COEI-1-FIBVEG Selective plant fibers

1. Object, origin and scope of application

Selective plant fibers come from the edible parts of certain plants, generally of cereal origin. The plant fibers undergo series of mechanical treatments and extractions that concentrate the active complex without damaging the structure of the plant fiber. The objective is to increase the adsorption capacity.

The activated plant fibers fix some pesticide residues that may be present in wine and ochratoxin A. They are used during the filtration of wines.

2. Labelling

The label should contain the following indications:

- the name or sales denomination,
- the statement 'Product for oenological use,
- the batch number and expiry date,
- the storage conditions,
- the origin and composition of the fibers,
- the name or company name of the manufacturer,
- the address of the manufacturer,
- the net quantity.

3. Characteristics

The product is insoluble and comes in the form of a very fine powder.

4. Composition

The selective plant fibers contain a minimum of 90% (in mass) insoluble parietal compounds (NDF fraction) in total, determined by the Van Soest method in Annex 1.

5. Trials

5.1. Desiccation-related loss

Place 5 g of product in a desiccator at 90 °C for 15 minutes. The weight loss should not exceed 8% of the initial weight.

All of the limits set below relate to dry products.

5.2. Ashes

Without going above 550 °C, progressively incinerate the residue left in the determination from the dessication loss.

The weight of the ashes should be less than 1%.

5.3. Products soluble in aqueous solution

Place 10 g of selective plant fibers in a 250 mL container then pour 100 mL of water slowly while mixing by hand to obtain a homogeneous suspension. Collect the selective plant fibers on a filter and rinse the container with distilled water to pick up the residues from the selective plant fibers. After 48 h at a temperature of 45 °C, the loss in soluble products should not exceed 3% of the initial dry matter weight.

5.4. Contaminant adsorption trial

5.4.1. Pesticides

The capacity for adsorption (KF) of 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide (Boscalid) by the selective plant fiber, determined according to the method described in Annex 2, should be over or equal to 1000 mg/kg for a dose of 2 g/L selective plant fibers.

5.4.2. Ochratoxin A

For a 2 g/L dose of selective plant fibers, their capacity for adsorption (KF) of ochratoxin A (OTA), determined according to the method described in Annex 3, should be over or equal to 1200 mg/kg.

5.5. Iron

Quantification by atomic absorption spectrometry according to the method described in chapter II of the *International Oenological Codex*.

The iron content should be below 100 mg/kg.

5.6. Copper

Quantification by atomic absorption spectrometry according to the method described in chapter II of the *International Oenological Codex*.

The copper content should be below 25 mg/kg.

5.7. Lead

Quantification by atomic absorption spectrometry according to the method described in chapter II of the *International Oenological Codex*.

The lead content should be below 5 mg/kg.

5.8. Mercury

Quantification by atomic absorption spectrometry according to the method described in chapter II of the *International Oenological Codex*.

The mercury content should be below 1 mg/kg.

5.9. Arsenic

Quantification by atomic absorption spectrometry according to the method described in chapter II of the *International Oenological Codex*.

The arsenic content should be below 1 mg/kg.

5.10. Cadmium

Quantification by atomic absorption spectrometry according to the method described in chapter II of the *International Oenological Codex*.

The cadmium content should be below 1 mg/kg.

5.11. Salmonella

Salmonella should be absent in 25 g of selective plant fibers.

Proceed with counting according to the method described in Chapter II of the *International Oenological Codex*.

5.12. Bacteriological control

Proceed with counting according to the method described in Chapter II of the *International Oenological Codex*.

The total viable microorganism content should be less than $3 \cdot 10^4$ CFU/g.

5.13. Escherichia Coli

Proceed with counting according to the method described in Chapter II of the *International Oenological Codex*.

Absence should be checked on a 1-g sample.

5.14. Yeasts

Proceed with counting according to the method described in Chapter II of the *International Oenological Codex*.

Limit: 10^3 CFU/g of preparation.

5.15. Moulds

Proceed with counting according to the method described in Chapter II of the *International Oenological Codex*.

Limit: 10^3 CFU/g of preparation.

Annex 1: Method of analysis of insoluble parietal compounds (NDF fraction) according to the so-called 'crucible' method (Van Soest)

1. Principle

Analysis of plant-cell-wall components (hemicellulose, cellulose and lignin) after solubilisation of proteins and starches by treatment with neutral detergent (ND).

2. Apparatus

1. Balance with precision of 0.001 g
2. Drying oven
3. Oven
4. Desiccator
5. Filter crucibles (40-100 µm porosity)
6. Fibertec-type (or equivalent) analyser, i.e. closed (semi-automatic / automatic) apparatus making it possible to treat up to 6 crucibles at the same time, including dispensing of reagents, extraction and its phases of boiling, rinsing and filtration.

3. Reagents

1. Heat-stable α-amylase, e.g. (Ref.: A3306) Sigma Chemical Co.
2. Neutral detergent solution (NDS); for 5 L solution:

- sodium lauryl sulphate ($CH_3(CH_2)_{11}OSO_3Na$, p. m.: 288.4) – 150 g,
- EDTA – disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$, p. m.: 372.23) – 93.05 g,
- disodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$, p. m.: 381.37) – 34.05 g,

- disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$, p.m.: 177.99) – 22.8 g,
- triethylene glycol ($C_6H_{14}O_4$, p.m.: 150.17) – 50 mL.

4. Procedure

1. Insoluble fibers in the neutral detergent

Prepare the crucibles.
For each sample of plant fibers, prepare 2 crucibles:
Crucible for isolating insoluble components (NDF)
Weigh 2 g plant fibers in a clean, dry crucible.
Make a note of the weight with a precision of 0.001 g (W = weight of the sample).
Crucible for measuring the ashes content of the sample
Weigh 1 g plant fibers in a clean, dry crucible.
Make a note of the weight with a precision of 0.001 g (W = weight of the sample).
Insert the crucibles (into the Fibertec-type system).
Add 100 mL of NDS solution (1.3.2) to each sample at room temperature.
Add 50- µl □-amylase (1.3.1).
Bring to and maintain at boiling point as follows:
heat for 5-10 minutes, until boiling; reduce the temperature and add an anti-foaming agent (such as octanoic acid) as it starts to boil; adjust the temperature to maintain boiling and continue to heat for 60 minutes.
After 1 hour of extraction, stop heating and remove the NDS solution using a suction system.
Rinse and filter.
Add 40 mL hot water (90-100 °C) to each crucible, mix/stir the samples and leave to infuse for 2 minutes.
filter under vacuum.
Repeat this operation 4 times.
Add acetone to each crucible and leave to infuse for 2 minutes. Filter under vacuum.
Repeat this operation 2 times.
Remove the crucibles.
Rinse crucible (A) twice with hot water (90-100 °C) and place at 105°C for 12 hours.
Place crucible (B) at 105 °C for 12 hours, cool in the desiccator and weigh, which gives W1

(W1 = crucible + NDF fraction+ total ashes [TA]).

Then place it at 500 °C for 3 hours, cool in the desiccator and weigh, which gives W4

(W4 = crucible + total ashes [TA]).

4.2. Determination of the dry matter (DM) content

Weigh, with a precision of 0.001 g, a watch glass (W_{DM}).

Weigh, with a precision of 0.001 g, 2 g plant fibers in a clean and dry watch glass, which gives W2

(W2 = weight before drying).

Place the watch glass at 105 °C for 16 hours, leave to cool in the desiccator and weigh, which gives W3

(W3 = weight after drying).

5. Calculations

1. Determination of dry matter (DM)

$$MS(\%) = \frac{P3 - W_{ms}}{P2} \times 100$$

5.2. Determination of the insoluble fraction of fibers in the neutral detergent fiber (NDF)

$$NDF(\%) = \frac{(P1 - P4)}{P \times \frac{\%MS}{100}} \times 100$$

Annex 2: Measurement of the pesticide adsorption capacity by selective plant fibers

1. Principle

The aim is to determine the adsorption capacity by selective plant fibers of a fungicide used for the treatment of vines, whose trade name is Boscalid.

- **IUPAC chemical name:** 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide
- **Chemical formula:** $C_{18}H_{12}CL_2N_2O$
- **CAS No.:** 188425-85-6

The proposed method refers to the determination of the Freundlich isotherm.

2. Safety precautions

Pesticides are potentially toxic and should be handled under safe conditions protecting the analysts, especially when preparing stock solutions from pure analytical standards. Operators should protect their hands and eyes, and work under an extraction hood.

3. Apparatus

3.1. Everyday laboratory glassware: calibrated flasks, pipettes, flasks
3.2. Balance with precision of 0.001 g
3.3. Magnetic stirrer
3.4. Centrifuge

4. Reagents

4.1. 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide analytical standard in powder form with a purity of > 99%
4.2. Quality acetone for residue analysis
4.3. Preparation of 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide standard solutions:
4.3.1. 1000-mg/L stock solution of 2-chloro-N-(4'-chlorobiphényl-2-yl) nicotinamide in acetone: dissolve precisely 50 mg pure analytical-standard powder in 50 mL acetone. The stock solution may be kept at -20 °C for up to a year
4.3.2. 100-, 10- and 1-mg/L working solutions of 2-chloro-N-(4'-chlorobiphényl-2-yl) nicotinamide in acetone: use successive dilutions of the stock solution in acetone. Working solutions may be kept at -20 °C for up to 6 months.

5. Procedure

A summary of the conditions used for the preparation of control wines and test wines is provided in Table 1 (see below).

5.1. Preparation of control wines

Prepare each control wine from a wine free from pesticides, adding 9 increasing concentrations of 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide to the control in order to obtain, for example, 500 mL of each supplemented wine (see Table 1). Carry out the additions using the working standard solutions (2.4.3.2). Conduct 2 repetitions per concentration. 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide is then analysed in the 9 control wines, in order to obtain the initial concentrations measured.

5.2. Preparation of test wines

Place the 9 wines supplemented with pesticide (2.5.1) in contact with the selective plant fiber.

Procedure:

Add 0.4 g selective plant fibers to a small volume of control wine supplemented with 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide, then pour the mixture into a 200-mL calibrated flask and make up to 200 mL with this same wine (the dose of plant fiber is 2 g/L).

Leave this wine in contact with the plant fibers in a stoppered flask with the magnetic stirrer on for 45 minutes. Centrifuge for 5 minutes at 4500 rpm (3600 g). Separate the supernatant from the centrifugation pellet and proceed with the analysis of the 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide residues in order to obtain the residual concentrations measured in the supernatant. Repeat this operation for the 9 control wines supplemented with 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide (2.5.1). Conduct 2 repetitions per concentration.

Table 1: summary of the conditions for the determination of the capacity for adsorption of 2-chloro-N-(4'chlorobiphenyl-2-yl) nicotinamide

Contact time	45 minutes
Wine used for testing	Wine free from pesticides (prior analysis)
Selective plant fibers	Dose of 2 g/L (test wines) Absence (control wines)
Pesticide molecule tested	2-chloro-N-(4'chlorobiphenyl-2-yl) nicotinamide (common name: Boscalid)

Concentrations of 2-chloro-N-(4'chlorobiphenyl-2-yl) nicotinamide added	5 µg/L 15 µg/L 30 µg/L 60 µg/L 120 µg/L 240 µg/L 480 µg/L 960 µg/L 1500 µg/L
Number of repetitions	2
Centrifugation – parameters	Room temperature 4500 rpm (round 3600 g) for 5 minutes
Method of analysis of 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide residue	Determination of pesticide residues in wine after extraction using the QuEChERS method (OIV-MA-AS323-08-type II), then analysis of the extracts by UPLC/MS/MS

6. Calculations

The determination of the capacity for adsorption of 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide is calculated using the following Freundlich equation:

$$CAds = KF * CRes^{1/n}$$

or its linear form: $\text{Log } CAds = 1/n \text{ Log } CRes + \text{Log } KF$

where

- KF = the selective plant fiber's capacity for adsorption of the molecule in µg/g of fiber,
- n = the affinity of the selective plant fiber for the molecule,
- **CRes = the residual concentration** of chloro-N-(4'chlorobiphenyl-2-yl) nicotinamide measured in the wine, in µg/mL, after contact with the selective plant fibers,

- **CAds = the concentration adsorbed** by the selective plant fibers, in µg/g:
- CAds in µg/g = CAds in µg /L / 2 (where the adsorbent dose = 2 g fiber/L wine),
- **CAds in µg/L = the initial concentration** measured in the supplemented control wine, in µg/L, before contact with the selective plant fibers **– CRes in µg/L**.

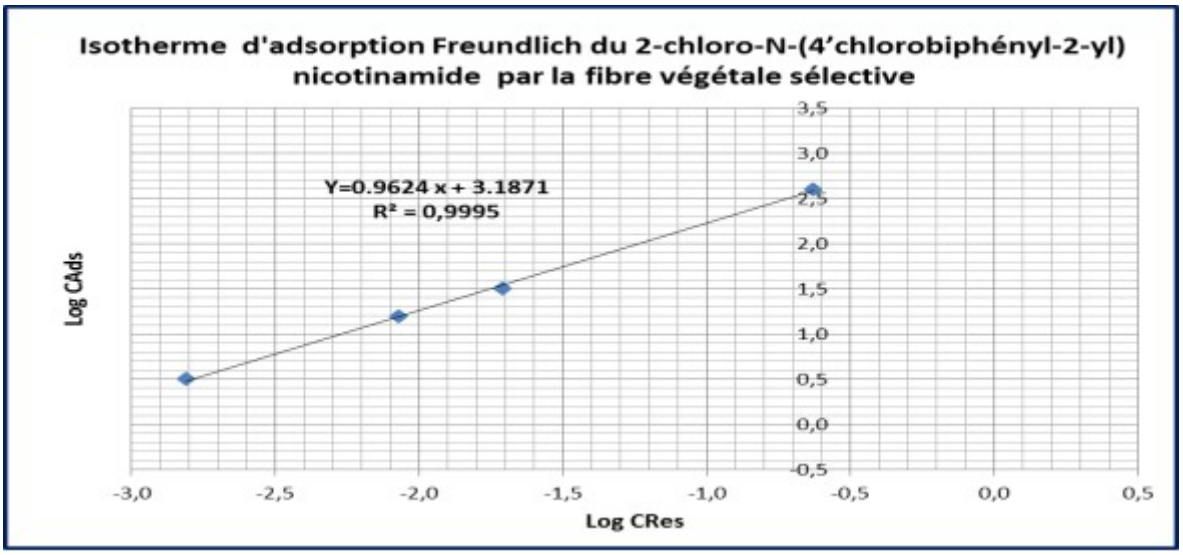
Based on the residual concentrations (µg/L) measured, the concentrations of adsorbed 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide (µg/L) are thus calculated for each initial concentration and

the regression curve **Log CAds = 1/n Log CRes + Log KF** is traced.

The Freundlich adsorption regression of the pesticide by the selective plant fiber thus allows Freundlich's two constants to be calculated: the adsorption capacity in µg/g (KF) and the affinity of the fiber for pesticide (n). The equation of the line y = ax + b gives the slope a =1/n and b = Log KF.

E.g. Freundlich isotherm for 2-chloro-N-(4'chlorobiphenyl-2-yl) nicotinamide

Freundlich isotherm for 2-chloro-N-(4'chlorobiphenyl-2-yl) nicotinamide by semective plant fibers



As such, in the below example, the following may be calculated:

- b = Log KF = 3.1871, where KF = 10^b = 1538.54
- a = 1/n = 0.9624, where n = 1 /a = 1.04

The affinity (n) of the selective plant fiber for 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide is 1.04 and the adsorption capacity (KF) of 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide by the selective plant fibre is 1538.54 µg/g or mg/kg of fiber.

Annex 3: Measurement of the ochratoxin A adsorption capacity by selective plant fibers

1. Principle

The aim is to determine the adsorption capacity by selective plant fibers of a certain mycotoxin:

- **Commercial name:** Ochratoxin A (OTA)
- **IUPAC chemical name:** N-{[(3*R*)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1H-isochromen-7-yl] carbonyl}-L-phenylalanine
- **Chemical formula:** $C_{20}H_{18}ClNO_6$
- **CAS No.:** 303-47-9

The proposed method refers to the determination of the Freundlich isotherm.

2. Safety precautions

Ochratoxin A is a toxin classified by the International Agency for Research on Cancer (IARC) as category 2B (possibly carcinogenic to human). It should therefore be handled under safe conditions protecting the analysts, especially when preparing stock solutions from pure analytical standards. Operators should protect their hands and eyes, and work under an extraction hood.

3. Apparatus

1. Everyday laboratory glassware: calibrated flasks, pipettes, flasks
2. Balance with precision of 0.001 g
3. Magnetic stirrer
4. Centrifuge

4. Reagents

1. Ochratoxin A (OTA) analytical standard in powder form with a purity of > 99%
2. Pure toluene, methanol and ethanol (HPLC quality)
3. 0.1-mol/L sodium acetate buffer with pH 5.2: dissolve 13.061 g sodium acetate trihydrate into 900 mL of distilled water. Adjust the pH to 5.2 with acetic acid then make up to 1000 mL with distilled water.
4. Preparation of ochratoxin A standard solutions:
    1. 50-mg/L stock solution in the toluene-acetic acid mixture: dissolve precisely 5 mg pure ochratoxin (3.4.1) in 100 mL toluene-acetic acid mixture (99:1, v/v). The stock solution may be kept at -20°C for up to a year.
    2. 20 mg/L Working solution in methanol: evaporate, using a nitrogen flow, an aliquot portion (20 mL) of stock solution, then re-dissolve in 50 mL pure methanol. The working solution may be kept at -20°C for up to 6 months.
    3. Addition solutions of 10, 5 and 2 mg/L in ethanol: conduct successive dilutions of the working solution in absolute ethanol. The addition solutions may be kept at -20 °C for up to 2 months.

5. Procedure

A summary of the conditions used for the preparation of control solutions and test solutions is provided in Table 2 (see below).

5.1. Preparation of control solutions

Prepare each control solution from a sodium acetate buffer solution with a pH of 5.2 (3.4.3), adding 9 increasing concentrations of ochratoxin A to the control in order to obtain, for example, 50 mL of each supplemented control solution (see Table 1). Carry out the additions using the addition solutions (3.4.4.3). Conduct 2 repetitions per

concentration. Ochratoxin A is then analysed in the 9 control solutions in order to obtain the initial concentrations measured.

5.2. Preparation of test solutions

Place the 9 solutions supplemented with OTA (3.5.1) in contact with the selective plant fiber.

Procedure:

Add 0.05 g selective plant fibers to a small volume of acetate sodium buffer solution with a pH of 5.2 supplemented with OTA, then pour the mixture into a 25-mL calibrated flask and make up to 25 mL with this same buffer solution (the dose of plant fiber is 2 g/L). After 45 minutes of contact with the selective plant fibers while stirring, centrifuge the suspensions and separate the supernatant from the centrifugation pellet of fibers. Repeat this operation for the 9 control solutions supplemented with ochratoxin A (3.5.1). Ochratoxin A is then determined by HPLC, in order to obtain the residual concentrations measured in the supernatant. Conduct 2 repetitions per concentration.

Table 2: summary of the conditions for the determination of the capacity for adsorption of OTA

Contact time	45 minutes
Buffer used for testing	Sodium acetate (pH 5.2)
Selective plant fibres	Dose of 2 g/L (test solutions) Absence (control solutions)
Concentration of ochratoxin A added	2 µg/L 5 µg/L 20 µg/L 125 µg/L 450 µg/L 900 µg/L 2,000 µg/L 5,000 µg/L 10,000 µg/L

Number of repetitions	2
Centrifugation – parameters	Room temperature 10000 rpm (round 13000 g) for 2-3 minutes
Method of analysis of ochratoxin A	Determination of ochratoxin A in wine after going through an immunoaffinity column (OIV-MA-AS315-10), followed by analysis by HPLC with fluorometric detection

6. Calculations

The determination of the capacity for adsorption of ochratoxin A is calculated according to the following Freundlich equation:

$$CAds = KF * CRes^{1/n}$$

or its linear form: $LogCAds = 1/n Log\ CRes + Log\ KF$

where

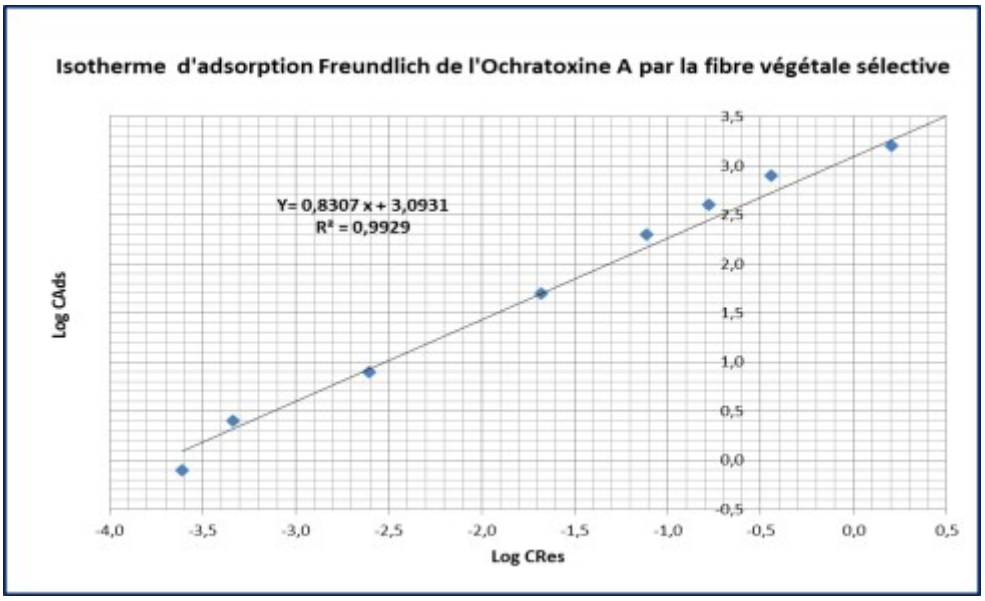
- KF = the selective plant fiber's capacity for adsorption of the molecule in µg/g of fiber,
- n = the affinity of the selective plant fiber for the molecule,
- **CRes = the residual concentration** of ochratoxin A measured in the test solution, in µg/mL, after contact with the selective plant fibers,
- **CAds = the concentration adsorbed** by the selective plant fibers, in µg/g:
- CAds in µg/g = CAds in µg /L / 2 (where the adsorbent dose = 2 g fibre/L buffer solution),
- **CAds in µg/L = the initial concentration** measured in the control solution, in µg/L, before contact with the selective plant fibers **- CRes in µg/L**.

Based on the residual concentrations (µg/L) measured, the concentrations of adsorbed ochratoxin A (µg/L) are thus calculated for each initial concentration and the regression curve **Log CAds = 1/n Log CRes + Log KF** is traced. The Freundlich

adsorption regression of ochratoxin A by the selective plant fiber thus allows Freundlich's two constants to be calculated: the adsorption capacity in µg/g (KF) and the affinity of the fibre for ochratoxin A (n). The equation of the line y = ax + b gives the slope a =1/n and b = Log KF.

E.g. Freundlich isotherm for ochratoxin A

Freundlich isotherm for ochratoxin A



As such, in the below example, the following may be calculated:

- b = Log KF = 3.0931, where KF =10^b = 1239.21
- a = 1/n = 0.8307, where n = 1 /a = 1.2

The affinity (n) of the selective plant fiber for ochratoxin A is 1.2 and the adsorption capacity (KF) of ochratoxin A by the selective plant fiber is 1239.21 µg/g or mg/kg fiber.