected in the same day and they were considered sufficiently representative of the lot of pomaces or lees because they were homogenised during maceration or sedimentation processes in the winery.

All solid samples were ground before OTA extraction, powders were directly extracted, liquid samples were used as such whereas PFS available as capsules were opened to recover their content that was weighed and analysed for OTA. Solid samples containing high humidity (grape pomaces and wine lees collected from distilleries) were dried before grinding. The list of the 49 samples analysed for OTA are reported in Tables 1, 2, and 3.

Determination of OTA. Each sample was analysed as reported previously.[12] In particular, solid and liquid samples (5 g) were extracted with 30 mL of acetonitrile/water (60:40, v/v) by shaking for

60 min. Some liquid samples containing high levels of sugars showed two liquid layers after the addition of the extraction solvent. New aliquots of these samples were extracted with acetonitrile/methanol/water (90:90:80, v/v/v). The addition of methanol to the extraction solvent mixture prevents the formation of two liquid layers. After centrifugation (4000 rpm, 5 min) and filtration through a Whatman No. 4 filter paper, 6 mL of filtrate were diluted with 44 mL of water solution containing PEG (1%) and $NaHCO_3$ (5%), mixed, and filtered through Whatman GF/A glass microfiber. A 10 mL volume of diluted extract (equivalent to 0.2 g of solid sample or 0.17 g of liquid sample) was passed through an OchraTest immunoaffinity column at a flow rate of about 1 drop/s. The column was washed with 10 mL of water solution containing NaCl (2.5%) and $NaHCO_3$ (0.5%), followed by 10 mL of distilled water at a flow rate of 1-2 drops/s. The eluates were discarded, and OTA was recovered in a vial by passing 2 × 1 mL of methanol through the column. The purified extract was dried under nitrogen stream at ca. 50 °C and reconstituted with 500 μL of the HPLC mobile phase. 100 μL, corresponding to 0.04 g of solid sample or 0.034 g of liquid sample, were injected into the HPLC apparatus by a full loop injection system. The HPLC determination and confirmation of OTA were performed according to the AOAC Official Method 2001.01.[16] The HPLC apparatus was an 1100 series equipped with a G1312A binary pump, a G1313A autosampler, a G1316A column thermostat set at 25 °C, a G1321A spectrofluorometric detector set at 333 nm (λ_{ex}) and 460 nm (λ_{em}), and a Chemstation G2170AA Windows 2000 operating system (Agilent, Waldbronn, Germany). The column used was a 150 mm × 3 mm i.d., 4 μm, Synergi Hydro-RP column (Phenomenex, Torrance, CA, USA) with a 3 mm, 0.45 μm pore size guard filter (Rheodyne, Cotati, CA, USA). The mobile phase was an isocratic mixture of acetonitrile/water/acetic acid (99:99:2, v/v/v) eluted at a flow rate of 1.0 mL/min.

The identity and concentration of OTA in the purified extracts of two food supplements (#23 and #24) containing low levels of OTA were confirmed by LC-MS/MS by using the apparatus and analytical conditions reported elsewhere.[17]

Recovery experiments. The performances of the analytical method were checked on blank samples of PFS based on red grape seeds (#1), food coloring agent based on grape powder (#32) and leavening agent containing tartrates obtained from grape (#39) spiked in triplicate with OTA at 5 and 20 μg/kg. Spiked samples were left overnight to allow solvent evaporation.

RESULTS AND DISCUSSION

Method performance. Analysis of spiked blank samples demonstrated that the method generated accurate and precise results. Recovery and repeatability results ranged between 87-102% and 2-4%, respectively (Table 4) and the chromatograms of blank and spiked samples did not show matrix interfering peaks at retention time of OTA (Figure 2). Values of limit of detection (LOD) and limit of quantitation (LOQ), calculated as signal-to-noise ratio of 3:1 and 6:1, were 0.50 μg/kg and 1.16 μg/kg, respectively. Figure 2 shows the chromatograms of a PFS blank sample (#10) and two PFS samples naturally contaminated with OTA at 20.23 μg/kg (#9) and 5.19 μg/kg (#11). The identity and levels of OTA were confirmed in the purified extracts of two samples of PFS (#23 and #24) that were analysed by LC-MS/MS.

Occurrence of OTA in commercial samples. Eighteen out of 24 PFS samples (75%) were found contaminated with OTA at levels ranging from <1.16 to 20.23 μg/kg (Table 1). For each sample of PFS we have reported the dose form, daily consumption, composition, percentage of derivatives of *V. vinifera*, OTA level and its calculated probable daily intake (PDI). Assuming that most of the OTA measured in positive PFS samples originated from *V. vinifera* used as ingredient, the calculated OTA level in this ingredient ranged between <1.16 – 809.20 μg/kg. These levels of OTA are not surprising since levels up to 849.1 μg/kg of OTA were reported for samples of grape pomaces collected in South Italy.[12] The percentages of *V. vinifera*, mainly extracts of grape pomaces, in the PFS considered in this study were quite variable ranging between 0.5 – 52.3% but no correlation was found between these percentages and OTA levels ($R^2 = 0.01$). This suggests that either OTA contamination of *V. vinifera* was variable or other ingredients used for PFS formulation could have contributed to OTA contamination. The calculated values of PDI of OTA for consumers

of PFS analysed in this study are reported in Table 1 and ranged from <0.007 to 0.54 ng/kg bw which correspond to <0.04 – 3.2% of TDI (17.14 ng/kg bw) for this mycotoxin.

The occurrence of OTA in other kind of PFS was previously investigated by several authors.[18-20] In particular, 6 out of 62 PFS (10%) were found positive for OTA at levels <0.3 – 6 µg/kg with a calculated PDI of 0.15 ng/kg bw for the sample containing the highest OTA level (6 µg/kg).[18] OTA was also reported in 18 out of 50 samples (36%) of green coffee bean extracts-based PFS at levels ranging between <1 – 136.9 µg/kg.[19] Thirty-two samples (63%) of brewer's yeast food supplements were found contaminated with 0.03 – 1.53 µg/kg OTA.[20] OTA was also found, at different range levels, in dried fruits and botanicals such as medicinal plants (43% positive, up to 2340 µg/kg OTA), ginseng (40% positive, 0.4 – 1.8 µg/kg OTA), ginger (100% positive, 1.8 – 2.9 µg/kg OTA), red pepper (31% positive, 10.6 – 66.2 µg/kg OTA), and liquorice (45% positive, 0.3 – 217 µg/kg OTA).[21]

The consumption of PFS across different countries is not homogeneous and their production, marketing and control is regulated by every national legislation, although anyone can freely purchase products marketed on worldwide websites.[2] People living in the USA and northern Europe are the major consumers of PFS, mainly by people who may really in good health and not by people who really need them.[22-23] Several research groups collected data on the habits of adult populations from different areas of the USA and EU to characterize the consumptions of PFS, but no clear information is yet available about average consumption in various age groups. [2, 23-25] One of the problems that still prevents the creation of a reliable database is the extreme variability of the ingredients in the PFS available on the market. Some types of PFS may contain toxic compounds which are constituents of plants used as ingredient of these types of PFS.[26] The results of our study indicate that OTA is widespread in PFS derived from *V. vinifera*, therefore it should be considered as an additional safety issue for PFS.

Grape pomaces and wine lees are also used to manufacture either food coloring agents or tartrates. Anthocyanins (E 163), a food additive belonging to the functional class food coloring agents can be

produced from grape skin extracts.[27] Anthocyanins (E 163) refers to the food additive which are not sufficiently characterized and may contain both the glycosides (anthocyanins) and the aglycones (anthocyanidins).[27] Tartrates can be manufactured from grape pomaces or wine lees.[28] The results on the contamination of OTA in commercial samples of 13 food coloring agents and 4 leavening agents containing tartrates are reported in Table 2 whereas the results of samples collected in two distilleries located in Puglia, a region of South Italy, are reported in Table 3. Within the 13 food coloring agents, 9 samples (69%) were contaminated with OTA at levels between <1.16 – 32.00 µg/kg whereas none of the 4 samples of leavening agents containing tartrates were contaminated by OTA. As far as we know this is the first report on the occurrence of OTA in food coloring agents manufactured from *V. vinifera*.

Occurrence of OTA in samples from distilleries. The results of the samples collected in the two distilleries that used grape pomaces or wine lees to produce tartrates, ethyl alcohol, grape skins, grape seeds and biogas are reported in Table 3. All samples were contaminated and the levels of OTA in tartrates were 2-4% of the level of OTA measured in the starting materials (wine lees or grape pomaces). The levels of OTA in samples of grape skins (72.20 µg/kg) and grape seeds (23.86 µg/kg) were comparable to that of the starting grape pomaces (46.50 µg/kg). These results confirm the stability of OTA during processing of grape pomaces to produce grape skins, ethyl alcohol and grape seeds. Moreover, the levels of OTA measured in grape skins and grape seeds account for the total amount of OTA originally present in starting grape pomaces. This suggests that the toxin does not pass into ethyl alcohol during distillation of pomaces as previously reported on a laboratory scale.[12]

As shown in Table 3 a low level of OTA (3.87 µg/kg) was measured in the mud sample collected from the purification plant in spite of the high levels of OTA present in the starting wine lees (240.93 µg/kg). A possible explanation of this result could be a OTA degradation made by some of microorganisms used for anaerobic and aerobic digestion. In this case, the materials present in the

purification plant could be a good source of OTA degrading microorganisms. Further investigations are necessary to support this hypothesis.

Maximum permitted levels of OTA are in force in Europe for some products derived from *V. vinifera* such as wine and grape juice (2 µg/kg), raisins and sultanas (10 µg/kg).[29] The results reported herein demonstrate the high incidence and, for some samples, the high level of OTA in PFS and food coloring agents derived from *V. vinifera* which require maximum permitted levels of OTA to be established also for these products.

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ABBREVIATIONS USED

OTA ochratoxin A; PFS plant food supplements; TWI tolerable weekly intake; TDI tolerable daily intake; PDI probable daily intake.

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Caption to Figure

Figure 1. Structure of Ochratoxin A (OTA).

Figure 2. Chromatograms of a blank sample #10 (black), sample #9 (green) and #11 (red), naturally contaminated with OTA (**1**) at 20.23 µg/kg and 5.19 µg/kg, respectively.

Table 1. Characteristics of Plant Food Supplements Tested Herein, Occurrence and Estimated Intake of OTA.

Sample No.	Dose form	Daily consumption (g)	Composition	*Vitis vinifera* (%)	OTA (μg/kg)	PDI[a] OTA (ng/kg bw)
1	Capsule (powder)	1	Dry extract of *Pinus pinaster* bark, dry extract of grape seed (*Vitis vinifera*)	29	nd[b]	<0.01
2	Capsule (solution)	3	*Perilla frutescens*, gelatin, glycerin, extract of red grape, hydrogenated soybean oil, soy lecithin, E171, E172, E120, E-306	10	5.23	0.26
3	Ampoule	15	Root fennel, yerba mate, grapes, pineapple	4.7	<LOQ[c]	<0.29
4	Capsule (powder)	0.78	Red grape seed, E 400, L-Glutathione, green tea, LDL Factors Plus, Coenzyme Q10	8.5	<LOQ	<0.01
5	Powder	2.4	Ash, petiole cherry, olive leaves, pansy, meadowsweet, grape pomace, green tea, yerba mate, *Citrus aurantium*, kola nut	2	<LOQ	<0.05
6	Powder	10	Pineapple fruit powder, black currant fruit powder, fructose, calcium citrate, quinoa sprout powder, magnesium citrate, acerola juice powder, açai juice powder, goji berry extract, xanthan, mixed plant tocopherols, promegranate peel extract, red grape extract, green tea extract, tagetes extract, turmeric extract, black elderberry extract, coenzyme Q10, aloe vera leaf gel concentrate, zinc gluconate, manganese gluconate, copper gluconate, chromium chloride	0.8	<LOQ	<0.19
7	Capsule (powder)	1	*Polygonum cuspidatum extract*, red grape seeds, vegetal magnesium stearate, silicon dioxide	10	<LOQ	<0.02
8	Tablet	1.16	Grape seed, rapeseed oil, gelatin, glycerin, sorbitol, glycerol monostearate, sunflower lecithin, tomato extract, lutein, extract flaxseed, bioretinol, vitamin E, rice flour, microcrystalline cellulose, dyes	7	nd	<0.01
9	Tablet	1.6	Dicalcium phosphate, microcrystalline cellulose, citrus bioflavonoids, vitamin C, potassium chloride, hydrolyzed marine collagen, blueberry, pineapple, vegetable fats, grape pomace (skins and seeds), red vine leaves, silicon dioxide, vitamin E, *Centella asiatica*, witch hazel, melioto, *Chrysanthemum american*, milk thistle, dandelion	5	20.23	0.54

10	Tablet	1.94	Cellulose, extract of grape skins and seeds, extract of Greek hay seed, extract of bean pod, extract of green tea leaves, extract of mate leaves, extract of kola nut, extract of green coffee bean, extract of dandelion root, extract of artichoke head, extract of cinnamon bark, carboxymethylcellulose sodium, iron oxide, magnesium stearate, silicon dioxide, hydroxypropylcellulose	11.5	nd	<0.02
11	Capsule (powder)	0.46	Dry extract of grape skins and seeds, dry extract of *Granata*, edible gelatin, maltodextrin, silicon dioxide and magnesium stearate, selenium yeast, iron oxide	29	5.19	0.04
12	Tablet	1.24	Dry extract of grape, leaves of *Lespedeza*, elderberry flowers, *Ortosifon* (leaves and flowers), microcrystalline cellulose, calcium phosphate, potassium phosphate, rutin, silicon dioxide, magnesium stearate, blueberry, vitamin B6, hydroxypropylmethylcellulose, microcrystalline cellulose, stearic acid, potassium silicate and aluminum, iron oxide.	4	2.85	0.06
13	Capsule (solution)	0.86	Dry extract of grape seeds, L-tyrosine, edible gelatin, borage oil, wheat germ oil, glycerol, palm oil, DL-α-tocoferol acetate, flava wax, soybean oil, soy lecithin, beta-carotene, iron oxide, sodium selenite	8.3	nd	<0.01
14	Syrup	4	Mate leaves, fennel seed, grape pomace, pomegranate powder, maltodextrin, water, sucralose, chromium chloride, potassium sorbate, sodium benzoate.	ni[d]	nd	<0.03
15	Tablet	12	Meadowsweet plant, extract of grape pomace, dandelion leaves, ash leaves, grapefruit, bitter orange pericarp, citric acid, sorbic acid, benzoic acid.	52.3	<LOQ	<0.23
16	Solution	0.69	Microcrystalline cellulose, silicon dioxide, extract of vine leaves, E468, E1202, calcium hydrogen phosphate, hydroxypropyl methylcellulose, talc, magnesium stearate, titanium dioxide, iron oxide, hydrogenated vegetable fat.	14	nd	<0.01
17	Powder	2.5	Grape pomace extract, grape juice concentrate, sorbitol, maltodextrin, fructooligosaccharides, natural aroma of black currants, citric acid, dry extracts of: stalk cherry, ash leaves, kola nut, maté leaves, olive leaves, orange peel, wild pansy flower, meadowsweet flowery, green tea leaves, saccharin	2	<LOQ	<0.05
18	Tablet	9.75	Grape pomace extract, sorbitol, dry extract of *Garcinia* fruit, maltodextrin, magnesium stearate, potassium iodide, chromium chloride	0.5	<LOQ	<0.19

19	Ampoule	15	Grape pomace, mate leaves, root of fennel, apple juice concentrate, pineapple juice concentrate	5	2.12	0.53
20	Solution	10	Grape pomace extract, purified water, glycerin, fluid extract of plants (cherry stem, nettle root, rhizome of Bermuda grass, leaves maté, marc), apple aroma, cocoa extract, acesulfame potassium, potassium sorbate, sodium benzoate.	ni	<LOQ	<0.19
21	Comfit	1.4	Grape seeds extract, talc, calcium phosphate, microcrystalline cellulose, vitamin C, sodium carboxymethylcellulose, polyvinylpyrrolidone, E133, E104, E171, magnesium stearate vegetable, shellac, silicon dioxide, blueberry extract, camauba wax	7.2	<LOQ	<0.03
22	Capsule (powder)	0.42	Dry extract of grape must (*Vitis vinifera*), cellulose, magnesium stearate, silica dioxide, dry extract of *Polygonum cuspidatum* root, maltodextrin, hydroxypropyl methylcellulose, iron oxide, titanium dioxide	47.6	<LOQ	<0.01
23	Tablet	1.10	Microcrystalline cellulose, potassium phosphate, Java tea *Orthosiphon stamineus* (leaves), melioto (*Meliotus officinalis*, leaves), bioflavonoids from lemon, corn starch, grape seeds (*Vitis vinifera*), rutin, hydroxypropyl methylcellulose, L-ascorbic acid, DL-α-tocopherol acetate, croscaramellose sodium, silicon dioxide, vegetable magnesium stearate, E171, E132	14.5	1.80	0.03
24	Capsule (solution)	1.20	Soybean oil (*Glycine max*), gelatin, grape seed (*Vitis vinifera*), glycerole, melioto flowers (*Melilotus officinalis*), rutin, hesperidin,soy lecithin, beeswax, colloidal silica, E172	25	1.94	0.04

[a]PDI: probable daily intake. [b]nd: not detected (LOD = 0.50 µg/kg). [c]LOQ: limit of quantitation (1.16 µg/kg). [d]ni: not indicated in the label.

Table 2. Occurrence of OTA in Food Coloring Agents and Leavening Agents Containing Tartrates Derived from *Vitis vinifera*.

Sample No.	Physical state	Product name	OTA (μg/kg)
25	Solid	107 Anthocyan A55P	16.80
26	Solid	Grape extract 25%	1.52
27	Solid	Enocyan 4% E163	32.00
28	Liquid	Enocyan 1% l-ws E163	<LOQ[a]
29	Solid	Red anthocyan E163 95-96%	4.28
30	Liquid	Exberry grape	nd[b]
31	Liquid	Exberry grape	nd
32	Solid	Exberry grape - powder	nd
33	Liquid	Enocyanin	nd
34	Liquid	Red anthocyan E163 0.5-1.5%	2.28
35	Liquid	Enocyanin	<LOQ
36	Liquid	Colour prep-grade skin extact	<LOQ
37	Liquid	Colour prep-grade skin extact	1.93
38	Solid	Cream of tartar E336	nd
39	Solid	Leavening agent	nd
40	Solid	Cream of tartar	nd
41	Solid	Tartaric acid	nd

[a]LOQ: limit of quantitation (1.16 μg/kg). [b]nd: not detected (LOD= 0.50 μg/kg).

Table 3. Occurrence of OTA in Samples from Distilleries.

Sample No.	Physical state	Composition	OTA (μg/kg)
Distillery 1			
42	Solid	Lees	240.93
43	Solid	Mud	3.87
44	Solid	Tartrates	7.33
45	Solid	Tartrates	10.16
Distillery 2			
46	Solid	Grape pomace	46.50
47	Solid	Dried grape seeds	23.86
48	Solid	Dried grape skins	72.20
49	Solid	Tartrates	<LOQ[a]

[a]LOQ: limit of quantitation (1.16 μg/kg).

Table 4. Recovery and Repeatability Results for OTA Determination in Spiked Samples. Results Are Mean of Three Replicates.

Sample	Spiking level (μg/kg)	Mean recovery (%)	SD[a]	RSD_r[b] (%)
PFS based on red grape seeds (#1)	5	88	2.9	3
	20	87	3.2	4
Food coloring based on grape powder (#32)	5	102	1.7	2
	20	103	4.1	4
Leavening agent containing tartrates derived from *V. vinifera* (#39)	5	99	3.8	4
	20	97	2.1	2

[a]SD: standard deviation. [b]RSD_r: within laboratory relative standard deviation

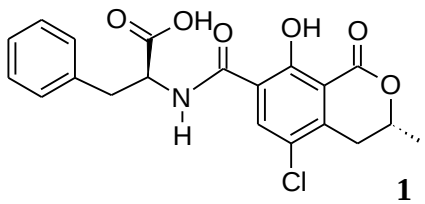

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Figure 1

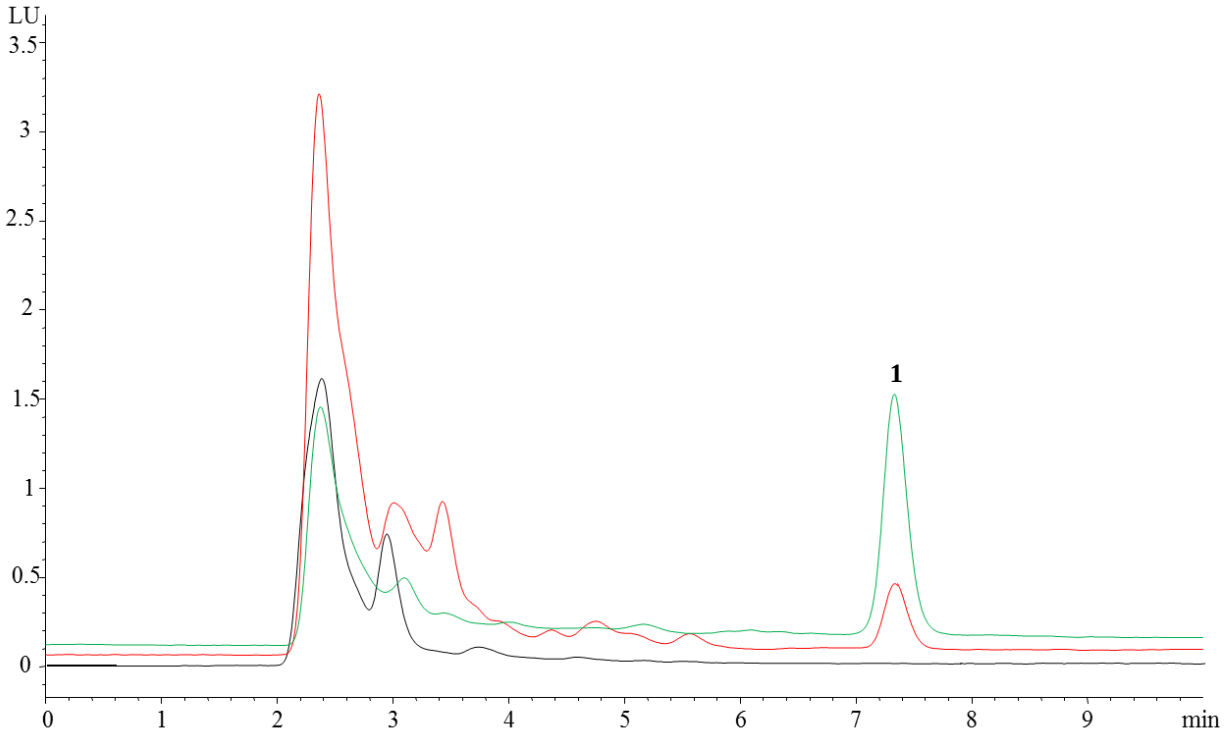
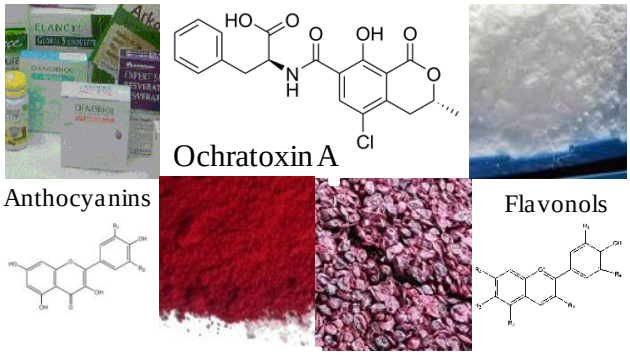


Figure 2



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