

03



Industria y Comercio

SUPERINTENDENCIA

Delegatura de Propiedad Industrial

División de Nuevas Creaciones

PCT
SOLICITUD

PATENTE DE INVENCION

05-116274

21. EXPEDIENTE No.

54. TITULO

51. CLASIFICACION INTERNACIONAL

71. SOLICITANTE VDF FUTURECENTICALS

DOMICILIO MAHEUCE, ILLINOIS, U.S.A.

74. APODERADO JADIE LUIS CUELLAR T.

22. BOGOTA, D.C., 16-11-05

PETITORIO



SUPERINDUSTRIA Y COMERCIO
 Radicación: 0516274 00000000 Folios: 155
 Fecha (PMD): 2003-11-16 17:56:57
 Trámite: 011 PCT-PAYEN 379 FASE NAL: 411 PRESENTAL
 Dependencia: 2002 DIVISION DE MARCAS ORCENOMES

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE

PCT ENTRADA EN FASE NACIONAL

1 SOLICITUD DE:			
☒ Patente de Invención.		☐ Patente de Modelo de Utilidad,	
☐ Capítulo I.		☒ Capítulo II	
2 SOLICITANTE (71)	Nombre: VDF FUTURECEUTICALS Dirección: 300 WEST 6TH STREET Nacionalidad o Domicilio: MOMENCE, ILLINOIS Lugar de Constitución: U.S.A. Teléfono: Fax: E-mail:	IDENTIFICACIÓN C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número _____	
3 REPRESENTANTE O APODERADO	Nombre: JAIME LUIS CUELLAR TRUJILLO Dirección: AVENIDA 13 No. 85-44, OF. 101 Teléfono: Fax: E-mail:	IDENTIFICACIÓN C.C. ☒ , NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número <u>19.360.271</u> TP <u>64.905</u>	
4 INVENTOR (ES) (72)	Nombre: MILJKOVIC DUSAN, DUELL BRAD, MILJKOVIC VUKOSAVA Dirección: 4655 Ramsay Avenue, San Diego, Ca, 92122, 1441 Santa Lucia RD. No. 1134, Chula Vista, Ca 91913 Nacionalidad o Domicilio: ESTADOS UNIDOS DE AMERICA		
5 PCT (86) Solicitud Internacional No. <u>PCT/US2003/11950</u> Fecha <u>16/04/2003</u> Publicación Internacional No. <u>3</u> Fecha _____			
6 Título (54) _____ _____ _____			
7 Clasificación Internacional (51) <u>A23F5/02; 5/16</u>			
8 Prioridad sí ☒ NO ☐	(33) País de Origen _____ _____ _____	(32) Fecha _____ _____ _____	(31) Número de Solicitud _____ _____ _____
9 Comprobante de pago No. _____ Fecha _____			

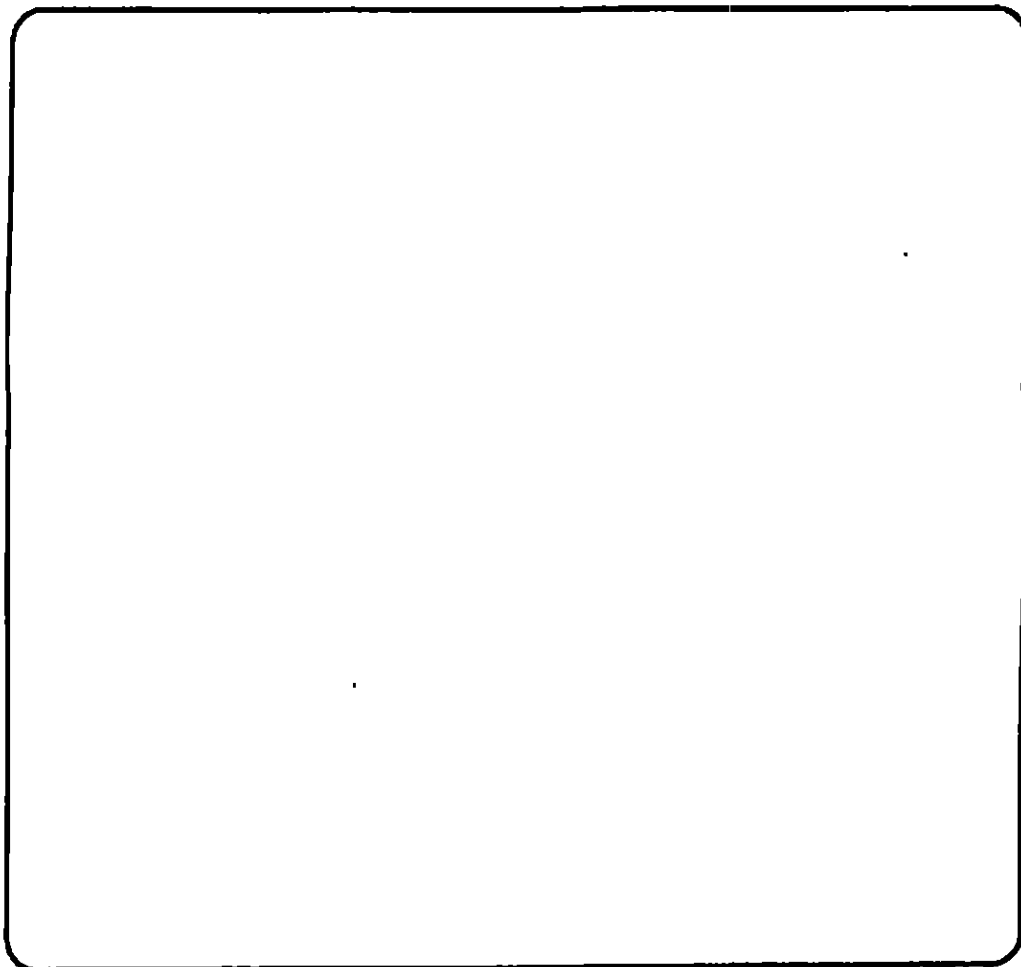
10

ANEXOS

- ☒ Comprobante de pago de la tasa de representación de la solicitud.
- ☐ Documento que acredite la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☐ Poderes, si fuere el caso.
- ☐ Documentos de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones.
- ☐ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos).
- ☐ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos).
- ☐ Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA)
- ☐ Traducción del examen preliminar internacional efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ De ser el caso, certificado de depósito del material biológico.
- ☐ Arte final 12x12.
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- ☐ De ser el caso, modificaciones efectuadas a la solicitud internacional.
- ☐ Otros.

11

FIGURA CARACTERÍSTICA:

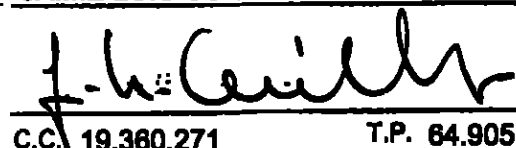


12

Solicito la concesión de la patente

NOMBRE: JAIME LUIS CUELLAR TRUJILLO

FIRMA:



C.C. 19.360.271

T.P. 64.905

Instrucciones para la presentación de los anexos, ver reverso de esta página.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800174089-2

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Radicación : 05116274 000000000
Fecha (PMS) : 2005-11-16 17:56:57 Folios: 155
Trámite : 011 PCT-PATENT 379 FASE NAL1 411 PRESEMIAC
Dependencia: 2000 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 05 - 82,610
FECHA : OCTUBRE 27 DE 2005

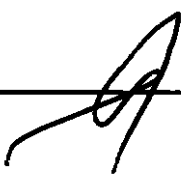
***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
TRIANA URIBE & NICHOLSEN CONSIGNACION		BANCO POPULAR	050-00110-6	42208431	27/10/2005	503,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	443,000.
		TOTAL :	\$ 443,000.00

SON: CUATROCIENTOS CUARENTA Y TRES MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

TRANSMITTAL LETTER TO THE UNITED STATES RECEIVING OFFICE

Date	16 April 2003
International Application No.	To be determined
Attorney Docket No.	100700.0024PCT

I. Certification under 37 CFR 1.10 (if applicable)

EV282599972US
Express Mail mailing number

16 April 2003
Date of Deposit

I hereby certify that the application/correspondence attached hereto is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.


Signature of person mailing correspondence

Erika Simpson
Typed or printed name of person mailing correspondence

II. ☒ New International Application

TITLE	Low-Mycotoxin Coffee Cherry Products
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Earliest priority date (Day/Month/Year)

SCREENING DISCLOSURE, INFORMATION: In order to assist in screening the accompanying international application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied. (Note: check as many boxes as apply):

- A. ☐ The invention disclosed was not made in the United States.
- B. ☒ There is no prior U.S. application relating to this invention.
- C. ☐ The following prior U.S. application(s) contain subject matter which is related to the invention disclosed in the attached international application. (NOTE: priority to these applications may or may not be claimed on form PCT/RO/101 (Request) and this listing does not constitute a claim for priority.)

application no.		filed on	
application no.		filed on	

- D. ☐ The present international application contains additional subject matter not found in the prior U.S. application(s) identified in paragraph C. above. The additional subject matter is found on pages and ☐ DOES NOT ALTER ☐ MIGHT BE CONSIDERED TO ALTER the general nature of the invention in a manner which would require the U.S. application to have been made available for inspection by the appropriate defense agencies under 35 U.S.C. 181 and 37 CFR 5.1. See 37 CFR 5.15

III. ☐ A Response to an Invitation from the RO/US. The following document(s) is(are) enclosed:

- A. ☐ A Request for An Extension of Time to File a Response
- B. ☐ A Power of Attorney (General or Regular)
- C. ☐ Replacement pages:

pages		of the request (PCT/RO/101)	pages		of the figures
pages		of the description	pages		of the abstract
pages		of the claims			

- D. ☐ Submission of Priority Documents

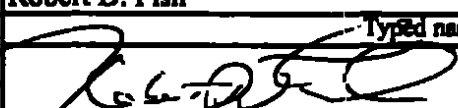
Priority document		Priority document	
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- E. ☐ Fees as specified on attached Fee Calculation sheet form PCT/RO/101 annex

IV. ☐ A Request for Rectification under PCT 91 ☐ A Petition ☐ A Sequence Listing Diskette

V. ☐ Other (please specify):

The person signing this form is the:

☐ Applicant	Robert D. Fish
☒ Attorney/Agent (Reg. No.) 33880	
☐ Common Representative	Signature

PCT REQUEST

Original (for SUBMISSION) - printed on 16.04.2003 09:03:19 AM

0	For receiving Office use only	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.92 (updated 01.10.2002)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	United States Patent and Trademark Office (USPTO) (RO/US)
0-7	Applicant's or agent's file reference	100700.24PCT
I	Title of invention	LOW-MYCOTOXIN COFFEE CHERRY PRODUCTS
II	Applicant	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	VDF FUTURECEUTICALS
II-5	Address:	300 West 6th Street Mokenca, IL 60954-0009 United States of America
II-6	State of nationality	US
II-7	State of residence	US
II-8	Telephone No.	815-472-3100
II-9	Facsimile No.	815-472-3850
III-1	Applicant and/or inventor	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	MILJKOVIC, Dusan
III-1-5	Address:	VDF FUTURECEUTICALS 4655 Ramsay Avenue San Diego, CA 92122 United States of America
III-1-6	State of nationality	YU
III-1-7	State of residence	US

PCT REQUEST

100700.24PCT

Original (for SUBMISSION) - printed on 18.04.2003 09:03:19 AM

III-2	Applicant and/or inventor	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	DUELL, Brad
III-2-5	Address:	VDF Futureceuticals 1441 Santa Lucia Rd. #1134 Chula Vista, CA 91913 United States of America
III-2-6	State of nationality	US
III-2-7	State of residence	US
III-3	Applicant and/or inventor	
III-3-1	This person is:	applicant and inventor
III-3-2	Applicant for	US only
III-3-4	Name (LAST, First)	MILJKOVIC, Vukosava
III-3-5	Address:	VDF Futureceuticals 4655 Ramsay Avenue San Diego, CA 92122 United States of America
III-3-6	State of nationality	YU
III-3-7	State of residence	US
IV-1	Agent or common representative; or address for correspondence	agent
	The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	
IV-1-1	Name	RUTAN & TUCKER LLP
IV-1-2	Address:	611 Anton Blvd., Suite 1400 Costa Mesa, CA 92626 United States of America
IV-1-3	Telephone No.	714-641-5100
IV-1-4	Facsimile No.	714-546-9035
IV-2	Additional agent(s)	additional agent(s) with same address as first named agent
IV-2-1	Name(s)	FISH, Robert(33880); ZOETWEY, David(45258); FESSENMAIER, Martin(46697)

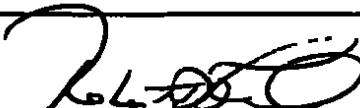
V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR IE IT LU MC NL PT SE SK TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT (patent and utility model) AU AZ BA BB BG BR BY BZ CA CH&LI CN CO CR CU CZ (patent and utility model) DE (patent and utility model) DK (patent and utility model) DM DZ EC EE (patent and utility model) ES FI (patent and utility model) GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SI SK (patent and utility model) SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
V-3	Precautionary Designation Statement In addition to the designations made under Items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under Item V-4 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-4	Exclusion(s) from precautionary designations	NONE
VI	Priority claim	NONE
VII-1	International Searching Authority Chosen	United States Patent and Trademark Office (USPTO) (ISA/US)

PCT REQUEST

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100700.24PCT

Original (for SUBMISSION) - printed on 16.04.2003 09:03:19 AM

VII	Declarations	Number of declarations	
VII-1	Declaration as to the identity of the inventor	-	
VII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	
IX	Check list	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	5	-
IX-2	Description	15	-
IX-3	Claims	2	-
IX-4	Abstract	1	EZABST00.TXT
IX-5	Drawings	0	-
IX-7	TOTAL	23	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-17	PCT-EASY diskette	-	Diskette
IX-19	Figure of the drawings which should accompany the abstract		
IX-20	Language of filing of the international application	English	
X-1	Signature of applicant, agent or common representative		
X-1-1	Name (LAST, First)	FISH, Robert	

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/US
10-6	Transmittal of search copy delayed until search fee is paid	

9

PCT REQUEST

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100700.24PCT

Original (for SUBMISSION) - printed on 16.04.2003 09:03:19 AM

FOR INTERNATIONAL BUREAU USE ONLY

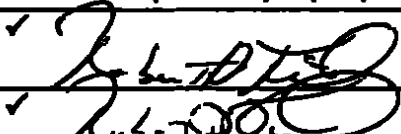
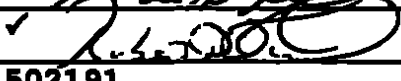
11-1	Date of receipt of the record copy by the International Bureau	
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PCT (ANNEX - FEE CALCULATION SHEET)

100700.24PCT

Original (for SUBMISSION) - printed on 16.04.2003 09:03:19 AM

(This sheet is not part of and does not count as a sheet of the international application)

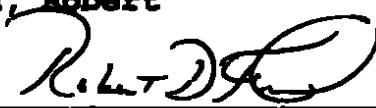
0	For receiving Office use only		
0-1	International Application No.		
0-2	Date stamp of the receiving Office		
0-4	Form - PCT/RO/101 (Annex) PCT Fee Calculation Sheet Prepared using	PCT-EASY Version 2.92 (updated 01.10.2002)	
0-8	Applicant's or agent's file reference	100700.24PCT	
2	Applicant	VDF FUTURECEUTICALS, et al.	
12	Calculation of prescribed fees	fee amount/multiplier	Total amounts (USD)
12-1	Transmittal fee T	⇒	240
12-2-1	Search fee S	⇒	700
12-2-2	International search to be carried out by	US	
12-3	International fee		
	Basic fee (first 30 sheets) b1	407	
12-4	Remaining sheets	0	
12-5	Additional amount (X)	9	
12-6	Total additional amount b2	0	
12-7	b1 + b2 = B	407	
12-8	Designation fees		
	Number of designations contained in international application	95	
12-9	Number of designation fees payable (maximum 5)	5	
12-10	Amount of designation fee (X)	88	
12-11	Total designation fees D	440	
12-12	PCT-EASY fee reduction R	-125	
12-13	Total international fee (B+D-R) I	⇒	722
12-17	TOTAL FEES PAYABLE (T+S+I+P)	⇒	1,662
12-19	Mode of payment	cheque	
12-20	Deposit account instructions		
	The receiving Office:	United States Patent and Trademark Office (USPTO) (RO/US)	
12-20-2	Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.		
12-20-3	Authorization to charge the fee for priority document.		
12-21	Deposit account No.	502191	
12-22	Date	16 April 2003 (16.04.2003)	

PCT (ANNEX - FEE CALCULATION SHEET)

2/2

100700.24PCT

Original (for SUBMISSION) - printed on 16.04.2003 09:03:19 AM

12-23	Name and signature	FISH, Robert 
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VALIDATION LOG AND REMARKS

13-2-4	Validation messages Priority	Green? No priority of an earlier application has been claimed. Please verify
13-2-7	Validation messages Contents	Yellow! The power of attorney or a copy of the general power of attorney will need to be furnished unless all applicants sign the request form.
		Green? The international application contains no drawings. Please verify.
13-2-8	Validation messages Fees	Green? Please confirm that fee schedule utilized is the latest available

ABSTRACT

12
A coffee cherry is harvested, preferably in a sub-ripe state, and quick-dried to provide a basis for numerous nutritional products. Such coffee cherries and portions thereof may be particularly characterized by their extremely low concentration of mycotoxins, including various aflatoxins, fumonisins, ochratoxins, and/or vomitoxin (DON, deoxynivalenol).

LOW-MYCOTOXIN COFFEE CHERRY PRODUCTS

Field of The Invention

The field of the invention is food products, and especially food products prepared from whole quick-dried sub-ripe coffee cherries, or fragments/portions thereof.

5 Background of The Invention

Various parts of the coffee tree have been used for nutritional purposes for a relatively long time (see *e.g.*, Pendergrast, M. *Uncommon Grounds*. Basic Books: New York, 1999). For example, coffee tree leaves and fresh, ripe coffee cherries were boiled to make tea. In other examples, the pulp of the coffee cherry can be fermented to produce wine as described in
10 Chinese Patent CN 1021949. In a still further well known example, the seeds (*i.e.*, the beans) of the coffee tree are extracted from the cherry, dried, roasted, ground, and extracted with hot water to provide the beverage that many users enjoy as coffee.

Unfortunately, coffee cherries, and especially the pulp and husk tend to rapidly spoil in the presence of molds, fungi, and other microorganisms, and therefore contain almost always
15 significant levels of mycotoxins (see *e.g.*, Pittet, A., Tornare, D., Huggett, A., Viani, R. Liquid Chromatographic Determination of Ochratoxin A in Pure and Adulterated Soluble Coffee Using an Immunoaffinity Column Cleanup Procedure. *J. Agric. Food Chem.* 1996, 44, 3564-3569; or Bucheli, P., Kanchanomai, C., Meyer I., Pittet, A. Development of Ochratoxin A during Robusta (*Coffea canephora*) Coffee Cherry Drying. *J. Agric. Food Chem.* 2000, 48, 1358-1362). Thus,
20 beverages produced from the coffee pulp, husk, mucilage, and/or whole coffee cherry generally failed to find acceptance as beverage ingredients (Although one product is advertised as "coffee cherry tea" [<http://www.paradisereolocation.com/paradisetogo/foodproducts.htm>], the product is actually made from coffee cherry pulp and was recently determined to have substantial quantities of mycotoxins).

25 Even in situations where the pulp, mucilage, and hull is removed, mycotoxins may still be present on and/or in the coffee bean. Consequently, considerable efforts have been undertaken to detoxify coffee beans and other food products. For example, where the mycotoxin is already present in the food product, selected mycotoxins can be extracted from the food product using

various solvents and procedures as described in U.S. Pat. No. 4,436,756 to Canella et al. On the other hand, various mycotoxins can be adsorbed from the food product onto a mineral carrier as described in U.S. Pat. No. 5,935,623 to Alonso-Debolt.

In still other methods, selected mycotoxins can be degraded using enzymes as described in U.S. Pat. No. 5,716,820 to Duvick et al. The inventors in the '820 reference even contemplate that the genes encoding for such enzymes may be cloned to produce transgenic plants that are then thought to be less contaminated with mycotoxins. Alternatively, microorganisms may be employed to destroy enzymatically mycotoxins found in food products as described in U.S. Pat. No. 6,025,188 to Duvick et al.

Where mycotoxins are not yet produced by a microorganism present on a plant or other food stuff, pesticides or other compositions that control microbial growth or production of mycotoxins in microorganisms may be employed. For example, Emerson et al. describe in U.S. Pat. No. 5,639,794 use of a saponin as a synergist to control colonization and/or growth of plant and animal pathogens. Alternatively, as described in U.S. Pat. No. 4,199,606 to Bland, propionic acid on a carrier may be employed as a diffusible growth inhibitor for various microorganisms. Further known compositions (see *e.g.*, U.S. Pat. No. 5,698,599 to Subbiah or U.S. Pat. No. 3,798,323 to Leary) may be employed to suppress or at least reduce synthesis of mycotoxins in a microorganism.

Alternatively, mycotoxin-containing food products may be blended with uncontaminated food products to a concentration that is acceptable and/or below the maximum allowable amount of mycotoxins in food products (see *e.g.*, Herrman, T. and Trigo-Stockli, D.; *Mycotoxins in Feed Grains and Ingredients*; Kansas State University, May 2002), or (at least potentially) mycotoxin-containing coffee cherry products may be employed in a non-food product. In still other uses, the mycotoxin content may not be considered relevant as the coffee cherry product is incinerated and thus the mycotoxins are at least partially destroyed as described in U.S. Pat. No. 4,165,752, GB 2026839, or CA 1104410. Here, the inventor teaches that the coffee cherries may be compressed, dehydrated, ground, and roasted to yield a smokable product.

However, while most of the known methods reduce the concentration of mycotoxins to at least some degree, numerous disadvantages remain. Among other things, additional processing

cherry has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins, and preferably has a polyphenol concentration of at least 10 mg/oz (most preferably at a chlorogenic acid to caffeine ratio of at least 2.7).

5 Thus, viewed from another perspective, it is contemplated that a quick-dried coffee cherry or portion thereof has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins, preferably having a chlorogenic acid content of at least 2% (wt/wt) and a polyphenol content of at least 3.2% (wt/wt).

10 Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention.

Detailed Description

15 The inventors have discovered that low-mycotoxin or even mycotoxin-free tea and other food products may be produced from whole, substantially non-damaged coffee cherries that are preferably picked at a sub-ripe stage, and wherein the coffee cherry is quick-dried after harvest. Among other advantages, the inventors discovered that such coffee cherries significantly reduce the likelihood of infestation of the coffee cherry with mold and fungi known to produce mycotoxins. Still further, the inventors discovered that sub-ripe coffee cherries (in contrast to completely unripe coffee cherries) provide a desirable level of flavor and an aroma profile in the
20 coffee cherry product (*e.g.*, coffee cherry tea) as well as relatively high levels of polyphenols, polysaccharides, and other nutrients.

The term "food product" as used herein refers to any product that is ingested by a human and/or animal for nutritional, health-maintenance, health-improvement, and/or recreational purpose. Particularly preferred food products include those consumed by human, wherein such
25 food products may be solid products (*e.g.*, dietary supplement, snack bar, bagged tea, etc.) or liquid products (*e.g.*, tea or other beverage, syrup or elixir, etc.).

As also used herein, the term "coffee cherry" refers to the fruit of the coffee tree (*Coffea spec.*, Family *Rubiaceae*) in which exocarp and outer mesocarp (*i.e.*, the pulp) surround the inner

mesocarp (*i.e.* the mucilage) and endocarp (*i.e.*, the hull), which in turn surround the seeds (*i.e.*, the beans). Thus, the term coffee cherry specifically refers to a whole coffee cherry, which may or may not include the stem of the cherry.

The term "sub-ripe coffee cherry" refers to a coffee cherry that has not yet reached the ripe stage, which is generally characterized by susceptibility to or presence of a fungal infection and/or presence of mycotoxins. Thus, a sub-ripe coffee cherry is at a ripeness stage in which the coffee cherry - when quick-dried - will exhibit mycotoxin levels that are below 20 ppb for total aflatoxins, below 5 ppm for total fumonisins, below 5 ppm for total vomitoxins, and below 5 ppb for ochratoxins. Consequently, quick-dried coffee cherries are typically dried within 0-48 hours (and more preferably between 6-24 hours) of the harvest such that the residual water content is no higher than 20% (wt/wt), and more typically no higher than 6-12% (wt/wt).

Viewed from an other perspective, sub-ripe coffee cherries generally have a complete or almost complete (at least 95% of the cherry) red color (or in some cases yellow color), and typically include various surface defects (*e.g.*, blemishes, cuts, and/or holes covering an area of more than 5% of the cherry). Thus, a sub-ripe coffee cherry will typically exhibit at least some green color (at least 5%, more typically at least 10%) and will typically be free of any surface defects (*e.g.*, blemishes, cuts, and/or holes covering an area of less than 5% of the cherry). Sub-ripe coffee cherries may also be characterized in that they will remain on the coffee tree for a subsequent round of picking where the coffee cherries are hand picked and used for the production of coffee beans. Alternatively, a color sorting machine with CCD equipment may be employed to identify and select sub-ripe coffee cherries on a quantitative color basis where the coffee cherries are mass-harvested and automatically sorted.

It should further be appreciated that while many of the following aspects and examples employ coffee cherries in a sub-ripe state, completely ripe coffee cherries are also contemplated suitable herein, especially where such ripe coffee cherries are substantially devoid of surface damage (*i.e.*, no more than 5% of surface area) or microbial infection (*i.e.*, infestation that results in mycotoxin levels of less than 20 ppb for total aflatoxins, less than 5 ppm for total fumonisins, less than 5 ppm for total vomitoxins, and less than 5 ppb for ochratoxins on a dry weight basis). Thus, all contemplated food products and/or coffee cherries may comprise completely ripe as

well as sub-ripe coffee cherries in varying proportions. For example, suitable proportions include 100% ripe : 0% sub-ripe, preferably 90% ripe : 10% sub-ripe, more preferably 75% ripe : 25% sub-ripe, even more preferably 50% ripe : 50% sub-ripe, and most preferably less than 25% ripe : more than 75% sub-ripe.

5 As further used herein, the term "quick-dried" coffee cherry means that the whole coffee cherry is dried under a protocol that limits growth of molds, fungi, and/or yeast to an extent such that the dried coffee cherry will exhibit mycotoxin levels that are below 20 ppb for total aflatoxins, below 5 ppm for total fumonisins, below 5 ppm for total vomitoxins, and below 5 ppb for ochratoxins. Consequently, quick-dried coffee cherries are typically dried within 0-48 hours
10 (and more preferably between 6-24 hours) of the harvest such that the residual water content is no higher than 20% (wt/wt), and more typically no higher than 6-12% (wt/wt).

As still further used herein, the term "mycotoxin" refers to any toxic product formed in a mold, fungus, and/or yeast that exhibits significant toxicity to a human or animal when ingested. Thus, specifically contemplated mycotoxins include aflatoxins (and particularly B1, B2, G1, and
15 G2), fumonisins (and particularly B1, B2, and B3), ochratoxin, deoxynivalenol (DON, vomitoxin), T-2 toxin, and zearalenone. The term "total aflatoxins" therefore refers to the sum of all aflatoxin variants, the term "total fumonisins" refers to the sum of all fumonisin variants, and the term "total ochratoxins" therefore refers to the sum of all ochratoxin variants.

In one exemplary aspect of the inventive subject matter, whole undamaged sub-ripe (e.g.,
20 semi ripe or almost ripe) coffee cherries are hand picked and within about one hour quick-dried using a dry air drier at about 140°F until constant weight is obtained. The so obtained coffee cherries typically possess significant storage stability, high resistance to infection by fungi, and lower shipping weight than wet cherry.

It is generally contemplated that the sub-ripe coffee cherries may be derived from various
25 sources, and the particular use of the sub-ripe coffee cherries will at least in part determine the particular source(s). However, it is preferred that the sub-ripe coffee cherries are derived from a single coffee species (e.g., *coffea arabica*), which is cultivated under similar growth conditions (e.g., shade-grown). Among other advantages, it is contemplated that a single source of coffee cherries will facilitate quick-drying the sub-ripe coffee cherries. It should be recognized,

however, that once the sub-ripe coffee cherries are quick-dried, various coffee species and/or coffee cherries from various growth conditions may be blended to achieve a mixture with particularly preferred characteristics.

Furthermore, it should be appreciated that depending on the particular product or use for the coffee cherry, the degree of ripeness of the coffee cherry may vary considerably. For example, where extraction of polyphenols and/or chlorogenic acid from the whole coffee cherry is desired, semi-ripe (stage 1 or stage 2) coffee cherries may be used. On the other hand, where the coffee cherry is used for the production of a coffee cherry tea and flavor and aroma are paramount, almost ripe coffee cherries may be picked. In still further contemplated aspects, unripe coffee cherries, or any reasonable mixtures of varying ripeness degrees may be used. Especially where the coffee cherry is a ripe coffee cherry, it is contemplated that the whole cherry is preferably free of surface defects, including cracks, splits, holes, or other openings. However, while not preferred, coffee cherries with surface defects may also be used. While not limiting to the inventive subject matter, it is generally preferred that the sub-ripe coffee cherries (or coffee cherry mixtures) are washed with water or other aqueous solution (e.g., diluted hypochlorite solution) to remove soil particles and other debris before drying.

Quick-drying is preferably performed immediately after picking to up to about two days after picking until a constant weight is obtained (or until the outer pulp of the cherry has dried). Thus, and depending on the particular heat source available, it is generally preferred that quick-drying is performed at a temperature of about 100° to about 180°F for a period of about 6-48 hours. For example, where electrical (or other) energy is readily available, the sub-ripe coffee cherries may be dried in a warm air drier in a stationary or rotating drum, or in a refractance window drying process. Alternatively, the sub-ripe coffee cherries may also be freeze dried. On the other hand, and especially where energy sources are not readily available, the sub-ripe coffee cherries may be sun dried. However, regardless of the particular drying method, it should be recognized that the sub-ripe coffee cherries are quick-dried to prevent production of mycotoxins from fungi, molds, and/or yeast that are already present and/or colonize (e.g., via infection or sporulation) the coffee cherry. Thus, sub-ripe coffee cherries are advantageously dried on a surface that is clean and free of sources of mycotoxin contamination. In further alternative

aspects of the inventive subject matter, the sub-ripe coffee cherries may also be frozen and stored/transported until quick-drying can be implemented.

The so obtained quick-dried sub-ripe coffee cherries may then without further mycotoxin detoxification be employed for various uses in numerous food products. For example, where the whole quick-dried sub-ripe coffee cherry is used in a food product, the coffee cherry may be admixed with another consumable (e.g., admixture with grain for animal feed, or coating with chocolate for human consumption). In another especially preferred example, the quick-dried sub-ripe coffee cherry is ground and used as a food additive or as a basis for brewing coffee cherry tea (e.g., for use as loose tea, grinding to a size of 500-3000 μm is preferred, or for bagged teas, grinding to a size of 200-1000 μm is preferred).

Alternatively, it should be recognized that only parts of the quick-dried sub-ripe coffee cherry may be employed in a food product. For example, where the sub-ripe coffee cherry is in an almost ripe state, it is contemplated that the pulp, mucilage, and/or hull may be separated from the seeds, which are then (optionally admixed with other seeds) roasted to commercial grade coffee beans. The remaining pulp, mucilage, and/or hull from the quick-dried sub-ripe coffee cherry may then be employed as food additive or basis for extraction of one or more desired components (e.g., polyphenols).

In still further contemplated uses of so obtained quick-dried sub-ripe coffee cherries, it is contemplated that the coffee cherries (or portions thereof) may be employed as starting material for extraction of various beneficial components. For example, the quick-dried sub-ripe coffee cherries may be extracted with an aqueous (e.g., water, water-ethanol mixture) or non-aqueous solvent (e.g., critical point CO_2 , dimethylformamide) to isolate one or more components that can be used in a food product. For example, quick-dried sub-ripe coffee cherries may provide an excellent source of polyphenols, chlorogenic acid, and/or caffeine.

The term "polyphenol" as used herein refers to a diverse group of compounds produced by a plant, wherein the compounds include a phenol ring to which at least one OH group, and more typically at least two OH groups are covalently attached. For example, representative polyphenols include ellagic acid, tannic acid, vanillin, caffeic acid, chlorogenic acid, ferulic acid, catechins (e.g., epicatechin gallate, epigallocatechin), flavonols (e.g., anthocyanidins, quercetin,

kaempferol), and various other flavonoids, and their glycosides and depsides. Furthermore, contemplated polyphenols may also be in oligomeric or polymeric form (e.g., oligomeric proanthocyanidins or condensed tannins).

5 In another preferred aspect of the inventive subject matter, the inventors contemplate use of whole quick-dried sub-ripe coffee cherries in the production of various beverages. For example, it was observed that teas produced from unripe (green) and semi-ripe stage 1 whole quick-dried coffee cherries possess relatively low aroma and flavor characteristics. Thus, extracts or at least partially condensed teas from unripe (green) and semi-ripe stage 1 whole quick-dried coffee cherries may be added as low-flavor additive to a commercially available beverage to
10 enhance the nutritional properties.

As ripeness increases, more aroma and flavor is evident in the quick-dried sub-ripe coffee cherries. Consequently, tea produced from semi-ripe stage 2 and almost ripe whole coffee cherries will possess high aroma and fruity flavor, and the inventors contemplate that semi-ripe and almost ripe coffee cherries could be used directly after quick-drying and grinding for a
15 "Whole Coffee Fruit Tea", either by itself or after placing into tea bags. As illustrated below, such a tea would provide high levels of polyphenols, typically 60-70 mg per 6 oz. cup. Furthermore, teas prepared from whole quick-dried sub-ripe coffee cherries generally possess relatively high ratios of chlorogenic acid to caffeine (typically about 2-4.5) as compared to beverages made from roasted coffee (typically about 0.4). Therefore, it should be appreciated
20 that tea prepared from quick-dried sub-ripe coffee cherries is much more nutritional (based upon polyphenols and chlorogenic acid) than roasted coffee.

Therefore, the inventors contemplate a food product that includes a preparation of a (preferably sub-ripe) coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than
25 5 ppm for total fumonisins. Alternatively, contemplated mycotoxin levels may also be in the range of 20-50 ppb, but more preferably less than 15 ppb, even more preferably less than 10 ppb, and most preferably less than 5 ppb for total aflatoxins. Similarly contemplated mycotoxin levels also include a range of 10-30 ppb, but more preferably less than 5 ppb, even more preferably less than 3 ppb, and most preferably less than 2 ppb for total ochratoxins. Likewise, contemplated

mycotoxin levels also include a range of 5-20 ppm, but more preferably less than 15 ppm, even more preferably less than 10 ppm, and most preferably less than 5 ppm for total fumonisins and/or vomitoxins.

As already discussed above, the preparation of the sub-ripe coffee cherry may include the whole coffee cherry, comprise a ground fragment of the whole coffee cherry, or include the bean, the pulp, the mucilage, and/or the hull of the quick-dried sub-ripe coffee cherry. Alternatively, it should be recognized that the preparation may also comprise an extract from the whole quick-dried sub-ripe coffee cherry (or fragment or portion thereof).

Contemplated food products especially include beverages prepared from contemplated quick-dried sub-ripe coffee cherries (or fragments or portions thereof), or beverages to which extracts or pieces from contemplated quick-dried sub-ripe coffee cherries (or fragments or portions thereof) have been added. Similarly, further contemplated food products include baked goods (e.g., bread, crackers, etc.), snacks (e.g., candy or energy bars), cereals, and other solid nutrients to which extracts or pieces from contemplated quick-dried sub-ripe coffee cherries (or fragments or portions thereof) have been added. Alternatively, contemplated food products also include a nutritional supplement in liquid or solid form that comprises an extract of the quick-dried sub-ripe coffee cherry.

Depending on the particular purpose, it should be recognized that such food products may be prepared from quick-dried sub-ripe coffee cherries having a primarily green color with less than 25% red color, more preferably with less than 25% green color, and most preferably from quick-dried sub-ripe coffee cherries having primarily (no less than 90%, most typically no less than 95%) red color with less than 5% blemished area. Still further contemplated compositions and methods are disclosed in our co-pending U.S. Patent application with the title "Methods for Coffee Cherry Products", filed on or about April 16, 2003, which is incorporated by reference herein.

Examples

The following examples are provided to enable a person of ordinary skill in the art to make and use compositions according to the inventive subject matter and to illustrate exemplary compositions and methods generally described herein.

Harvest Of Whole Coffee Cherries

The ripeness of the coffee cherries was determined by visually estimating the amount of green and red color (or yellow, where applicable) of the whole cherries. As the cherries ripen, the green cherries will typically increase in size and subsequently develop increasing amounts of red color. For the present examples, the coffee cherries were collected at four stages of ripeness: Completely, or almost completely green (unripe; typically less than 5% of the coffee cherry red or yellow), primarily green with some red (semi-ripe, stage 1; typically less than 25% of the coffee cherry red or yellow), primarily red with some green (semi-ripe, stage 2; typically less than 25% of the coffee cherry green), and unbroken, unblemished red (almost ripe; typically less than 10% of the coffee cherry green; area of blemishes, cuts, or otherwise broken surface less than 5%). As much as possible, whole, unbroken and uncut cherries were collected.

Quick-Drying Of The Whole Coffee Cherries

Whole coffee cherries for sample extraction were prepared by drying the cherries within 1-12 hours after harvest on separate trays of an air dryer according to the following procedure. Coffee cherries (400-600 g) were weighed into beakers and washed two times with tap water, followed by a single wash with distilled water. The so washed coffee cherries were placed on a tray of an air dryer to drain, and then dried at 150-160°F for 16-18 hours to constant weight. Drying was stopped when the weight at two consecutive one-hour intervals differed by less than 1 g. Typical yields of dried whole cherry were 160-220 g. Further analysis indicated 6-12% residual water content in the dried cherry.

Mycotoxin Analysis

In order to determine the viability of the whole coffee cherry at the unripe, semi-ripe, and almost ripe stages (see above) for use in a nutritional product (and especially for use in tea), the level of selected mycotoxins was measured and compared against comparative products and red, ripe coffee cherry by-product from coffee production. As can be clearly seen in Table 1 below, quick-dried coffee cherries of all sub-ripe harvest stages had a mycotoxin level below the detection limit of 1 ppb (as measured for aflatoxin and ochratoxin).

The mycotoxin concentration was determined in an independent laboratory by both ELISA and HPLC analysis. Based on the below results, the inventors conclude that all samples from the different sub-ripe harvest stages are suitable for direct use in a nutritional product for both human and veterinary consumption. In contrast, the by-product of coffee production (predominantly consisting of pulp, mucilage, and hull from coffee cherries) from ripe cherries of red color with blemishes (typically greater than 20% of the cherry surface) had a substantial content in both aflatoxins and ochratoxins. Similarly, the comparative product "Paradise to Go Tea" (made from coffee cherry pulp) exhibited mycotoxins in double-digit concentrations.

RIPENESS	COLOR	AFLATOXIN	OCHRATOXIN
Unripe, quick-dried	Green	< 1ppb	< 1ppb
Semi-ripe Stage 1, quick-dried	Mostly green with some red	< 1ppb	< 1ppb
Semi-ripe Stage 2, quick-dried	Mostly red with some green	< 1ppb	< 1ppb
Almost ripe, quick-dried	Red, Blemished Area <5%	< 1ppb	< 1ppb
Ripe (by-product of Coffee Production)	Red, Blemished Area >20%	> 200 ppb	> 500 ppb
Paradise to Go Tea (dry matter)	N/A	> 25 ppb	> 40 ppb

Table 1

Polyphenol(PP), Chlorogenic Acid (CG), and Caffeine (CF) Analysis for Quick-dried Sub-ripe Whole Coffee Cherries

In a further series of experiments, the levels of total polyphenols, chlorogenic acid, and caffeine from quick-dried whole coffee cherry at various sub-ripe stages were measured and compared against green and roasted coffee beans. Table 2 summarizes the results of this analysis.

Interestingly, while the polyphenol (PP) level of quick-dried coffee cherries of all sub-ripe harvest stages was somewhat less than the level of green or roasted coffee beans, significant quantities of polyphenols in quick-dried sub-ripe coffee cherries still remain. Similarly, the chlorogenic acid (CG) content of quick-dried whole coffee cherry at various sub-ripe stages remained at substantial high levels as compared to roasted coffee, but was somewhat lower as

compared to green beans. The caffeine (CF) level of quick-dried coffee cherries of all sub-ripe harvest stages was substantially within the caffeine level of green and roasted coffee beans (It should be pointed out that all data given are on a dry matter basis and are not normalized to the dry weight of the bean).

5 Polyphenol analysis: Dried whole coffee cherry (or green beans or roasted beans) (1.00 g) were ground in a rotating steel knife coffee grinder for 30 seconds to produce a ground sample. The ground sample was added to 100 mL distilled water and the resulting mixture heated to boiling in an Erlenmeyer flask for 30 minutes. The heat was removed and the mixture allowed to cool to room temperature. The resulting suspension was transferred to a 100 mL graduated
10 cylinder and water added to bring the volume to 100 mL. The mixture was then transferred back to the Erlenmeyer flask, stirred briefly, and the solids allowed to settle. An aliquot (~3 mL) of the supernatant solution was filtered through an 0.45 µm Acrodisc filter, and the resulting clear solution was diluted 1:10 with distilled water using a volumetric flask (1.00 mL diluted with 9.00 mL distilled water).

15 The Folin-Ciocalteu method was used to measure the polyphenol content of the diluted solution as follows. One mL of the diluted solution was added to a test tube, mixed with 1 mL of 0.2N Folin-Ciocalteu's Phenol reagent (Sigma solution, 2N, diluted 1:10 with water), and allowed to stand 5 minutes at room temperature. One mL of 1N NaHCO₃ was added and the reaction mixture left at room temperature for 2 hours. The polyphenol level was determined
20 using a UV-visible spectrophotometer standardized against catechin, at λ_{max}=750 nm against distilled water as blank.

Chlorogenic acid: Determination of chlorogenic acid was done using HPLC separation of the filtered clear solution prepared above using standard analytical and separation protocols well known in the art. Similarly, determination of caffeine was done using HPLC separation of the
25 filtered clear solution prepared above using standard analytical and separation protocols well known in the art (for exemplary protocols see e.g., Bispo M.S., et al. in J. Chromatogr. Sci.; 2002, Jan;40(1):45-8, or Nakakuki, H. et al. in J. Chromatogr. A.;1999, Jul 2;848(1-2):523-7).

RIPENESS	COLOR	% PP	%CG	% CF	CG/CF
Unripe, quick-dried	Green	3.80	2.64	1.03	2.56

Semi-ripe stage 1, quick-dried	Mostly green with some red	3.28	2.70	1.00	2.70
Semi-ripe stage 2, quick-dried	Mostly red with some green	3.54	2.00	0.70	2.86
Almost ripe, quick-dried	Red, Blemished Area <5%	3.35	N/D	N/D	N/D
Green Coffee beans	Green	4.58	3.31	0.95	3.48
Roasted Coffee beans	Brown	3.93	0.50	1.20	0.42

Table 2

Polyphenol(PP), Chlorogenic Acid (CG), and Caffeine (CF) Analysis for a Tea brewed from Quick-dried Sub-ripe Whole Coffee Cherries

Quick-dried sub-ripe coffee cherry were ground in a rotating steel knife coffee grinder for 10-30 seconds to produce a ground sample. To the ground sample (1.00 g) was added 90 mL (approx. 3 fluid oz.) boiling distilled water and the resulting mixture allowed to stand in an Erlenmeyer flask for 10 minutes to produce a coffee cherry tea. An aliquot (~3 mL) of the supernatant solution was filtered through a 0.45 µm Acrodisc filter, and the resulting clear solution was diluted 1:10 with distilled water using a volumetric flask (1.00 mL diluted with 9.00 mL distilled water). The Folin-Ciocalteu method as described above was used to measure the polyphenol content (on dry matter basis, catechin equivalents) of the so prepared coffee cherry tea. Table 3 summarizes the results.

RIPENESS	SOLVENT	% PP	%CG	% CF	CG/CF
Unripe, quick-dried	Water	10.93	8.61	3.04	2.83
Semi-ripe stage 1, quick-dried	Water	9.38	7.58	2.72	2.78
Semi-ripe stage 2, quick-dried	Water	8.51	6.74	1.71	3.95
Almost ripe, quick-dried	Water	6.92	1.34	0.29	4.61

Table 3

Brewing Of Teas From Whole Coffee Cherry Of Different Ripeness

The following procedure was used to prepare teas from whole coffee cherry of different ripeness for aroma and taste testing. Whole dried coffee cherry (10-20 g) was ground in a rotating steel knife coffee grinder for 10-30 seconds to produce a ground sample. The ground

coffee cherry (2.0 g) was placed in a ceramic cup and of nearly boiling water (6 oz., 190-200°F) added. The slurry was stirred and allowed to stand for 3 minutes, at which time the aroma and taste of the supernatant liquid were noted. Results are shown in Table 4.

RIPENESS	COLOR	AROMA	TASTE	PP/6 OZ. TEA
Unripe, quick-dried	Green	None	Almost no taste	76 mg
Semi-ripe stage 1, quick-dried	Mostly green with some red	Very mild fruity	Slight fruit flavor	66 mg
Semi-ripe stage 2, quick-dried	Mostly red with some green	Mild fruity	Mild fruit flavor	71 mg
Almost ripe, quick-dried	Red, Blemished Area <5%	Fruity	Rich fruit flavor	67 mg

Table 4

Thus, specific embodiments and applications of low-mycotoxin coffee cherry products have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

CLAIMS**What is claimed is:**

1. A food product comprising a preparation of a coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
2. The food product of claim 1 wherein the preparation of the coffee cherry comprises a ground fragment of the coffee cherry.
3. The food product of claim 2 wherein the preparation of the coffee cherry comprises an extract from a ground fragment of the coffee cherry.
4. The food product of claim 1 wherein the preparation of the coffee cherry comprises at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
5. The food product of claim 1 wherein the preparation of the coffee cherry comprises an extract from at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
6. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily green color with less than 25% red color.
7. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 25% green color.
8. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 5% blemished area.
9. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using heated air.
10. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using solar radiation.

11. The food product of claim 1 wherein the coffee cherry is quick dried by exposing the coffee cherry to at least one of ambient air and sun light.
12. The food product of claim 1 wherein the food product is a tea brewed from the coffee cherry.
- 5 13. The food product of claim 1 wherein the food product is a beverage comprising an extract of the coffee cherry.
14. The food product of claim 1 wherein the food product is nutritional supplement in liquid or solid form and comprising an extract of the coffee cherry.
15. A tea brewed from a comminuted quick-dried coffee cherry or portion thereof.
- 10 16. The tea of claim 15 wherein the coffee cherry has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
17. The tea of claim 16 having a polyphenol concentration of at least 10 mg/oz.
18. The tea of claim 15 wherein the coffee cherry is a sub-ripe coffee cherry.
- 15 19. A quick-dried coffee cherry or portion thereof having a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
20. The quick-dried coffee cherry of claim 19 wherein the coffee cherry is a sub-ripe coffee cherry.

20

ABSTRACT

A coffee cherry is harvested, preferably in a sub-ripe state, and quick-dried to provide a basis for numerous nutritional products. Such coffee cherries and portions thereof may be particularly characterized by their extremely low concentration of mycotoxins, including various
5 aflatoxins, fumonisins, ochratoxins, and/or vomitoxin (DON, deoxynivalenol).

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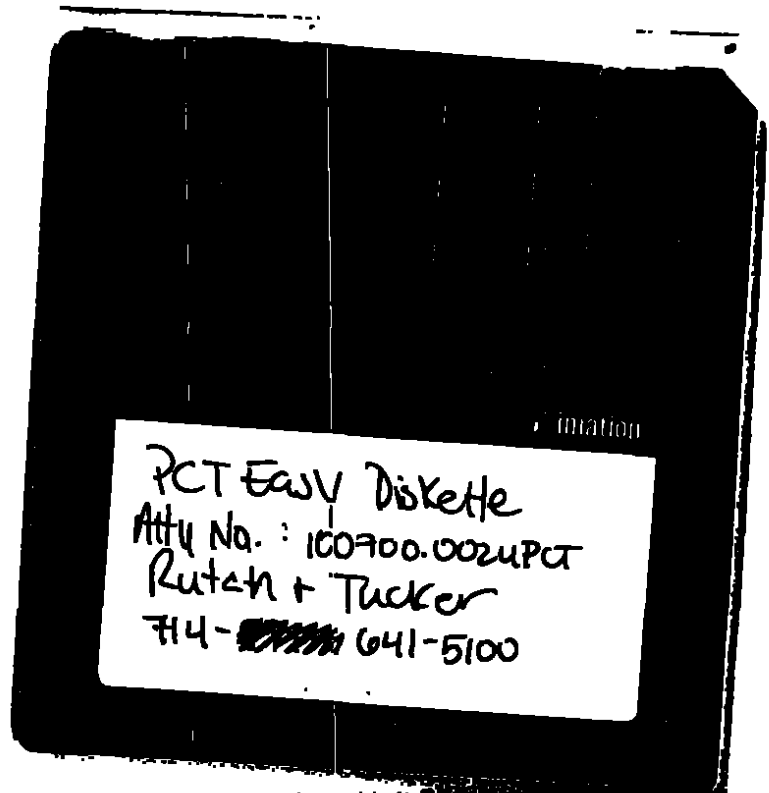
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From the INTERNATIONAL BUREAU

PCT**NOTICE INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION TO THE DESIGNATED OFFICES**

(PCT Rule 47.1(c), first sentence)

To:

RUTAN & TUCKER LLP
611 Anton Blvd., Suite 1400
Costa Mesa CA 92626
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 18 November 2004 (18.11.2004)		IMPORTANT NOTICE
Applicant's or agent's file reference 100700.24PCT		
International application No. PCT/US2003/011950	International filing date (day/month/year) 16 April 2003 (16.04.2003)	Priority date (day/month/year)
Applicant VDF FUTURECEUTICALS et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

AU, AZ, BY, CH, CN, CO, DZ, EP, HU, KG, KP, KR, MD, MK, MZ, RU, TM, US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

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The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 18 November 2004 (18.11.2004) under No. WO 2004/098303

4. **TIME LIMITS** for filing a demand for international preliminary examination and for entry into the national phase

The applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be 30 MONTHS from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of 19 months from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see *PCT Gazette* No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the *PCT Newsletter*, October and November 2001 and February 2002 issues.

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(54) Title: LOW-MYCOTOXIN COFFEE CHERRY PRODUCTS

(57) Abstract: A coffee cherry is harvested, preferably in a sub-ripe state, and quick-dried to provide a basis for numerous nutritional products. Such coffee cherries and portions thereof may be particularly characterized by their extremely low concentration of mycotoxins, including various aflatoxins, fumonisins, ochratoxins, and/or vomitoxin (DON, deoxynivalenol).

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LOW-MYCOTOXIN COFFEE CHERRY PRODUCTS

Field of The Invention

The field of the invention is food products, and especially food products prepared from whole quick-dried sub-ripe coffee cherries, or fragments/portions thereof.

5 Background of The Invention

Various parts of the coffee tree have been used for nutritional purposes for a relatively long time (see *e.g.*, Pendergrast, M. *Uncommon Grounds*. Basic Books: New York, 1999). For example, coffee tree leaves and fresh, ripe coffee cherries were boiled to make tea. In other examples, the pulp of the coffee cherry can be fermented to produce wine as described in
10 Chinese Patent CN 1021949. In a still further well known example, the seeds (*i.e.*, the beans) of the coffee tree are extracted from the cherry, dried, roasted, ground, and extracted with hot water to provide the beverage that many users enjoy as coffee.

Unfortunately, coffee cherries, and especially the pulp and husk tend to rapidly spoil in the presence of molds, fungi, and other microorganisms, and therefore contain almost always
15 significant levels of mycotoxins (see *e.g.*, Pittet, A., Tomare, D., Huggett, A., Viani, R. Liquid Chromatographic Determination of Ochratoxin A in Pure and Adulterated Soluble Coffee Using an Immunoaffinity Column Cleanup Procedure. *J. Agric. Food Chem.* 1996, 44, 3564-3569; or Bucheli, P., Kanchanomai, C., Meyer I., Pittet, A. Development of Ochratoxin A during Robusta (*Coffea canephora*) Coffee Cherry Drying. *J. Agric. Food Chem.* 2000, 48, 1358-1362). Thus,
20 beverages produced from the coffee pulp, husk, mucilage, and/or whole coffee cherry generally failed to find acceptance as beverage ingredients (Although one product is advertised as "coffee cherry tea" [<http://www.paradiserelocation.com/paradisetogo/foodproducts.htm>], the product is actually made from coffee cherry pulp and was recently determined to have substantial quantities of mycotoxins).

25 Even in situations where the pulp, mucilage, and hull is removed, mycotoxins may still be present on and/or in the coffee bean. Consequently, considerable efforts have been undertaken to detoxify coffee beans and other food products. For example, where the mycotoxin is already present in the food product, selected mycotoxins can be extracted from the food product using

various solvents and procedures as described in U.S. Pat. No. 4,436,756 to Canella et al. On the other hand, various mycotoxins can be adsorbed from the food product onto a mineral carrier as described in U.S. Pat. No. 5,935,623 to Alonso-Debolt.

5 In still other methods, selected mycotoxins can be degraded using enzymes as described in U.S. Pat. No. 5,716,820 to Duvick et al. The inventors in the '820 reference even contemplate that the genes encoding for such enzymes may be cloned to produce transgenic plants that are then thought to be less contaminated with mycotoxins. Alternatively, microorganisms may be employed to destroy enzymatically mycotoxins found in food products as described in U.S. Pat. No. 6,025,188 to Duvick et al.

10 Where mycotoxins are not yet produced by a microorganism present on a plant or other food stuff, pesticides or other compositions that control microbial growth or production of mycotoxins in microorganisms may be employed. For example, Emerson et al. describe in U.S. Pat. No. 5,639,794 use of a saponin as a synergist to control colonization and/or growth of plant and animal pathogens. Alternatively, as described in U.S. Pat. No. 4,199,606 to Bland, propionic
15 acid on a carrier may be employed as a diffusible growth inhibitor for various microorganisms. Further known compositions (see e.g., U.S. Pat. No. 5,698,599 to Subbiah or U.S. Pat. No. 3,798,323 to Leary) may be employed to suppress or at least reduce synthesis of mycotoxins in a microorganism.

20 Alternatively, mycotoxin-containing food products may be blended with uncontaminated food products to a concentration that is acceptable and/or below the maximum allowable amount of mycotoxins in food products (see e.g., Herrman, T. and Trigo-Stockli, D.; Mycotoxins in Feed Grains and Ingredients; Kansas State University, May 2002), or (at least potentially) mycotoxin-containing coffee cherry products may be employed in a non-food product. In still other uses, the mycotoxin content may not be considered relevant as the coffee cherry product is incinerated and
25 thus the mycotoxins are at least partially destroyed as described in U.S. Pat. No. 4,165,752, GB 2026839, or CA 1104410. Here, the inventor teaches that the coffee cherries may be compressed, dehydrated, ground, and roasted to yield a smokable product.

However, while most of the known methods reduce the concentration of mycotoxins to at least some degree, numerous disadvantages remain. Among other things, additional processing

steps will require dedicated equipment, thereby increasing processing time and costs. Moreover, and especially where pesticides and/or fungicides are used, new problems with residual toxic chemicals may arise.

Thus, despite numerous beneficial properties of coffee cherries and its components, whole coffee cherries are generally not used as food products as mycotoxins are typically present in substantial quantities in the ripe and overripe fruit. Therefore, there is still a need to provide improved methods and compositions for coffee cherries, and especially for products comprising coffee cherries with low or no mycotoxin content for human and veterinary consumption.

Summary of the Invention

The present invention is directed to compositions and methods that include quick-dried (preferably sub-ripe) coffee cherries or portions thereof, wherein the coffee cherries are substantially devoid of, or have a very low content of mycotoxins.

In one aspect of the inventive subject matter, a food product comprises a preparation of a coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins. Preferred preparations in such food products include the bean, pulp, mucilage, and/or hull of the quick-dried coffee cherry, or ground fragments of the coffee cherry, or an extract thereof. It is further preferred that the coffee cherry is a sub-ripe coffee cherry. Preferred food products include a tea brewed from the quick-dried (preferably sub-ripe) coffee cherries, or a beverage comprising an extract of the coffee cherry. Alternatively, suitable food products also include nutritional supplements in liquid or solid form comprising an extract of the coffee cherry.

Contemplated sub-ripe coffee cherries have a primarily green color with less than 25% red color, more preferably a primarily red color with less than 25% green color, and even more preferably a primarily red color with less than 5% blemished area. The (sub-ripe) coffee cherries may be quick-dried using various methods, however, it is generally preferred that the coffee cherries are quick dried using heated air or exposure to sun and/or ambient air.

In another aspect of the inventive subject matter, a tea is brewed from a comminuted or ground quick-dried (preferably sub-ripe) coffee cherry or portion thereof, wherein the coffee

cherry has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins, and preferably has a polyphenol concentration of at least 10 mg/oz (most preferably at a chlorogenic acid to caffeine ratio of at least 2.7).

5 Thus, viewed from another perspective, it is contemplated that a quick-dried coffee cherry or portion thereof has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins, preferably having a chlorogenic acid content of at least 2% (wt/wt) and a polyphenol content of at least 3.2% (wt/wt).

10 Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention.

Detailed Description

15 The inventors have discovered that low-mycotoxin or even mycotoxin-free tea and other food products may be produced from whole, substantially non-damaged coffee cherries that are preferably picked at a sub-ripe stage, and wherein the coffee cherry is quick-dried after harvest. Among other advantages, the inventors discovered that such coffee cherries significantly reduce the likelihood of infestation of the coffee cherry with mold and fungi known to produce mycotoxins. Still further, the inventors discovered that sub-ripe coffee cherries (in contrast to completely unripe coffee cherries) provide a desirable level of flavor and an aroma profile in the
20 coffee cherry product (e.g., coffee cherry tea) as well as relatively high levels of polyphenols, polysaccharides, and other nutrients.

25 The term "food product" as used herein refers to any product that is ingested by a human and/or animal for nutritional, health-maintenance, health-improvement, and/or recreational purpose. Particularly preferred food products include those consumed by human, wherein such food products may be solid products (e.g., dietary supplement, snack bar, bagged tea, etc.) or liquid products (e.g., tea or other beverage, syrup or elixir, etc.).

As also used herein, the term "coffee cherry" refers to the fruit of the coffee tree (*Coffea spec.*, Family *Rubiaceae*) in which exocarp and outer mesocarp (i.e., the pulp) surround the inner

mesocarp (*i.e.* the mucilage) and endocarp (*i.e.*, the hull), which in turn surround the seeds (*i.e.*, the beans). Thus, the term coffee cherry specifically refers to a whole coffee cherry, which may or may not include the stem of the cherry.

The term "sub-ripe coffee cherry" refers to a coffee cherry that has not yet reached the ripe stage, which is generally characterized by susceptibility to or presence of a fungal infection and/or presence of mycotoxins. Thus, a sub-ripe coffee cherry is at a ripeness stage in which the coffee cherry - when quick-dried - will exhibit mycotoxin levels that are below 20 ppb for total aflatoxins, below 5 ppm for total fumonisins, below 5 ppm for total vomitoxins, and below 5 ppb for ochratoxins. Consequently, quick-dried coffee cherries are typically dried within 0-48 hours (and more preferably between 6-24 hours) of the harvest such that the residual water content is no higher than 20% (wt/wt), and more typically no higher than 6-12% (wt/wt).

Viewed from an other perspective, sub-ripe coffee cherries generally have a complete or almost complete (at least 95% of the cherry) red color (or in some cases yellow color), and typically include various surface defects (*e.g.*, blemishes, cuts, and/or holes covering an area of more than 5% of the cherry). Thus, a sub-ripe coffee cherry will typically exhibit at least some green color (at least 5%, more typically at least 10%) and will typically be free of any surface defects (*e.g.*, blemishes, cuts, and/or holes covering an area of less than 5% of the cherry). Sub-ripe coffee cherries may also be characterized in that they will remain on the coffee tree for a subsequent round of picking where the coffee cherries are hand picked and used for the production of coffee beans. Alternatively, a color sorting machine with CCD equipment may be employed to identify and select sub-ripe coffee cherries on a quantitative color basis where the coffee cherries are mass-harvested and automatically sorted.

It should further be appreciated that while many of the following aspects and examples employ coffee cherries in a sub-ripe state, completely ripe coffee cherries are also contemplated suitable herein, especially where such ripe coffee cherries are substantially devoid of surface damage (*i.e.*, no more than 5% of surface area) or microbial infection (*i.e.*, infestation that results in mycotoxin levels of less than 20 ppb for total aflatoxins, less than 5 ppm for total fumonisins, less than 5 ppm for total vomitoxins, and less than 5 ppb for ochratoxins on a dry weight basis). Thus, all contemplated food products and/or coffee cherries may comprise completely ripe as

well as sub-ripe coffee cherries in varying proportions. For example, suitable proportions include 100% ripe : 0% sub-ripe, preferably 90% ripe : 10% sub-ripe, more preferably 75% ripe : 25% sub-ripe, even more preferably 50% ripe : 50% sub-ripe, and most preferably less than 25% ripe : more than 75% sub-ripe.

5 As further used herein, the term "quick-dried" coffee cherry means that the whole coffee cherry is dried under a protocol that limits growth of molds, fungi, and/or yeast to an extent such that the dried coffee cherry will exhibit mycotoxin levels that are below 20 ppb for total aflatoxins, below 5 ppm for total fumonisins, below 5 ppm for total vomitoxins, and below 5 ppb for ochratoxins. Consequently, quick-dried coffee cherries are typically dried within 0-48 hours
10 (and more preferably between 6-24 hours) of the harvest such that the residual water content is no higher than 20% (wt/wt), and more typically no higher than 6-12% (wt/wt).

As still further used herein, the term "mycotoxin" refers to any toxic product formed in a mold, fungus, and/or yeast that exhibits significant toxicity to a human or animal when ingested. Thus, specifically contemplated mycotoxins include aflatoxins (and particularly B1, B2, G1, and
15 G2), fumonisins (and particularly B1, B2, and B3), ochratoxin, deoxynivalenol (DON, vomitoxin), T-2 toxin, and zearalenone. The term "total aflatoxins" therefore refers to the sum of all aflatoxin variants, the term "total fumonisins" refers to the sum of all fumonisin variants, and the term "total ochratoxins" therefore refers to the sum of all ochratoxin variants.

In one exemplary aspect of the inventive subject matter, whole undamaged sub-ripe (e.g.,
20 semi ripe or almost ripe) coffee cherries are hand picked and within about one hour quick-dried using a dry air drier at about 140°F until constant weight is obtained. The so obtained coffee cherries typically possess significant storage stability, high resistance to infection by fungi, and lower shipping weight than wet cherry.

It is generally contemplated that the sub-ripe coffee cherries may be derived from various
25 sources, and the particular use of the sub-ripe coffee cherries will at least in part determine the particular source(s). However, it is preferred that the sub-ripe coffee cherries are derived from a single coffee species (e.g., *coffea arabica*), which is cultivated under similar growth conditions (e.g., shade-grown). Among other advantages, it is contemplated that a single source of coffee cherries will facilitate quick-drying the sub-ripe coffee cherries. It should be recognized,

however, that once the sub-ripe coffee cherries are quick-dried, various coffee species and/or coffee cherries from various growth conditions may be blended to achieve a mixture with particularly preferred characteristics.

Furthermore, it should be appreciated that depending on the particular product or use for the coffee cherry, the degree of ripeness of the coffee cherry may vary considerably. For example, where extraction of polyphenols and/or chlorogenic acid from the whole coffee cherry is desired, semi-ripe (stage 1 or stage 2) coffee cherries may be used. On the other hand, where the coffee cherry is used for the production of a coffee cherry tea and flavor and aroma are paramount, almost ripe coffee cherries may be picked. In still further contemplated aspects, unripe coffee cherries, or any reasonable mixtures of varying ripeness degrees may be used. Especially where the coffee cherry is a ripe coffee cherry, it is contemplated that the whole cherry is preferably free of surface defects, including cracks, splits, holes, or other openings. However, while not preferred, coffee cherries with surface defects may also be used. While not limiting to the inventive subject matter, it is generally preferred that the sub-ripe coffee cherries (or coffee cherry mixtures) are washed with water or other aqueous solution (e.g., diluted hypochlorite solution) to remove soil particles and other debris before drying.

Quick-drying is preferably performed immediately after picking to up to about two days after picking until a constant weight is obtained (or until the outer pulp of the cherry has dried). Thus, and depending on the particular heat source available, it is generally preferred that quick-drying is performed at a temperature of about 100° to about 180°F for a period of about 6-48 hours. For example, where electrical (or other) energy is readily available, the sub-ripe coffee cherries may be dried in a warm air drier in a stationary or rotating drum, or in a refractance window drying process. Alternatively, the sub-ripe coffee cherries may also be freeze dried. On the other hand, and especially where energy sources are not readily available, the sub-ripe coffee cherries may be sun dried. However, regardless of the particular drying method, it should be recognized that the sub-ripe coffee cherries are quick-dried to prevent production of mycotoxins from fungi, molds, and/or yeast that are already present and/or colonize (e.g., via infection or sporulation) the coffee cherry. Thus, sub-ripe coffee cherries are advantageously dried on a surface that is clean and free of sources of mycotoxin contamination. In further alternative

aspects of the inventive subject matter, the sub-ripe coffee cherries may also be frozen and stored/transported until quick-drying can be implemented.

The so obtained quick-dried sub-ripe coffee cherries may then without further mycotoxin detoxification be employed for various uses in numerous food products. For example, where the whole quick-dried sub-ripe coffee cherry is used in a food product, the coffee cherry may be admixed with another consumable (*e.g.*, admixture with grain for animal feed, or coating with chocolate for human consumption). In another especially preferred example, the quick-dried sub-ripe coffee cherry is ground and used as a food additive or as a basis for brewing coffee cherry tea (*e.g.*, for use as loose tea, grinding to a size of 500-3000 μm is preferred, or for bagged teas, grinding to a size of 200-1000 μm is preferred).

Alternatively, it should be recognized that only parts of the quick-dried sub-ripe coffee cherry may be employed in a food product. For example, where the sub-ripe coffee cherry is in an almost ripe state, it is contemplated that the pulp, mucilage, and/or hull may be separated from the seeds, which are then (optionally admixed with other seeds) roasted to commercial grade coffee beans. The remaining pulp, mucilage, and/or hull from the quick-dried sub-ripe coffee cherry may then be employed as food additive or basis for extraction of one or more desired components (*e.g.*, polyphenols).

In still further contemplated uses of so obtained quick-dried sub-ripe coffee cherries, it is contemplated that the coffee cherries (or portions thereof) may be employed as starting material for extraction of various beneficial components. For example, the quick-dried sub-ripe coffee cherries may be extracted with an aqueous (*e.g.*, water, water-ethanol mixture) or non-aqueous solvent (*e.g.*, critical point CO_2 , dimethylformamide) to isolate one or more components that can be used in a food product. For example, quick-dried sub-ripe coffee cherries may provide an excellent source of polyphenols, chlorogenic acid, and/or caffeine.

The term "polyphenol" as used herein refers to a diverse group of compounds produced by a plant, wherein the compounds include a phenol ring to which at least one OH group, and more typically at least two OH groups are covalently attached. For example, representative polyphenols include ellagic acid, tannic acid, vanillin, caffeic acid, chlorogenic acid, ferulic acid, catechins (*e.g.*, epicatechin gallate, epigallocatechin), flavonols (*e.g.*, anthocyanidins, quercetin,

kaempferol), and various other flavonoids, and their glycosides and depsides. Furthermore, contemplated polyphenols may also be in oligomeric or polymeric form (e.g., oligomeric proanthocyanidins or condensed tannins).

In another preferred aspect of the inventive subject matter, the inventors contemplate use of whole quick-dried sub-ripe coffee cherries in the production of various beverages. For example, it was observed that teas produced from unripe (green) and semi-ripe stage 1 whole quick-dried coffee cherries possess relatively low aroma and flavor characteristics. Thus, extracts or at least partially condensed teas from unripe (green) and semi-ripe stage 1 whole quick-dried coffee cherries may be added as low-flavor additive to a commercially available beverage to enhance the nutritional properties.

As ripeness increases, more aroma and flavor is evident in the quick-dried sub-ripe coffee cherries. Consequently, tea produced from semi-ripe stage 2 and almost ripe whole coffee cherries will possess high aroma and fruity flavor, and the inventors contemplate that semi-ripe and almost ripe coffee cherries could be used directly after quick-drying and grinding for a "Whole Coffee Fruit Tea", either by itself or after placing into tea bags. As illustrated below, such a tea would provide high levels of polyphenols, typically 60-70 mg per 6 oz. cup. Furthermore, teas prepared from whole quick-dried sub-ripe coffee cherries generally possess relatively high ratios of chlorogenic acid to caffeine (typically about 2-4.5) as compared to beverages made from roasted coffee (typically about 0.4). Therefore, it should be appreciated that tea prepared from quick-dried sub-ripe coffee cherries is much more nutritional (based upon polyphenols and chlorogenic acid) than roasted coffee.

Therefore, the inventors contemplate a food product that includes a preparation of a (preferably sub-ripe) coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins. Alternatively, contemplated mycotoxin levels may also be in the range of 20-50 ppb, but more preferably less than 15 ppb, even more preferably less than 10 ppb, and most preferably less than 5 ppb for total aflatoxins. Similarly contemplated mycotoxin levels also include a range of 10-30 ppb, but more preferably less than 5 ppb, even more preferably less than 3 ppb, and most preferably less than 2 ppb for total ochratoxins. Likewise, contemplated

mycotoxin levels also include a range of 5-20 ppm, but more preferably less than 15 ppm, even more preferably less than 10 ppm, and most preferably less than 5 ppm for total fumonisins and/or vomitoxins.

As already discussed above, the preparation of the sub-ripe coffee cherry may include the whole coffee cherry, comprise a ground fragment of the whole coffee cherry, or include the bean, the pulp, the mucilage, and/or the hull of the quick-dried sub-ripe coffee cherry. Alternatively, it should be recognized that the preparation may also comprise an extract from the whole quick-dried sub-ripe coffee cherry (or fragment or portion thereof).

Contemplated food products especially include beverages prepared from contemplated quick-dried sub-ripe coffee cherries (or fragments or portions thereof), or beverages to which extracts or pieces from contemplated quick-dried sub-ripe coffee cherries (or fragments or portions thereof) have been added. Similarly, further contemplated food products include baked goods (e.g., bread, crackers, etc.), snacks (e.g., candy or energy bars), cereals, and other solid nutrients to which extracts or pieces from contemplated quick-dried sub-ripe coffee cherries (or fragments or portions thereof) have been added. Alternatively, contemplated food products also include a nutritional supplement in liquid or solid form that comprises an extract of the quick-dried sub-ripe coffee cherry.

Depending on the particular purpose, it should be recognized that such food products may be prepared from quick-dried sub-ripe coffee cherries having a primarily green color with less than 25% red color, more preferably with less than 25% green color, and most preferably from quick-dried sub-ripe coffee cherries having primarily (no less than 90%, most typically no less than 95%) red color with less than 5% blemished area. Still further contemplated compositions and methods are disclosed in our co-pending U.S. Patent application with the title "Methods for Coffee Cherry Products", filed on or about April 16, 2003, which is incorporated by reference herein.

Examples

The following examples are provided to enable a person of ordinary skill in the art to make and use compositions according to the inventive subject matter and to illustrate exemplary compositions and methods generally described herein.

Harvest Of Whole Coffee Cherries

The ripeness of the coffee cherries was determined by visually estimating the amount of green and red color (or yellow, where applicable) of the whole cherries. As the cherries ripen, the green cherries will typically increase in size and subsequently develop increasing amounts of red color. For the present examples, the coffee cherries were collected at four stages of ripeness: Completely, or almost completely green (unripe; typically less than 5% of the coffee cherry red or yellow), primarily green with some red (semi-ripe, stage 1; typically less than 25% of the coffee cherry red or yellow), primarily red with some green (semi-ripe, stage 2; typically less than 25% of the coffee cherry green), and unbroken, unblemished red (almost ripe; typically less than 10% of the coffee cherry green; area of blemishes, cuts, or otherwise broken surface less than 5%). As much as possible, whole, unbroken and uncut cherries were collected.

Quick-Drying Of The Whole Coffee Cherries

Whole coffee cherries for sample extraction were prepared by drying the cherries within 1-12 hours after harvest on separate trays of an air dryer according to the following procedure. Coffee cherries (400-600 g) were weighed into beakers and washed two times with tap water, followed by a single wash with distilled water. The so washed coffee cherries were placed on a tray of an air dryer to drain, and then dried at 150-160°F for 16-18 hours to constant weight. Drying was stopped when the weight at two consecutive one-hour intervals differed by less than 1 g. Typical yields of dried whole cherry were 160-220 g. Further analysis indicated 6-12% residual water content in the dried cherry.

Mycotoxin Analysis

In order to determine the viability of the whole coffee cherry at the unripe, semi-ripe, and almost ripe stages (see above) for use in a nutritional product (and especially for use in tea), the level of selected mycotoxins was measured and compared against comparative products and red, ripe coffee cherry by-product from coffee production. As can be clearly seen in Table 1 below, quick-dried coffee cherries of all sub-ripe harvest stages had a mycotoxin level below the detection limit of 1 ppb (as measured for aflatoxin and ochratoxin).

The mycotoxin concentration was determined in an independent laboratory by both ELISA and HPLC analysis. Based on the below results, the inventors conclude that all samples from the different sub-ripe harvest stages are suitable for direct use in a nutritional product for both human and veterinary consumption. In contrast, the by-product of coffee production (predominantly consisting of pulp, mucilage, and hull from coffee cherries) from ripe cherries of red color with blemishes (typically greater than 20% of the cherry surface) had a substantial content in both aflatoxins and ochratoxins. Similarly, the comparative product "Paradise to Go Tea" (made from coffee cherry pulp) exhibited mycotoxins in double-digit concentrations.

RIPENESS	COLOR	AFLATOXIN	OCHRATOXIN
Unripe, quick-dried	Green	< 1ppb	< 1ppb
Semi-ripe Stage 1, quick-dried	Mostly green with some red	< 1ppb	< 1ppb
Semi-ripe Stage 2, quick-dried	Mostly red with some green	< 1ppb	< 1ppb
Almost ripe, quick-dried	Red, Blemished Area <5%	< 1ppb	< 1ppb
Ripe (by-product of Coffee Production)	Red, Blemished Area >20%	> 200 ppb	> 500 ppb
Paradise to Go Tea (dry matter)	N/A	> 25 ppb	> 40 ppb

Table 1

Polyphenol(PP), Chlorogenic Acid (CG), and Caffeine (CF) Analysis for Quick-dried Sub-ripe Whole Coffee Cherries

In a further series of experiments, the levels of total polyphenols, chlorogenic acid, and caffeine from quick-dried whole coffee cherry at various sub-ripe stages were measured and compared against green and roasted coffee beans. Table 2 summarizes the results of this analysis.

Interestingly, while the polyphenol (PP) level of quick-dried coffee cherries of all sub-ripe harvest stages was somewhat less than the level of green or roasted coffee beans, significant quantities of polyphenols in quick-dried sub-ripe coffee cherries still remain. Similarly, the chlorogenic acid (CG) content of quick-dried whole coffee cherry at various sub-ripe stages remained at substantial high levels as compared to roasted coffee, but was somewhat lower as

compared to green beans. The caffeine (CF) level of quick-dried coffee cherries of all sub-ripe harvest stages was substantially within the caffeine level of green and roasted coffee beans (It should be pointed out that all data given are on a dry matter basis and are not normalized to the dry weight of the bean).

5 Polyphenol analysis: Dried whole coffee cherry (or green beans or roasted beans) (1.00 g) were ground in a rotating steel knife coffee grinder for 30 seconds to produce a ground sample. The ground sample was added to 100 mL distilled water and the resulting mixture heated to boiling in an Erlenmeyer flask for 30 minutes. The heat was removed and the mixture allowed to cool to room temperature. The resulting suspension was transferred to a 100 mL graduated
10 cylinder and water added to bring the volume to 100 mL. The mixture was then transferred back to the Erlenmeyer flask, stirred briefly, and the solids allowed to settle. An aliquot (~3 mL) of the supernatant solution was filtered through an 0.45 μ m Acrodisc filter, and the resulting clear solution was diluted 1:10 with distilled water using a volumetric flask (1.00 mL diluted with 9.00 mL distilled water).

15 The Folin-Ciocalteu method was used to measure the polyphenol content of the diluted solution as follows. One mL of the diluted solution was added to a test tube, mixed with 1 mL of 0.2N Folin-Ciocalteu's Phenol reagent (Sigma solution, 2N, diluted 1:10 with water), and allowed to stand 5 minutes at room temperature. One mL of 1N NaHCO₃ was added and the reaction mixture left at room temperature for 2 hours. The polyphenol level was determined
20 using a UV-visible spectrophotometer standardized against catechin, at λ_{max} =750 nm against distilled water as blank.

Chlorogenic acid: Determination of chlorogenic acid was done using HPLC separation of the filtered clear solution prepared above using standard analytical and separation protocols well known in the art. Similarly, determination of caffeine was done using HPLC separation of the
25 filtered clear solution prepared above using standard analytical and separation protocols well known in the art (for exemplary protocols see e.g., Bispo M.S., et al. in J. Chromatogr. Sci.; 2002, Jan;40(1):45-8, or Nakakuki, H. et al. in J. Chromatogr. A.;1999, Jul 2;848(1-2):523-7).

RIPENESS	COLOR	% PP	%CG	% CF	CG/CF
Unripe, quick-dried	Green	3.80	2.64	1.03	2.56

Semi-ripe stage 1, quick-dried	Mostly green with some red	3.28	2.70	1.00	2.70
Semi-ripe stage 2, quick-dried	Mostly red with some green	3.54	2.00	0.70	2.86
Almost ripe, quick-dried	Red, Blemished Area <5%	3.35	N/D	N/D	N/D
Green Coffee beans	Green	4.58	3.31	0.95	3.48
Roasted Coffee beans	Brown	3.93	0.50	1.20	0.42

Table 2

Polyphenol(PP), Chlorogenic Acid (CG), and Caffeine (CF) Analysis for a Tea brewed from Quick-dried Sub-ripe Whole Coffee Cherries

Quick-dried sub-ripe coffee cherry were ground in a rotating steel knife coffee grinder for 10-30 seconds to produce a ground sample. To the ground sample (1.00 g) was added 90 mL (approx. 3 fluid oz.) boiling distilled water and the resulting mixture allowed to stand in an Erlenmeyer flask for 10 minutes to produce a coffee cherry tea. An aliquot (~3 mL) of the supernatant solution was filtered through a 0.45 µm Acrodisc filter, and the resulting clear solution was diluted 1:10 with distilled water using a volumetric flask (1.00 mL diluted with 9.00 mL distilled water). The Folin-Ciocalteu method as described above was used to measure the polyphenol content (on dry matter basis, catechin equivalents) of the so prepared coffee cherry tea. Table 3 summarizes the results.

RIPENESS	SOLVENT	% PP	%CG	% CF	CG/CF
Unripe, quick-dried	Water	10.93	8.61	3.04	2.83
Semi-ripe stage 1, quick-dried	Water	9.38	7.58	2.72	2.78
Semi-ripe stage 2, quick-dried	Water	8.51	6.74	1.71	3.95
Almost ripe, quick-dried	Water	6.92	1.34	0.29	4.61

Table 3

Brewing Of Teas From Whole Coffee Cherry Of Different Ripeness

The following procedure was used to prepare teas from whole coffee cherry of different ripeness for aroma and taste testing. Whole dried coffee cherry (10-20 g) was ground in a rotating steel knife coffee grinder for 10-30 seconds to produce a ground sample. The ground

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coffee cherry (2.0 g) was placed in a ceramic cup and of nearly boiling water (6 oz., 190-200°F) added. The slurry was stirred and allowed to stand for 3 minutes, at which time the aroma and taste of the supernatant liquid were noted. Results are shown in Table 4.

RIPENESS	COLOR	AROMA	TASTE	PP/6 OZ. TEA
Unripe, quick-dried	Green	None	Almost no taste	76 mg
Semi-ripe stage 1, quick-dried	Mostly green with some red	Very mild fruity	Slight fruit flavor	66 mg
Semi-ripe stage 2, quick-dried	Mostly red with some green	Mild fruity	Mild fruit flavor	71 mg
Almost ripe, quick-dried	Red, Blemished Area <5%	Fruity	Rich fruit flavor	67 mg

Table 4

5 Thus, specific embodiments and applications of low-mycotoxin coffee cherry products have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms

10 should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

15

CLAIMS

What is claimed is:

1. A food product comprising a preparation of a coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
2. The food product of claim 1 wherein the preparation of the coffee cherry comprises a ground fragment of the coffee cherry.
3. The food product of claim 2 wherein the preparation of the coffee cherry comprises an extract from a ground fragment of the coffee cherry.
4. The food product of claim 1 wherein the preparation of the coffee cherry comprises at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
5. The food product of claim 1 wherein the preparation of the coffee cherry comprises an extract from at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
6. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily green color with less than 25% red color.
7. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 25% green color.
8. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 5% blemished area.
9. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using heated air.
10. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using solar radiation.

11. The food product of claim 1 wherein the coffee cherry is quick dried by exposing the coffee cherry to at least one of ambient air and sun light.
12. The food product of claim 1 wherein the food product is a tea brewed from the coffee cherry.
- 5 13. The food product of claim 1 wherein the food product is a beverage comprising an extract of the coffee cherry.
14. The food product of claim 1 wherein the food product is nutritional supplement in liquid or solid form and comprising an extract of the coffee cherry.
15. A tea brewed from a comminuted quick-dried coffee cherry or portion thereof.
- 10 16. The tea of claim 15 wherein the coffee cherry has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
17. The tea of claim 16 having a polyphenol concentration of at least 10 mg/oz.
18. The tea of claim 15 wherein the coffee cherry is a sub-ripe coffee cherry.
- 15 19. A quick-dried coffee cherry or portion thereof having a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
20. The quick-dried coffee cherry of claim 19 wherein the coffee cherry is a sub-ripe coffee cherry.

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AMENDED CLAIMS

Received by the International Bureau on 08 September 2003 (08.09.03);
Original claims 1-20 replaced by amended claims 1-20 (2pages)

1. A food product that comprises a preparation of a coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
2. The food product of claim 1 wherein the preparation of the coffee cherry comprises a ground fragment of the coffee cherry.
3. The food product of claim 2 wherein the preparation of the coffee cherry comprises an extract from a ground fragment of the coffee cherry.
4. The food product of claim 1 wherein the preparation of the coffee cherry comprises at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
5. The food product of claim 1 wherein the preparation of the coffee cherry comprises an extract from at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
6. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily green color with less than 25% red color.
7. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 25% green color.
8. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 5% blemished area.
9. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using heated air.
10. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using solar radiation.
11. The food product of claim 1 wherein the coffee cherry is quick dried by exposing the coffee cherry to at least one of ambient air and sun light.

12. The food product of claim 1 wherein the food product is a tea brewed from the coffee cherry.
13. The food product of claim 1 wherein the food product is a beverage comprising an extract of the coffee cherry.
14. The food product of claim 1 wherein the food product is nutritional supplement in liquid or solid form and comprising an extract of the coffee cherry.
15. A tea that is brewed from a comminuted quick-dried coffee cherry or portion thereof.
16. The tea of claim 15 wherein the coffee cherry has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
17. The tea of claim 16 having a polyphenol concentration of at least 10 mg/oz.
18. The tea of claim 15 wherein the coffee cherry is a sub-ripe coffee cherry.
19. A quick-dried coffee cherry or portion thereof that has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
20. The quick-dried coffee cherry of claim 19 wherein the coffee cherry is a sub-ripe coffee cherry.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/11950

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A23F 5/02, 5/16

US CL : 426/595, 596, 433

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/595, 596, 433

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6376001 A (FABIAN) 23 April 2002 (23.04.02), see entire document.	1-20
A	US 5178832 A (PHILLIPS et al) 12 January 1993 (12.01.1993), see entire document.	1-20

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A" document member of the same patent family

Date of the actual completion of the international search

13 June 2003 (13.06.2003)

Date of mailing of the international search report

11 JUL 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
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Authorized officer

Anthony Weier

Telephone No. 703-308-0651

45
45

Attorney Docket No. 100700.0024PCT

**IN THE UNITED STATES INTERNATIONAL PRELIMINARY EXAMINATION
AUTHORITY (IPEA/US)**

International Application Number	International Filing Date	International Earliest Priority Date
PCT/US03/11950	16 April 2003	

Title of Invention: **Low-Mycotoxin Coffee Cherry Products**
Applicant: **VDF Futurechemicals**

**Mail Stop PCT - IPEA
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450**

**VERIFIED CERTIFICATION OF EXPRESS MAILING DATE
(INTERNATIONAL APPLICATION (37 CFR 1.10(c)))**

I declare that, on 12 November, 2004, I deposited, with the United States Postal Service, in an envelope "Express Mail, Post Office to Addressee," bearing Label Number EV389271813US, addressed to the "Commissioner for Patents, Alexandria VA 22313," and having an express mail certification that I executed, the following papers:

Chapter II Demand
Copy of Article 19 Amendments (5 pages)
Return Receipt Postcard

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date November 12, 2004


Barbara Hayashi

IPEA/US

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PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA		Date of receipt of DEMAND	
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 100700.0024P	
International application No. PCT/US03/11950	International filing date (day/month/year) 16 April 2003 (16.04.2003)	(Earliest) Priority date (day/month/year)	
Title of invention Low-Mycotoxin Coffee Cherry Products			
Box No. II APPLICANT(S)			
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) VDF FUTURECEUTICALS 300 West 6th Street Mokena, IL 60954-0009 US		Telephone No. 815-472-3100	
		Facsimile No. 815-472-3850	
		Teleprinter No.	
		Applicant's registration No. with the Office	
State (that is, country) of nationality: US		State (that is, country) of residence: US	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) MILJKOVIC, Dusan 4655 Ramsay Avenue San Diego, CA 92122 US			
State (that is, country) of nationality: Serbia		State (that is, country) of residence: US	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) DUELL, Brad L. 1441 Santa Lucia Rd. #1134 Chula Vista, CA 91913 US			
State (that is, country) of nationality: US		State (that is, country) of residence: US	
☒ Further applicants are indicated on a continuation sheet.			

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCEThe following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

FESSENMAIER, Martin; FISH, Robert D.

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Costa Mesa, CA 92626-1931

US

Telephone No.

714-641-5100

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714-546-9035

Teleprinter No.

Agent's registration No. with the Office

46697

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION****Statement concerning amendments:***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filedthe description ☒ as originally filed☐ as amended under Article 34the claims ☐ as originally filed☒ as amended under Article 19 (together with any accompanying statement)☐ as amended under Article 34the drawings ☒ as originally filed☐ as amended under Article 342. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of the applicable time limit under Rule 69.1(d).4. ☐ The applicant expressly wishes the international preliminary examination to start earlier than at the expiration of the applicable time limit under Rule 54bis.1(a).

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English☒ which is the language in which the international application was filed.☐ which is the language of a translation furnished for the purposes of international search.☐ which is the language of publication of the international application.☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.**Box No. V ELECTION OF STATES**

The filing of this demand constitutes the election of all Contracting States which are designated and are bound by Chapter II of the PCT.

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | | |
|--|---|-------|--------|
| 1. translation of international application | : | _____ | sheets |
| 2. amendments under Article 34 | : | _____ | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | 2 | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | 3 | sheets |
| 5. letter | : | _____ | sheets |
| 6. other (specify) | : | _____ | sheets |

For international Preliminary Examining Authority use only

received	not received
----------	--------------

☐	☐
☐	☐
☐	☐
☐	☐
☐	☐
☐	☐

The demand is also accompanied by the item(s) marked below:

- | | |
|--|---|
| 1. ☒ fee calculation sheet | 5. ☐ statement explaining lack of signature |
| 2. ☐ original separate power of attorney | 6. ☐ sequence listings in computer readable form |
| 3. ☐ original general power of attorney | 7. ☐ tables in computer readable form related to sequence listings |
| 4. ☐ copy of general power of attorney; reference number, if any: | 8. ☐ other (specify): |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).


Martin Fessenmaier, Agent

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

- | | |
|--|--|
| 3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.
☐ The applicant has been informed accordingly. | 6. ☐ The date of receipt of the demand is AFTER the expiration of the time limit under Rule 54bis.1(a) and item 7 or 8, below, does not apply. |
| 4. ☐ The date of receipt of the demand is WITHIN the time limit of 19 months from the priority date as extended by virtue of Rule 80.5. | 7. ☐ The date of receipt of the demand is WITHIN the time limit under Rule 54bis.1(a) as extended by virtue of Rule 80.5. |
| 5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82. | 8. ☐ Although the date of receipt of the demand is after the expiration of the time limit under Rule 54bis.1(a), the delay in arrival is EXCUSED pursuant to Rule 82. |

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Demand received from IPEA on:

PCT

FEE CALCULATION SHEET

Annex to the Demand

International application No. PCT/US03/11950		For International Preliminary Examining Authority use only	
Applicant's or agent's file reference 100700.0024P		Date stamp of the IPEA	
Applicant VDF FUTURECEUTICALS, et al.			
CALCULATION OF PRESCRIBED FEES			
1. Preliminary examination fee		600.00	P
2. Handling fee (<i>Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.</i>)		162.00	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box		USD 762.00	
		TOTAL	
MODE OF PAYMENT			
☒ authorization to charge deposit account with the IPEA (see below)	☐ cash		
☐ cheque	☐ revenue stamps		
☐ postal money order	☐ coupons		
☐ bank draft	☐ other (specify):		
AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT (This mode of payment may not be available at all IPEAs)			
☒ Authorization to charge the total fees indicated above.		IPEA/ US	
☐ (This check-box may be marked only if the conditions for deposit accounts of the IPEA so permit) Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.		Deposit Account No.: 502191	
		Date: 12 November 2004	
		Name: Martin Fessenmayer	
		Signature: 	

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*
MILJKOVIC, Vukosava
4655 Ramsay Avenue
San Diego, CA 92122
US

State *(that is, country)* of nationality:
Serbia

State *(that is, country)* of residence:
US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

☐

Further applicants are indicated on another continuation sheet.

IN THE INTERNATIONAL BUREAU (WIPO)

International Application Number	International Filing Date	International Earliest Priority Date
PCT/US03/11950	16 April 2003	-/-

Title of Invention: **LOW-MYCOTOXIN COFFEE CHERRY PRODUCTS**
Applicant: **VDF Futurecenticals**

International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20
Switzerland

**LETTER FOR PCT ARTICLE 19
(PCT SECTION 205)**

1. Applicant herewith submits replacement sheets numbered 7-8 to replace sheets numbered 7-8 originally filed for this application.
2. In respect of each claim appearing in the international application based on the replacement sheets submitted herewith, and in accordance with PCT Section 205, the following claim(s) is/are:
 - (i) unchanged: claim(s) 2-14, 16-18, and 20
 - (ii) cancelled: claim(s) -/-
 - (iii) new: claim(s) -/-
 - (iv) replacement of one or more claims as filed, as follows: 1, 15, and 19
 - (v) the result of the division of one or more claims as filed, as follows: -/-

Dear Sir:

The Search Report dated 11 July, 2003 designated one reference as being relevant to patentability. In response, claims 1, 15, and 19 have been revised. The revised claims and reference are addressed below *seriatim*.

Revised Claims

1. (Amended) A food product that comprises [comprising] a preparation of a coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
15. (Amended) A tea that is brewed from a comminuted quick-dried coffee cherry or portion thereof.
19. (Amended) A quick-dried coffee cherry or portion thereof that has [having] a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.

Fabian (U.S. Patent 6,376,001)

The Office considers claims 1-20 to be obvious over Fabian. The applicant disagrees, especially in view of the amendments herein.

Amended claim 1, and claims 2-14 by virtue of their dependence on amended claim 1 expressly require "...a preparation of a coffee cherry that is quick-dried such that [the] mycotoxin level of the coffee cherry is less than..." Fabian fails to teach these elements. There is also no suggestion or motivation in the '001 patent to modify Fabian's process such as to arrive at the subject matter as presently claimed. On the contrary, Fabian clearly teaches against the currently pending claims.

Fabian employs green coffee beans, and not a coffee cherry as expressly required by each of claims 1-14. A coffee bean is inconsistent with a coffee cherry, and particularly in view of the process taught by Fabian. Furthermore, Fabian teaches a protocol in which water is added to the beans prior to solvent extraction to remove the mycotoxin, and not quick-drying of whole cherry preparations as presently claimed. Clearly, Fabian teaches away, and in some respect even against the subject matter of claims 1-15.

Amended claim 15, and claims 16-18 by virtue of their dependence on amended claim 15 expressly require "...a tea..." that is brewed from "...a comminuted quick-dried coffee cherry ..." Once more, Fabian fails to teach these elements. There is also no suggestion or motivation in the Fabian reference to modify Fabian's process to a tea product as presently claimed. Again, Fabian clearly teaches against the currently pending claims.

First, Fabian employs green coffee beans, but not a coffee cherry, let alone a comminuted coffee cherry as expressly recited in amended claim 15 and dependent claims 16-18. Use of green coffee beans is at least inconsistent with a coffee cherry, but clearly teaches against use of comminuted coffee cherries as expressly required in claims 15-18. Furthermore, a tea is simply not a coffee brew, and there is nothing in the '001 reference that would indicate any other use for the green coffee beans than that commonly known in the art (i.e., roasting, grinding, and hot-extraction with water).

Amended claim 19, and claim 20 by virtue of its dependence on amended claim 19 expressly require "...a quick-dried coffee cherry or portion thereof that has a mycotoxin level of less than [specified values]..." Again, Fabian fails to teach these elements, and there is no suggestion or motivation in the '001 patent to modify Fabian's process to arrive at the subject matter as presently claimed.

Once more, Fabian employs green coffee beans, but not a coffee cherry, let alone a quick-dried coffee cherry as expressly recited in amended claim 19 and dependent claim 20. Furthermore, the addition of water to green coffee beans clearly teaches against quick-drying a coffee cherries as expressly required in claims 19-20.

Therefore, and at least for these reasons, Fabian not only fails to teach each of the elements recited in each of claims 1-20, but also fails to provide any suggestion or motivation to modify the teachings such as to arrive at the presently pending claims. Consequently, claims 1-20 are not obvious over Fabian.

Respectfully submitted,



Martin Fessenmaler, Ph.D.

Attorneys for Applicant(s)
Rutan & Tucker, LLP
Post Office Box 1950
Costa Mesa, CA 92628-1950
Tel: (714) 641-5100
Fax: (714) 546-9035

CLAIMS

What is claimed is:

1. A food product that comprises a preparation of a coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
2. The food product of claim 1 wherein the preparation of the coffee cherry comprises a ground fragment of the coffee cherry.
3. The food product of claim 2 wherein the preparation of the coffee cherry comprises an extract from a ground fragment of the coffee cherry.
4. The food product of claim 1 wherein the preparation of the coffee cherry comprises at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
5. The food product of claim 1 wherein the preparation of the coffee cherry comprises an extract from at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
6. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily green color with less than 25% red color.
7. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 25% green color.
8. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 5% blemished area.
9. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using heated air.
10. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using solar radiation.
11. The food product of claim 1 wherein the coffee cherry is quick dried by exposing the coffee cherry to at least one of ambient air and sun light.

12. The food product of claim 1 wherein the food product is a tea brewed from the coffee cherry.
13. The food product of claim 1 wherein the food product is a beverage comprising an extract of the coffee cherry.
14. The food product of claim 1 wherein the food product is nutritional supplement in liquid or solid form and comprising an extract of the coffee cherry.
15. A tea that is brewed from a comminuted quick-dried coffee cherry or portion thereof.
16. The tea of claim 15 wherein the coffee cherry has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
17. The tea of claim 16 having a polyphenol concentration of at least 10 mg/oz.
18. The tea of claim 15 wherein the coffee cherry is a sub-ripe coffee cherry.
19. A quick-dried coffee cherry or portion thereof that has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
20. The quick-dried coffee cherry of claim 19 wherein the coffee cherry is a sub-ripe coffee cherry.

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Please acknowledge receipt of the following by affixing hereon the Official date stamp and returning this card to our office.

Title:	Low-Mycotoxin Coffee Cherry Products
Inventor:	VDF - Dusan Miljkovic, et al.
Filing Date:	4/16/03
Serial Number:	PCT/US03/11950
Matter Type:	Patent - PCT
Date of Deposit:	12 November 2004
Matter #:	100700.0024PCT
Enclosures:	(A) Certificate of Express Mail; (B) Chapter II Demand; (C) Copy of Article 19 Amendments (5 pages); (D) Return Receipt Postcard

EV389271813US

PATENT COOPERATION TREATY

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From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
MARTIN FESSENMAIER
RUTAN & TUCKER
611 ANTON BOULEVARD, 14TH FLOOR
COSTA MESA, CA 92626-1931

PCT

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Rule 71.1)

Date of mailing
(day/month/year)

29 MAR 2005

Applicant's or agent's file reference

100700.0024P

IMPORTANT NOTIFICATION

International application No.

PCT/US03/11950

International filing date (day/month/year)

16 April 2003 (16.04.2003)

Priority date (day/month/year)

Applicant

VDF FUTURECEUTICALS

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the *PCT Applicant's Guide*.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed invention is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the IPEA/ US

Mail Stop PCT, Attn: IPEA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer

Anthony Weier

Telephone No. 571-272-1700



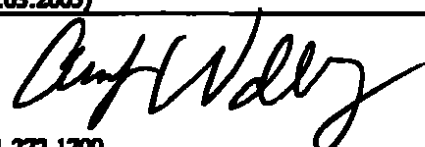
PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 100700.0024P		FOR FURTHER ACTION		See Form PCT/IPEA/416																
International application No. PCT/US03/11950		International filing date (day/month/year) 16 April 2003 (16.04.2003)		Priority date (day/month/year)																
International Patent Classification (IPC) or national classification and IPC IPC(7): A23F 5/02, 5/16 and US Cl.: 426/595, 596, 433																				
Applicant VDF FUTURECEUTICALS																				
1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of <u>5</u> sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. ☒ (sent to the applicant and to the International Bureau) a total of <u>2</u> sheets, as follows: ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions). ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box. b. ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions). 4. This report contains indications relating to the following items: <table border="0" style="width: 100%;"> <tr> <td>☒ Box No. I</td> <td>Basis of the report</td> </tr> <tr> <td>☐ Box No. II</td> <td>Priority</td> </tr> <tr> <td>☐ Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td>☐ Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td>☒ Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td>☐ Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td>☐ Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td>☐ Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </table>					☒ Box No. I	Basis of the report	☐ Box No. II	Priority	☐ Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	☐ Box No. IV	Lack of unity of invention	☒ Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	☐ Box No. VI	Certain documents cited	☐ Box No. VII	Certain defects in the international application	☐ Box No. VIII	Certain observations on the international application
☒ Box No. I	Basis of the report																			
☐ Box No. II	Priority																			
☐ Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability																			
☐ Box No. IV	Lack of unity of invention																			
☒ Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement																			
☐ Box No. VI	Certain documents cited																			
☐ Box No. VII	Certain defects in the international application																			
☐ Box No. VIII	Certain observations on the international application																			
Date of submission of the demand 12 November 2004 (12.11.2004)		Date of completion of this report 15 March 2005 (15.03.2005)																		
Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230		Authorized officer Anthony Weier  Telephone No. 571-272-1700																		

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US03/11950

Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language _____, which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:
- ☐ the international application as originally filed/furnished
- ☒ the description:
- pages 1-15 as originally filed/furnished
- pages* NONE received by this Authority on _____
- pages* NONE received by this Authority on _____
- ☒ the claims:
- pages NONE as originally filed/furnished
- pages* 16 and 17 as amended (together with any statement) under Article 19
- pages* NONE received by this Authority on _____
- pages* NONE received by this Authority on _____
- ☐ the drawings:
- pages NONE as originally filed/furnished
- pages* NONE received by this Authority on _____
- pages* NONE received by this Authority on _____
- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages _____
 - ☐ the claims, Nos. _____
 - ☐ the drawings, sheets/figs _____
 - ☐ the sequence listing (specify): _____
 - ☐ any table(s) related to the sequence listing (specify): _____
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages _____
 - ☐ the claims, Nos. _____
 - ☐ the drawings, sheets/figs _____
 - ☐ the sequence listing (specify): _____
 - ☐ any table(s) related to the sequence listing (specify): _____

* If item 4 applies, some or all of those sheets may be marked "superseded."

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US03/11950

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement			
Novelty (N)	Claims 1-20		YES
	Claims NONE		NO
Inventive Step (IS)	Claims 1-20		YES
	Claims NONE		NO
Industrial Applicability (IA)	Claims 1-20		YES
	Claims NONE		NO

2. Citations and Explanations (Rule 70.7)

Claims 1-20 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a food product comprising coffee cherry with the amount of total aflatoxins, total ochratoxins and total fumonisins called for in the instant claims and a tea in view of the meaning derived from the specification wherein same is prepared from a comminuted quick-dried coffee cherry or portion thereof.

Claims 1-20 meet the criteria set out in PCT Article 33(4), and thus the food product and tea have industrial applicability because the subject matter claimed can be made or used in industry.

NEW CITATIONS

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/US03/11930

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, Item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion was established on the basis of:

a. type of material

- ☐ a sequence listing
☐ table(s) related to the sequence listing

b. format of material

- ☐ in written format
☐ in computer readable form

c. time of filing/furnishing

- ☐ contained in the international application as filed
☐ filed together with the international application in computer readable form
☐ furnished subsequently to this Authority for the purposes of search and/or examination
☐ received by this Authority as an amendment* on _____

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US03/11950

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

What is claimed is:

1. A food product that comprises a preparation of a coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
2. The food product of claim 1 wherein the preparation of the coffee cherry comprises a ground fragment of the coffee cherry.
3. The food product of claim 2 wherein the preparation of the coffee cherry comprises an extract from a ground fragment of the coffee cherry.
4. The food product of claim 1 wherein the preparation of the coffee cherry comprises at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
5. The food product of claim 1 wherein the preparation of the coffee cherry comprises an extract from at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
6. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily green color with less than 25% red color.
7. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 25% green color.
8. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 5% blemished area.
9. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using heated air.
10. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using solar radiation.
11. The food product of claim 1 wherein the coffee cherry is quick dried by exposing the coffee cherry to at least one of ambient air and sun light.

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12. The food product of claim 1 wherein the food product is a tea brewed from the coffee cherry.
 13. The food product of claim 1 wherein the food product is a beverage comprising an extract of the coffee cherry.
 14. The food product of claim 1 wherein the food product is nutritional supplement in liquid or solid form and comprising an extract of the coffee cherry.
 15. A tea that is brewed from a comminuted quick-dried coffee cherry or portion thereof.
 16. The tea of claim 15 wherein the coffee cherry has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
 17. The tea of claim 16 having a polyphenol concentration of at least 10 mg/oz.
 18. The tea of claim 15 wherein the coffee cherry is a sub-ripe coffee cherry.
 19. A quick-dried coffee cherry or portion thereof that has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
 20. The quick-dried coffee cherry of claim 19 wherein the coffee cherry is a sub-ripe coffee cherry.



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UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

JANUARY 21, 2004

PTAS



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RUTAN & TUCKER LLP
ROBERT D. FISH
611 ANTON BLVD., SUITE 1400
COSTA MESA, CA 92626

**UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT**

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 07/14/2003

REEL/FRAME: 014270/0395
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
MILJKOVIC, DUSAN

DOC DATE: 10/18/2002

ASSIGNOR:
DUELL, BRAD L.

DOC DATE: 10/18/2002

ASSIGNOR:
MILJKOVIC, VUKOSAVA

DOC DATE: 10/18/2002

ASSIGNEE:
VDF FUTURECEUTICALS INC.
300 W. 6TH STREET
MOMENCE, ILLINOIS 60954

SERIAL NUMBER:
PATENT NUMBER:
PCT NUMBER: US0311950

FILING DATE:
ISSUE DATE:

66

014270/0395 PAGE 2

THERESA FREDERICK, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

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7.1403

07-18-2003



Form PTO-1595
(Rev. 10/02)
OMB No. 0651-0027 (exp. 07/30/2005)
Tab settings $\Rightarrow \Rightarrow \Rightarrow$

RE
102500900

U.S. DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office

To the Honorable Commissioner of Patents and Trademarks: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies): Dusan Miljkovic Brad L. Duell Vukosava Miljkovic Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No		2. Name and address of receiving party(ies) Name: <u>VDF Futureceuticals, Inc.</u> Internal Address: <u>300 W. 6th Street</u> Street Address: _____ City: <u>Momence</u> State: <u>IL</u> Zip: <u>60954</u> Additional name(s) & address(es) attached? ☐ Yes ☒ No	
3. Nature of conveyance: ☒ Assignment ☐ Merger ☐ Security Agreement ☐ Change of Name ☐ Other _____ Execution Date: <u>October 18, 2002</u>			
4. Application number(s) or patent number(s): If this document is being filed together with a new application, the execution date of the application is: _____ A. Patent Application No.(s) <u>PCT/US03/11950</u> B. Patent No.(s) _____ Additional numbers attached? ☐ Yes ☒ No			
5. Name and address of party to whom correspondence concerning document should be mailed: Name: <u>Robert D. Fish</u> Internal Address: <u>Rutan & Tucker LLP</u> Street Address: <u>611 Anton Blvd., Suite 1400</u> City: <u>Costa Mesa</u> State: <u>CA</u> Zip: <u>92626</u>		6. Total number of applications and patents involved: ☒ 1 7. Total fee (37 CFR 3.41).....\$40.00 ☐ Enclosed ☒ Authorized to be charged to deposit account 8. Deposit account number: <u>502191</u> (Attach duplicate copy of this page if paying by deposit account)	
DO NOT USE THIS SPACE			
9. Statement and signature. <i>To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.</i> Robert D. Fish Name of Person Signing Signature 7/10/03 Date Total number of pages including cover sheet, attachments, and documents: <u>4</u>			

Mail documents to be recorded with required cover sheet information to:
Commissioner of Patents & Trademarks, Box Assignments
Washington, D.C. 20231

07/17/2003
01 FC-M21

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ASSIGNMENT

WHEREAS, the undersigned, **Dusan Miljkovic, Brad L. Duell, and Vukosava Miljkovic**, all individuals employed by **VDF Futureceuticals, 300 W. 6th Street, Momence, Illinois, 60954-0009** (individually and collectively referred to hereinafter as "**ASSIGNORS**") has invented a certain invention entitled "**Low-Mycotoxin Coffee Cherry Products**", for which a PCT application for Letters of Patent of the United States of America was filed on April 16, 2003, serial number PCT/US03/11950; which together with related experimental data, trade secret, and other know-how is referred to hereinafter as the **INVENTION**;

WHEREAS, **VDF Futureceuticals** a company having its principal place of business at 300 W. 6th Street, Momence, Illinois, 60954-0009 (referred to hereinafter as "**ASSIGNEE**"), is desirous of acquiring all entire right, title and interest in, to and under said **INVENTION**, and in, to and under Letters Patent or similar legal protection to be, or having been, obtained therefor in the United States of America, its territorial possessions and in any and all countries foreign thereto;

NOW, THEREFORE, for good and valuable consideration, the receipt of which is hereby acknowledged, **ASSIGNORS** hereby sell, assign, transfer and set over unto the **ASSIGNEE**, its successors and assigns the entire title, right and interest in and to the **INVENTION**, and to all Letters Patent or similar legal protection arising therefrom, not only in the United States and its territorial possessions, but in all countries foreign thereto to be obtained for said **INVENTION** by said application or any continuation, continuation-in-part, divisional, renewal, substitute, reissue or reexamination thereof or any legal equivalent thereof in a foreign country for the full term or terms for which the same may be granted, including all priority rights under any International Convention.

ASSIGNORS hereby covenant that no assignment, sale, agreement or encumbrance has been or will be made or entered into which would conflict with this assignment;

ASSIGNORS further covenant that **ASSIGNEE** will, upon its request, be provided promptly with all pertinent facts and documents relating to said **INVENTION**, including the patent application listed above, and any Letters Patent and legal equivalents in foreign countries issuing therefrom as may be known and accessible to **ASSIGNORS**, and will testify as to the same in any interference or litigation related thereto and will promptly execute and deliver to **ASSIGNEE** or its legal representatives any and all papers, instruments or affidavits required to apply for, obtain, maintain, issue and enforce said invention and said Letters Patent and said equivalents thereof in any foreign country which may be necessary or desirable to carry out the purposes thereof.

Expected Performance. Each party herein agrees to perform all acts and execute and deliver all documents as may be necessary or appropriate to carry out the intent and purposes of This Agreement.

Entire Agreement. This Agreement embodies the entire understanding of The Parties and supersedes and replaces any and all pre-existing agreements or understandings between Assignee and Assignor. No amendment or modification of this Agreement shall be valid or binding upon

Expected Performance. Each party herein agrees to perform all acts and execute and deliver all documents as may be necessary or appropriate to carry out the intent and purposes of This Agreement.

Entire Agreement. This Agreement embodies the entire understanding of The Parties and supersedes and replaces any and all pre-existing agreements or understandings between Assignee and Assignor. No amendment or modification of this Agreement shall be valid or binding upon Assignee and Assignor unless made in writing and signed on behalf of each of The Parties by their respective duly authorized representative.

This Agreement may be executed in any number of counterparts, each of which shall be deemed an original but all of which together shall constitute one and the same instrument.

WITNESS my hand at Momence, IL, this 29 day of April 2003,
City State Month

Dusan Miljkovic
Dusan Miljkovic

WITNESS my hand at Momence, IL, this 29 day of April 2003,
City State Month

Bradley L. Duell
Brad L. Duell

WITNESS my hand at San Diego, CA, this 1 day of MAY 2003,
City State Month

Vukosava Miljkovic
Vukosava Miljkovic



D. 1/170

Please type a plus sign (+) inside this box → ☒

PTO/SB/21 (8-88)
Approved for use through 09/30/2000. OMB 0851-0031
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM (to be used for all correspondence after initial filing)	Application Number	PCT/US03/11950
	Filing Date	April 16, 2003
	First Named Inventor	Dusan Miljkovic
	Group Art Unit	N/A
	Examiner Name	N/A
Total Number of Pages in This Submission	4	Attorney Docket Number 100700.24PCT

ENCLOSURES (check all that apply)		
☐ Fee Transmittal Form	☒ Assignment Papers (for an Application)	☐ After Allowance Communication to Group
☐ Fee Attached	☐ Drawing(s)	☐ Appeal Communication to Board of Appeals and Interferences
☐ Amendment / Response	☐ Licensing-related Papers	☐ Appeal Communication to Group (Appeal Notice, Brief, Reply Brief)
☐ After Final	☐ Petition Routing Slip (PTC/SB/88) and Accompanying Petition	☐ Proprietary Information
☐ Affidavits/declaration(s)	☐ Petition to Convert to a Provisional Application	☐ Status Letter
☐ Extension of Time Request	☐ Power of Attorney, Revocation Change of Correspondence Address	☐ Additional Enclosure(s) (please identify below):
☐ Express Abandonment Request	☐ Terminal Disclaimer	
☐ Information Disclosure Statement	☐ Small Entity Statement	
☐ Certified Copy of Priority Document(s)	☐ Request for Refund	
☐ Response to Missing Parts/Incomplete Application	Remarks	
☐ Response to Missing Parts under 37 CFR 1.52 or 1.53		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT	
Firm or Individual name	Robert D. Fish; Fish & Associates, LLP
Signature	
Date	7/10/03

CERTIFICATE OF MAILING	
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on this date: 07/10/2003	
Typed or printed name	Erika E. Simpson
Signature	
Date	7/10/03

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

PATENT COOPERATION TREATY

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From the INTERNATIONAL SEARCHING AUTHORITY

To: ROBERT FISH RUTAN & TUCKER LLP 611 ANTON BLVD., SUITE 1400 COSTA MESA, CA 92626
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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 100700.24PCT	Date of Mailing <i>(day/month/year)</i> 11 JUL 2003
International application No. PCT/US03/11950	International filing date <i>(day/month/year)</i> 16 April 2003 (16.04.2003)
Applicant VDF FUTURECEUTICALS	

1.	☒	The applicant is hereby notified that the international search report has been established and is transmitted herewith. Filing of amendments and statement under Article 19: The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46): When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report. Where? Directly to the International Bureau of WIPO, 34, chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 740.14.35 For more detailed instructions, see the notes on the accompanying sheet.
2.	☐	The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.
3.	☐	With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that: ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices. ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.
4.		Reminders Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90 bis.1 and 90 bis.3, respectively, before the completion of the technical preparations for international publication. Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices. In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months. See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the <i>PCT Applicant's Guide</i>, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer Anthony Weier Telephone No. 703-308-0651
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Form PCT/ISA/220 (April 2002)

(See notes on accompanying sheet)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 100700.24PCT	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US03/11950	International filing date (<i>day/month/year</i>) 16 April 2003 (16.04.2003)	(Earliest) Priority Date (<i>day/month/year</i>)
Applicant VDF FUTURECEUTICALS		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the Report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (See Box II).

4. With regard to the title,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the abstract,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No. _____



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/11950

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A23F 5/02, 5/16

US CL : 426/595, 596, 433

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/595, 596, 433

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6376001 A (FABIAN) 23 April 2002 (23.04.02), see entire document.	1-20
A	US 5178832 A (PHILLIPS et al) 12 January 1993 (12.01.1993), see entire document.	1-20

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" documents defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

13 June 2003 (13.06.2003)

Date of mailing of the international search report

11 JUL 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US

Commissioner for Patents

P.O. Box 1430

Alexandria, Virginia 22313-1430

Facsimile No. (703)305-3230

Authorized officer

Anthony Weier

Telephone No. 703-308-0651

NOTES TO FORM PCT/ISA/220 (continued)

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 15 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

The statement should be brief, it should not exceed 500 words if in English or if translated into English.

It should not be confounded with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)".

It should not contain any disparaging comments on the international search report or the relevance of citations contained in that report. References to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

In what language ?

The amendments must be made in the language in which the international application is published. The letter and any statement accompanying the amendments must be in the same language as the international application if that language is English or French; otherwise, it must be in English or French, at the choice of the applicant.

Consequence if a demand for international preliminary examination has already been filed ?

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(e), first sentence).

Consequence with regard to translation of the international application for entry into the national phase ?

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty and of the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule" and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purpose of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended ?

The claims only.

The description and the drawings may only be amended during international preliminary examination under Chapter II.

When ? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments ?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How ? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or cancellation, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

What documents must/may accompany the amendments ?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confounded with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

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US006376001B1

(12) **United States Patent**
Fabian

(10) **Patent No.: US 6,376,001 B1**
(45) **Date of Patent: Apr. 23, 2002**

(54) **PROCESS FOR REMOVING MICOTOXINS
FROM A LOAD OF GREEN COFFEE**

5,288,511 A 2/1994 Kachas et al.
6,048,559 A 4/2000 Fabian

(75) **Inventor: Massimo Fabian, Trieste (IT)**

FOREIGN PATENT DOCUMENTS

(73) **Assignee: Demas S.p.A., Trieste (IT)**

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EP	0158381 A1	10/1985
FR	688638	8/1930
WO	WO9742831	11/1997

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) **Appl. No.: 09/330,782**

OTHER PUBLICATIONS

(22) **PCT Filed: Oct. 24, 1998**

(86) **PCT No.: PCT/EP98/06768**

§ 371 Date: May 5, 2000

§ 102(e) Date: May 5, 2000

(87) **PCT Pub. No.: WO99/23898**

PCT Pub. Date: May 28, 1999

(30) **Foreign Application Priority Data**

Nov. 7, 1997 (IT) M97A2496

(51) **Int. Cl.⁷ A23F 5/02**

(52) **U.S. Cl. 426/430; 426/425; 426/432;
426/434**

(58) **Field of Search 426/425, 430,
426/432, 434**

(56) **References Cited**

U.S. PATENT DOCUMENTS

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Hisaya Terada et al., "Liquid Chromatographic Determination of Ochratoxin A in Coffee Beans and Coffee Products," *Journal of the Association Analytical Chemists*, vol. 69, No. 6, 1986, pp. 960-964.

* cited by examiner

Primary Examiner—Anthony J. Weir

(74) *Attorney, Agent, or Firm*—Young & Thompson

(57) **ABSTRACT**

Green coffee is introduced into a container where it is subjected to continuous mixing in temperature continuous mixing in temperature ranging between environment and values close to 120° C. and water vapor is introduced into said container until the single beans of coffee have become porous and permeable; micotoxins are removed by extraction with a solvent in an acid environment obtained with a given acid or with a buffer solution with a given acid pH.

12 Claims, No Drawings

PROCESS FOR REMOVING MICOTOXINS FROM A LOAD OF GREEN COFFEE

The present invention concerns a process for removing micotoxins from a load of green coffee, intending as micotoxins in this case at least those micotoxins known as ochratoxins and aflatoxins that may be present in green coffee. One of the purposes of this process is to remove the micotoxins though maintaining the quantity of caffeine within the values of a non decaffeinated coffee. It is well known that the quantities of these micotoxins in coffee are very small in an absolute sense (for instance only a few—ppb—parts per billion, that is mg/ton) and that their presence may depend on the geographic area in which coffee grows, on possible unfavourable weather conditions and on possible negligence in storage and handling.

The applicant filed patent application PCT/EP97/02014 for an industrial process suitable to removing the above mentioned micotoxins from green coffee, said process essentially comprising the following stages:

- a) introducing a load of green coffee containing micotoxins into a container in which the coffee will preferably be subjected to continuous mixing at temperatures ranging from room temperature to about 105° C.;
- b) introducing water vapour into the container where it is kept until the coffee beans have become porous and permeable;
- c) introducing into the container a solvent suited to removing waxes and micotoxins and extraction of the solvent as soon as it has removed the waxes and most of the micotoxins from the coffee;
- d) recovering the solvent and separation from the latter of the micotoxins contained therein.

A first alternative to the above said stage c) is stage:

- c') introducing into the container a solvent saturated with caffeine leaving long enough to remove the totality of the micotoxins.

In a second alternative, the process contemplates the repetition of the stage c) in order to increase the level of removal of the micotoxins.

A first drawback of the above said process lies in the difficulty, from a plant viewpoint, of operating with a solvent saturated with caffeine.

A second drawback is given by the fact that to saturate the solvent with caffeine large quantities of the latter are required.

A further drawback of said process is given by the fact that the repetition of stage c) in order to increase to a higher level the removal of the micotoxins causes the removal of further quantities of caffeine from the coffee.

The applicant has furthered experiments until finding out that, by creating an acid environment obtained by means of a chosen acid or buffer solution with a given acid pH, in some of the process stages the removal of micotoxins is increased, while at the same time, the removal of caffeine is reduced compared to the known process. Note that the Known process takes place in a neutral environment.

The suitable acid environment for the above results is to be chosen by the operator between pH=7 and pH=2 as a function of the contact time between coffee and the acid mixed with the solvent used or else the contact time between the coffee and the buffer solution as a function of the temperature at which said contact takes place.

Such an acid environment can be achieved as follows:

introducing into the process an acid mixed with the solvent for the removal of micotoxins or, as an alternative;

introducing into the process a buffer solution with an acid pH and leaving it in contact with the coffee for the required time;

later discharging said buffer solution and introducing the solvent for the removal of the micotoxins.

On the basis of the results of the further experiments the process that is the subject of the present patent application comprises the following known stages:

- a) introducing a load of green coffee containing micotoxins into a container in which the coffee is preferably subjected to continuous mixing in temperature conditions ranging from room temperature to about 105° C.;
- b) introducing into the container water vapour to be kept there for the time required for the coffee beans to become porous and permeable;
- and newly includes the following subsequent stages:
- c) introducing into the container a quantity of solvent equal to at least 50% of the weight of the coffee, the solvent containing a suitable quantity of acid, and leaving it in contact with the coffee for at least 10 minutes at a temperature that ranges from room temperature to about 105° C., such higher value being raised up to 120° C. when using kinds of solvent adapted to it.

This stage c) may be repeated until most of the micotoxins have been removed. Note that "suitable quantity of acid" means that amount as chosen by the operator depending on the contact time between the coffee and the acid mixed with the solvent and on the temperature at which the removal takes place;

- d) recovering the micotoxin removal material and separating the micotoxins contained therein.

An alternative to the above stage (c) is a stage:

- c') introducing a buffer solution featuring a chosen acid pH, the acidity being a function of the contact time of the same buffer solution with the coffee and as a function of the temperature at which this stage of the process takes place, the buffer solution being a variable quantity between 50 and 200% of the weight of the coffee load; the acid contact time ranging between 10 and 60 minutes;

- d') discharging the buffer solution;

- e) introducing into the container a quantity of solvent, equal to at least 50% of the weight of the coffee load, and then extracting it after the removal of the greater part of the micotoxins from the coffee;

- f) recovering the micotoxin removal solvent and separating the micotoxins contained therein.

A further improvement defined by the experiments is that of adding to the water vapour in the stage (b) a quantity of water ranging between 5 and 50% of the weight of the coffee load at a temperature ranging from room temperature to 90° C. so that the coffee beans become porous and permeable more rapidly.

In order to better understand the invention some examples of embodiment of the process are described herebelow.

EXAMPLE 1

Description of a process in which the removal stage takes place at room temperature and the acid is previously mixed with the solvent:

- a) as defined above;
- b) as defined above;
- c) introducing into the container a quantity of dichloro methane equal to at least 50% of the weight of the

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- coffee load, the dichloro methane containing 2% of formic acid, and leaving it in contact with the coffee for 30 minutes this stage being repeatable until most of the micotoxins have been removed;
- d) Recovering the micotoxin removal material and separating the micotoxins contained therein.

EXAMPLE 2

Description of a process in which a container brought to room temperature is previously filled with an acid pH buffer solution:

- a) as defined above;
- b) as defined above;
- c) introducing into the container a quantity of phosphate buffer solution with pH4 , ranging from 50% to 200% of the weight of the coffee load, and leaving it in contact with the coffee for 15 minutes;
- d) Discharging the buffer solution;
- e) Introducing into the container dichloro methane, in a quantity equal to at least 50% of the weight of the coffee load, in order to remove the micotoxins and extracting the solvent after the removal of most of the micotoxins from the coffee;
- f) Recovering the solvent and separating the micotoxins contained therein.

What is claimed is:

1. A process for removing micotoxins from a load of green coffee without substantially reducing the caffeine content of the coffee, comprising maintaining the load of green coffee with water vapor in a container until the coffee has become porous and permeable, thereafter exposing the coffee to an acid environment external to the coffee, exposing the coffee to a solvent that dissolves micotoxins from the coffee, and thereafter separating the coffee and the micotoxin-containing solvent.

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2. A process as claimed in claim 1, wherein said exposure to acid environment is effected by admixing acid with said solvent.

3. A process as claimed in claim 1, wherein exposure to said acid environment is effected prior to exposing the coffee to the solvent.

4. A process as claimed in claim 1, wherein said exposure of the coffee to the solvent is carried out at a temperature from room temperature to 120° C.

5. A process as claimed in claim 1, in which said acid environment has a pH between 2 and 7.

6. A process as claimed in claim 1, wherein said solvent is dichloromethane.

7. A process as claimed in claim 1, wherein the solvent is in a quantity equal to at least 50% of the weight of the coffee and the coffee is left in contact with the acid environment for a contact time of at least 10 minutes.

8. A process as claimed in claim 1, wherein said acid environment is achieved by introducing into the container the solvent admixed with 2% formic acid.

9. A process as claimed in claim 1, and repeating the contacting of the coffee with solvent until most of the micotoxins have been removed from the coffee.

10. A process as claimed in claim 1, wherein the exposure of the coffee to acid is achieved by contacting the coffee with the buffer solution in a quantity ranging from 50 to 200% of the weight of the coffee, said buffer solution having an acid pH, for a time from 10 to 60 minutes, and then separating the coffee from the buffer solution prior to contacting the coffee with the solvent.

11. A process as claimed in claim 9, wherein the buffer solution is a phosphate buffer solution of about pH 4.

12. A process as claimed in claim 1, wherein, in addition to said water vapor, a quantity of liquid water from 5 to 50% of the weight of the coffee is introduced into the container, at a temperature from room temperature to 90° C.

• • • • •

[54] SELECTIVE IMMOBILIZATION AND DETECTION OF MYCOTOXINS IN SOLUTION

[75] Inventors: Timothy D. Phillips; Beverly A. Clement; Norman D. Haddadhaugh, all of College Station; William C. Gordon; Linda J. Gordon, both of Bryan, all of Tex.

[73] Assignee: The Texas A&M University System, College Station, Tex.

[21] Appl. No.: 677,361

[22] Filed: Mar. 26, 1991

Alkylphenone Retention Index, Anal. Chem. vol. 56, No. 13, pp. 2576-2579 (1984).

Charles E. Holaday, Rapid Screening Method for Zearalenone in Corn, Wheat and Sorghum, J. Am. Oil Chem. Soc., 491A (1980).

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(List continued on next page.)

Related U.S. Application Data

[63] Continuation of Ser. No. 319,844, Mar. 7, 1989, abandoned, which is a continuation of Ser. No. 101,597, Sep. 28, 1987, abandoned.

[51] Int. Cl.⁵ C07D 313/00

[32] U.S. Cl. 422/60; 422/69; 422/88; 436/122; 436/174; 436/178; 435/29

[38] Field of Search 436/128, 178, 174; 435/4, 29; 422/60, 69, 88

Primary Examiner—Thi Dang
Attorney, Agent, or Firm—Arnold, White & Durkee

[57] ABSTRACT

It has been discovered that certain minerals, particularly various naturally occurring forms of aluminum oxide, will preferentially bind selective mycotoxins from a mixture of mycotoxins. These adsorbents, when used in various combinations and/or in conjunction with the adsorbents of the prior art, permit the construction of detector tubes which can resolve mycotoxins in solution and provide a semi-quantitative fluorescent determination of their concentration in feed or foodstuff samples. The detector tubes comprise transparent tubes packed with isolated layers of selected minerals. A solvent extract from a sample potentially contaminated with mycotoxins is passed through the column. As the mycotoxin mixture passes through the detector tube and is contacted by the various mineral adsorbents, selected mycotoxins are immobilized on a specific mineral while other mycotoxins and co-extracted organic compounds pass through that layer to be immobilized on subsequent downstream mineral layers. The presence of mycotoxins is determined by examining the developed detector tube under a long wave uv light source.

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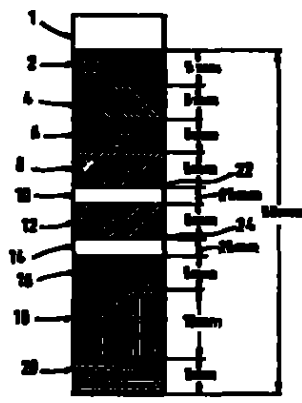
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22 Claims, 2 Drawing Sheets



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Fig. 1

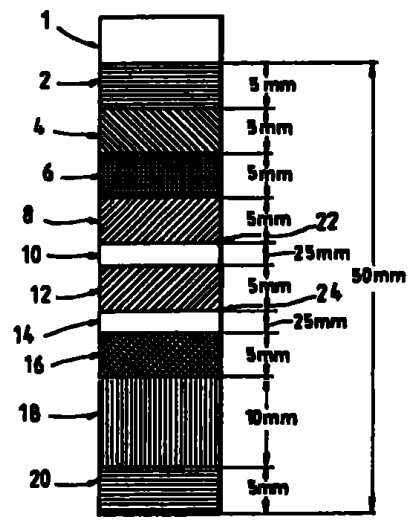


Fig. 2

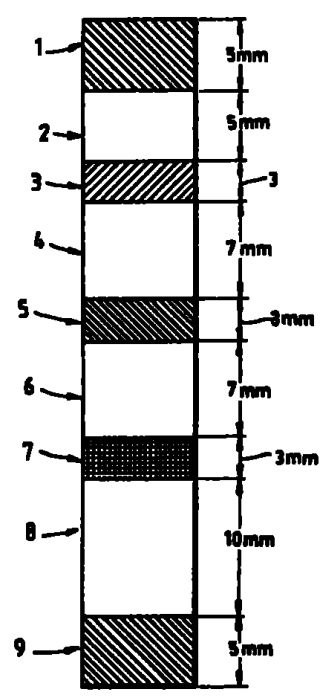


Fig. 3

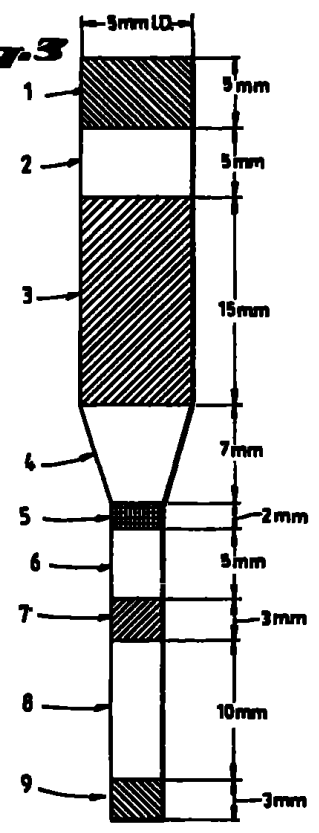


Fig. 4

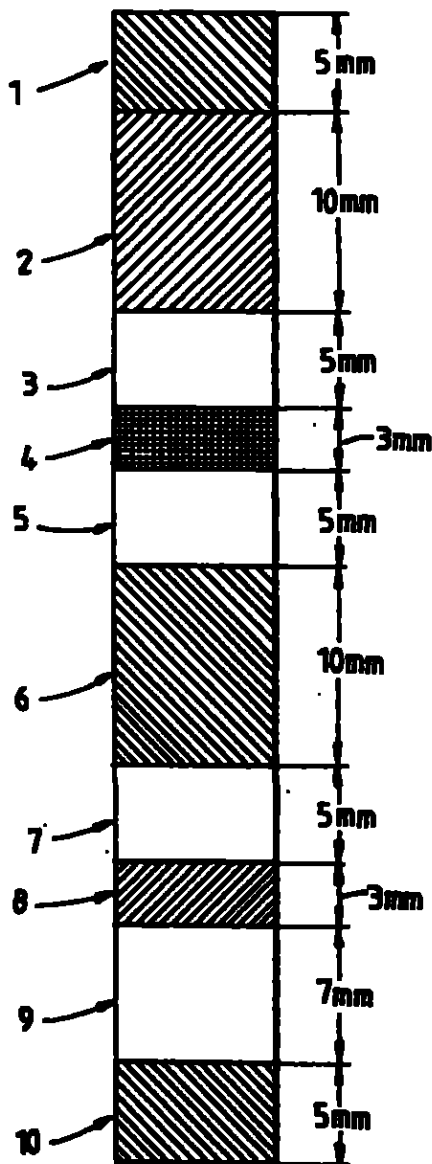
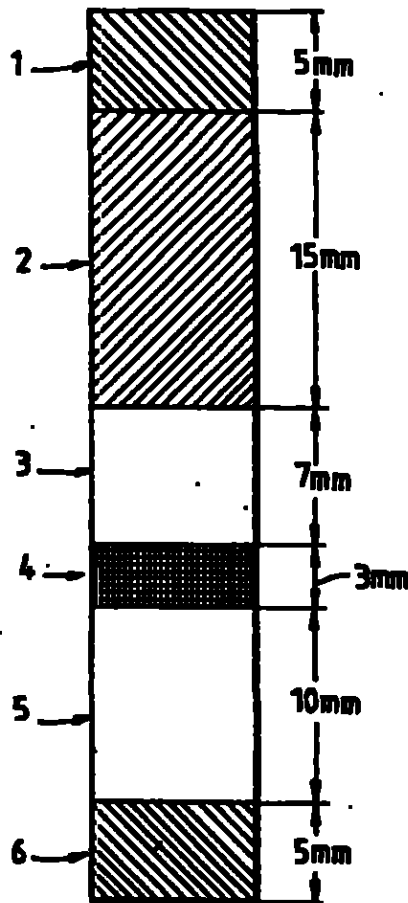


Fig 5



SELECTIVE IMMOBILIZATION AND DETECTION OF MYCOTOXINS IN SOLUTION

This is a continuation of copending application Ser. No. 07/319,844 filed on Mar. 7, 1989, now abandoned, which is continuation of Ser. No. 07/101,597, filed on Sep. 28, 1987, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

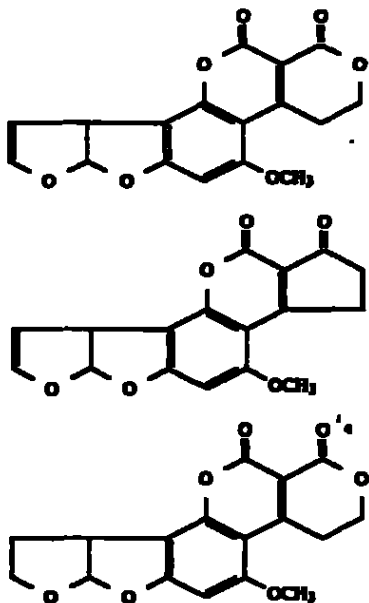
This invention relates to the separation and detection of mycotoxins (e.g., aflatoxins) occurring in grains, nuts, milk, and other foodstuffs. More particularly, the invention concerns the use of various minerals to selectively immobilize certain mycotoxins in a solution comprising one or more mycotoxins and other organic molecules.

2. Description of the Related Art

Mycotoxins are toxic compounds elaborated by fungi. Although the existence of mycotoxins has been known for many years, they have been studied less intensively than bacterial toxins in foods. Interest in the effects of mycotoxins was renewed in recent years after the discovery that aflatoxins were the causative agents in turkey X disease which killed thousands of turkeys in England in 1960.

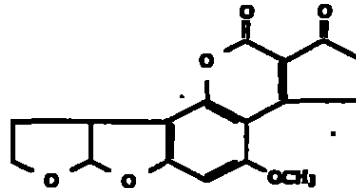
Aflatoxins

Aflatoxins are fluorescent toxic factors elaborated by the common mold *Aspergillus flavus*, among others. Originally two families were isolated; they were named aflatoxin B and G, because they exhibited blue and green fluorescence, respectively. More recently, at least eight compounds have been shown to belong to the aflatoxin group. The toxins have similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds that fluoresce upon exposure to ultraviolet light. The homologs which comprise the aflatoxins commonly designated B₁, B₂, G₁, and G₂ have the following chemical structures:



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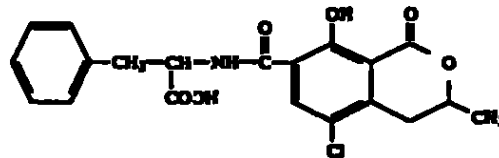
-continued



Ochratoxins

Members of the ochratoxin group of mycotoxins (i.e., ochratoxins A and B and corresponding methyl and ethyl esters and 4-hydroxyochratoxin A) are structurally related derivatives of 1,4-dihydro-3-methylisocoumarin linked by an amide bond to L-beta-phenylalanine at the 7-carboxy group. These chemicals can be produced by several species of the fungal genera *Aspergillus* and *Penicillium*. Ochratoxin A has been reported to occur as a natural contaminant in a variety of food and feedstuffs (e.g., corn, wheat, barley, dried beans, sorghum, moldy peanuts, mixed feed, green coffee beans, etc.) as well as residues in liver, kidneys and adipose tissue of pigs. The toxicity of ochratoxin A has been well documented in numerous experimental animals. Although this mycotoxin has drawn the greatest attention for its potent nephrotoxicity, it is also capable of eliciting other toxic effects including teratogenesis.

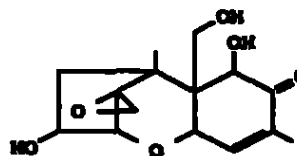
Ochratoxin A, a toxic metabolite of *Aspergillus ochraceus*, has the following chemical structure:



Ochratoxin A, when present in combination with aflatoxins, may bias the determination of aflatoxins when the subcolumn analytical test methods of the prior art are employed. The present invention eliminates this bias by providing a means to resolve a mixture of mycotoxins comprising aflatoxins and ochratoxin A.

Trichothecenes

Trichothecenes such as deoxynivalenol (DON) and related sesquiterpene alcohols are another very important class of mycotoxins due to their toxicity and frequent occurrence in grains. They generally have the carbon skeleton:



The trichothecenes are a group of some 50 biologically active sesquiterpenes produced by various species of fungi. They are chemically characterized by a 12,13-epoxy-trichothec-9-ene ring system. These compounds have been implicated as causal agents in both human and animal poisonings. As a group, the trichothecenes

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exhibit a wide spectrum of toxicity. Clinically, the ingestion of trichothecenes can result in vomiting, diarrhea, anorexia, stasis, hematuria, leukocytosis and severe leukopenia, inflammation of the GI tract, degeneration of nerve cells, degeneration of cardiac muscle, and lesions of the lymph nodes, testes and thymus. Topical contact can result in several dermal necroses. The trichothecenes have been strongly linked to the etiopathogenesis of natural intoxications (i.e., moldy corn toxicosis of cattle, swine and poultry in the U.S.; Akababi-Byo disease in Japan; Stachydotryo-toxicosis and alimentary toxic alekbia in the U.S.S.R.; and dendrodochitoxico-

sis). The minicolumn techniques of the prior art do not have the ability to separate trichothecenes from aflatoxin or even detect them on the same column in one step.

Zearalenone

Zearalenone, ((6-(10-Hydroxy-6-oxo-trans-1-undecenylo)-beta-resorcylic acid lactone)), is a commonly occurring mycotoxin produced by *Fusarium moniliforme* (F. Gramineum) and other *Fusarium* species on corn, sorghum, barley, wheat and other cereals. There have been numerous reports of zearalenone contamination of corn and commercial preparations of mixed feeds. In the case of wet-milled products from corn contaminated with zearalenone, it has been demonstrated that this mycotoxin becomes concentrated in those fractions generally used for animal feeds. When fed to animals (particularly swine), zearalenone causes a condition commonly referred to as hyperestrogenism. In swine, this syndrome involves primarily the genital system (e.g., swollen and edematous vulva, rectal and vaginal prolapse, enlarged uterus in the female pig, and a feminizing effect with atrophy of the testes and enlargement of the mammary glands in the young male). Besides the estrogenic symptoms, other clinical signs of zearalenone intoxication can include: diarrhea, emesis, refusal of feed, loss of weight and hemorrhage.

Sterigmatocystin

Sterigmatocystin, (2a,12a-Dihydro-8-hydroxy-6-methoxyfuro[3,2'-4,5]furo[3,2-c]azathien-7-one), is a mycotoxin produced by a variety of *Aspergillus* fungi and is also produced as an intermediate product in the biosynthesis of aflatoxins by *A. parasiticus* and *A. flavus* in foods and feeds. This mycotoxin produces liver and kidney damage in experimental animals. In monkeys, sterigmatocystin is reported to cause chronic hepatitis and hyperplasia. It has been hypothesized that like aflatoxin, sterigmatocystin is involved in the etiology of chronic liver disease of humans in Africa.

Patulin

Patulin (4-Hydroxy-4H-furo[3,2-c]pyran-2(6H)-one) is a toxic lactone produced by several fungi belonging to the genera *Aspergillus* and *Penicillium*. *Penicillium expansum* is the cause of common storage rot of apples and can result in as much as 17 mg of patulin in a single rotten apple. Of practical interest to the food industry is the fact that patulin is stable in apple juice and grape juice and thus occurs frequently as a contaminant of these foods. Patulin has been reported to be moderately toxic to animals and potentially carcinogenic in the male rat. In humans, oral administration of patulin has resulted in nausea and stomach irritation. Applications of 1% patulin to the skin has caused edema. Additionally, patulin has been shown to be cytotoxic (inhibiting

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cell division) and toxic to plants, fungi, yeasts, bacteria, protozoa and bacterial viruses.

Citrinin

Citrinin, [(3R-trans)-4,6-Dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid], is produced by a variety of *Penicillium* and *Aspergillus* fungi. It has been reported to co-occur with ochratoxin A and may play a role in field outbreaks of porcine nephropathy. The renal toxicity of citrinin in swine has been characterized by lesions and degeneration of renal epithelial cells of the proximal convoluted tubules, dilation of tubules, thickening of basal membranes and proliferation of cells in the interstitium. Citrinin has been shown to produce irritation of the nasal passages in humans following inhalation. In addition to its potent nephrotoxicity, citrinin has a depressant effect on respiration and the cardiovascular system in experimental animals.

Ergots

Ergot alkaloids (the most potent of the ergots) are principally metabolites of a group of closely related ergot fungi of the genus *Claviceps* or *Sphaeria*. Chemically, these alkaloids are amide or cyclic tripeptide derivatives of lysergic acid and can occur naturally only in the sclerotia produced during parasitism of grass and cereal host plants. The toxicity of ergot alkaloids results from their wide range of pharmacological activities involving the central nervous system, antagonism toward adrenaline and 5-hydroxytryptamine, and peripheral action expressed through the stimulation of smooth muscle (e.g., uterus, arteriole walls, or sphincter pupillae). Poisoning, due to ingestion of ergo alkaloid mycotoxins, has been reported in humans, cattle, sheep, horses, swine and poultry.

Although other mycotoxins have been and are being discovered, the aflatoxins retain a position of major importance because of their high toxicity and their common natural occurrence in such foods as cereal grains, oil-seeds, and oilseed meals stored under adverse conditions. The ability of aflatoxins to cause liver cancer has probably been the key factor in worldwide concern over their presence in foods and feeds. Although the effects of aflatoxins on humans are not clear at present, the mycotoxins are highly potent carcinogens to a number of animals and should be regarded as potentially hazardous to humans. Many agricultural commodities support the production of aflatoxins if conditions are favorable for the growth of *A. flavus* or other toxin-producing molds.

Generally, most aflatoxin production occurs during the harvest period after cereals, oilseeds or nuts have begun to dry but before they attain the moisture level best suited for storage. Storage of the foodstuffs under proper temperature and humidity conditions will prevent further contamination. Thus, it is recognized that one very effective step to ensure a wholesome product is to divert from edible use any contaminated lots as early in the food processing chain as possible. Accordingly, a need exists for simple, rapid, and convenient tests for mycotoxin contamination for use in the field. More especially, a need exists for test devices and procedures capable of resolving and separately detecting specific mycotoxins in a mixture comprising other mycotoxins and co-extracted organic compounds.

Chromatographic procedures for aflatoxins using classical minicolumns have been described by a number

of researchers. These techniques are reputed to be suitable for the field analysis of corn and peanuts. See, e.g., Holaday, C. E., "A Rapid Screening Method for the Aflatoxins and Ochratoxin A," J. Am. Oil Chem. Soc. 53:603 (1976); Holaday, C. E., "Rapid Screening Method for Zearalenone in Corn, Wheat and Sorghum," J. Am. Oil Chem. Soc. 49:1A (June 1980); Shotwell, O. L. and C. E. Holaday, "Minicolumn Detection Methods for Aflatoxin in Raw Peanuts: Collaborative Study," J. Assoc. Off. Anal. Chem. 64:674 (1981); Holaday, C., "Rapid Screening Method for Aflatoxin M₁ in Milk," J. Assoc. Off. Anal. Chem. 64:1064 (1981); Holaday, C. E., "Minicolumn Chromatography: State of the Art," J. Am. Oil Chem. Soc. 93:1A (December 1981).

In general, two methods of detecting aflatoxins using minicolumns are described in the prior art. In one, silica gel is the primary packing of the minicolumn. The minicolumn (or "millicolumn") is developed in a chloroform-methanol extract of a grain or oilseed sample. If aflatoxin is present, a blue fluorescent band at the lower end of the column is observed when the column is exposed to long-wave ultraviolet radiation. [See, e.g., Holaday, J. Am. Oil Chem. Soc. 45:680 (1968)]. The other general method employs Florisil® brand activated magnesium silicate (see U.S. Pat. No. 2,393,625) to bind the aflatoxins in the column. Extracts of the sample are purified and then applied to the column in a chloroform-acetone solution. The column is then filled with chloroform and allowed to drain. If aflatoxins are present above the detection limit, they will be detected as a fluorescent glow in the Florisil® layer when examined under longwave uv light. [See, e.g., Velasco, J. Am. Oil Chem. Soc. 49:141 (1972)]

Holaday has additionally described a screening method for the aflatoxins and ochratoxin A using minicolumns packed with Florisil®. Two columns are used. The aflatoxin minicolumn has a layer of alumina upstream from the Florisil® layer. The ochratoxin A minicolumn is packed only with Florisil®. The sample is blended in a methanol-water solution; treated with an aqueous solution of zinc sulfide and phosphotungstic acid; and extracted with benzene before being applied to the columns. The aflatoxin column is developed by pulling a hexane-acetone solution through it. Methanol is used to develop the column for ochratoxin A. Both analytes are detected by visual observation of the mycotoxins' fluorescence under uv light. [Holaday, J. Am. Oil Chem. Soc. 53:603 (1976)]

There are other types of chromatography which will perform the same function as the invention disclosed herein—the separation and detection of various mycotoxins. These include analytical methods employing high performance liquid chromatography (HPLC), thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and gas chromatography/mass spectrometry (GC/MS). Unfortunately, these techniques are not well suited for performing analyses in the field inasmuch as they require complex instruments and a relatively high degree of skill on the part of the person performing the analysis.

SUMMARY OF THE INVENTION

It has been discovered that certain minerals, particularly various naturally occurring forms of aluminum oxide (Al₂O₃), will preferentially bind selective mycotoxins in a mixture of mycotoxins. These adsorbents, when used in various combinations and/or in conjunction with the adsorbents of the prior art, permit the

construction of devices which can resolve mycotoxins in solution and provide a semi-quantitative fluorescent determination of their concentration in foodstuff samples. The devices of the present invention are easy to fabricate using readily available, relatively inexpensive materials and the test methods which employ these devices are rapid and easily performed in the field.

The test devices of the present invention comprise transparent tubes packed with isolated layers of selected minerals. In use, a solvent extract from a sample potentially contaminated with mycotoxins is passed through the detector tube or is transported through the tube by a different solvent or solvent mixture. As the mycotoxin mixture passes through the device and is contacted by the various mineral adsorbents, selected mycotoxins are immobilized on a specific mineral while other mycotoxins pass through that layer to be immobilized on subsequent downstream mineral layers. If the mycotoxins for which the device is specific are present in the sample above the detection limit they will appear as fluorescent bands near the top of the mineral layers which are selective for those particular mycotoxins when the developed detector tube is exposed to uv radiation. For some mycotoxins, it is necessary to heat the test device to convert the mycotoxin to a fluorescent product. Specially treated minerals may be employed to facilitate this conversion.

Semi-quantitative results may be obtained by comparing the fluorescence emission intensity of the mycotoxins immobilized in the test device to that of standard test devices or other fluorescent controls.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts in cross section a portion of a test device embodying the present invention. The particular device illustrated permits the immobilization and detection of ochratoxin A and aflatoxins.

FIG. 2 depicts in cross-section a portion of a test device which embodies the present invention. The device illustrated may be used to detect zearalenone, ochratoxin A, and aflatoxins.

FIG. 3 is a cross-sectional view of a portion of the test devices disclosed in Examples 3 and 4.

FIG. 4 is a cross-sectional view of a portion of a test device for the immobilization and detection of aflatoxins and deoxynivalenol which embodies the present invention.

FIG. 5 is a cross-sectional view of a portion of the test devices disclosed in Examples 6, 7, 8, and 9.

DETAILED DESCRIPTION

The present invention employs adsorbents having structure-selective affinities to various mycotoxins commonly occurring in agricultural commodities and foodstuffs. The selective adsorption properties of these materials enables the construction of a test device which can immobilize selected mycotoxins at certain physical locations in the test device thereby permitting the resolution and subsequent detection of mycotoxins extracted from samples of grain, oilseeds, and the like.

This immobilization phenomenon also enables one to physically separate the mycotoxin(s) of interest from interfering compounds. Interfering compounds are most commonly other fluorescent species. However, substances which quench the fluorescence of the analyte and/or block the exciting uv radiation on the emitted fluorescence of the analyte in the visible portion of the

spectrum are also considered interfering compounds for the purposes of this disclosure.

For the purposes of this disclosure, to "bind" or "immobilize" the mycotoxin analyte(s) or interfering compounds means that the substance is not appreciably eluted from the selective adsorbent by the sample solution or wash solvent(s). It should be appreciated that this differs fundamentally from chromatographic methods of analysis. Chromatography is based on the separation which obtains when substances having different affinities for the stationary phase are dissolved in a mobile phase and passed through a bed of or across a layer

occurs abundantly in nature, most often as impure hydroxides which are the essential constituents of bauxites and laterites. The numerous purer grades of alumina manufactured from these ores are major chemical products.

The nomenclature used for the various crystallized hydrates of alumina has not been completely standardized and these materials are differently named in various countries. An effort toward world uniformity has been made in recent years. Table 1 provides a summary of some of the more commonly used names and synonyms.

TABLE 1 Nomenclature of Crystallized Hydrates of Alumina			
Composition	Alcon	European synonym	CAS Registry No.
Al(OH) ₃	α-alumina trihydrate	hydrogibbsite or gibbsite	[14763-49-3]
	β-alumina trihydrate	bayerite	[20257-30-9] and [12152-73-1]
AlO(OH)	new β-trihydrate	senckleyite	[13640-05-4]
	α-alumina monohydrate	boehmite	[1318-23-4]
	β-alumina monohydrate	disopore	[14457-34-3]
	monohydrate		

of the stationary phase. In column chromatography, the compounds of interest are typically detected (or collected) as they exit the column, having been eluted by the mobile phase. In contrast, the affinities of the present invention are such that for all practical purposes, no significant elution of the mycotoxin from its selective adsorbent takes place when the recommended types and quantities of dissolving solvents and transporting (or "wash") solvents are used and detection takes place with the mycotoxin within the test device.

The minerals and the mycotoxins which they selectively bind or otherwise immobilize are: Florisil® for aflatoxins; bayerite for ochratoxin A, sterigmatocystin, and citrinin; pseudoboehmite for ochratoxin A, zearalenone, and citrinin; alumina for zearalenone, deoxynivalenol, patulin, sterigmatocystin and ergots; and silica gel for patulin, sterigmatocystin and ergots.

For binding and immobilizing certain mycotoxins, the following minerals have been found to be effective: for ochratoxin A, pseudoboehmite and other aluminum oxides including bayerite, gibbsite, boehmite, bauxite and alumina (activity grade IV or V); for zearalenone, neutral alumina and other aluminum oxides (of activity grade I or II), including acidic or basic alumina, bayerite, bauxite, boehmite, pseudoboehmite, and gibbsite; for patulin, acidic alumina and other aluminum oxides including bayerite, gibbsite, boehmite, pseudoboehmite, and bauxite, and silica gel; for sterigmatocystin, neutral alumina, grade I, including boehmite, pseudoboehmite, bauxite, gibbsite, and bayerite; for deoxynivalenol, neutral alumina and other aluminum oxides including bayerite, boehmite, pseudoboehmite, bauxite, gibbsite, and acidic alumina, and silica gel; for citrinin, bayerite, pseudoboehmite, bauxite, gibbsite, boehmite, and alumina; and, for ergots, alumina and silica gel.

Florisil®, a hard, porous, granular substance, is a brand of activated magnesium silicate typically used in vitamin analysis, chromatography, and antibiotic processing [see Shiono, U.S. Pat. No. 2,393,625 (1946 to Floridin)]. As noted above, its use in selectively binding aflatoxins from solutions comprising aflatoxins has been described in the literature.

Bayerite, pseudoboehmite, and alumina are all forms of aluminum oxide (Al₂O₃). Aluminum oxide (alumina)

The structural properties of gibbsite, bayerite, and boehmite are set out in Table 2.

TABLE 2 Structural Properties of Oxides and Hydroxides				
	Minerals			
	Gibbsite	Gibbsite	Bayerite	Boehmite
Formula	Al(OH) ₃	Al(OH) ₃	Al(OH) ₃	AlO(OH)
Crystal system	Monoclinic	Triclinic	Monoclinic	Orthorhombic
Space group	C _{2h} ²		C _{2h} ²	D _{2h} ¹⁷
Molecules per unit cell	4	16	2	2
Unit Axis Length, mμ				
a	0.866	1.713	0.806	0.286
b	0.807	1.028	0.867	1.222
c	0.971	0.973	0.671	0.370
Angle	94°34'	14°10'	90°18'	
		107°08'		
		107°		
Density, g/cm ³	2.42		2.33	3.01

Boehmite is a grayish, brownish, or reddish orthorhombic mineral: AlO(OH)—i.e., a mineral consisting of an orthorhombic form of aluminum oxide and hydroxide. It is a major constituent of some bauxites and it represents the gamma phase dimorphous with disopore. Disopore, also known as β-alumina monohydrate, is a well-crystallized modification of AlO(OH) which occurs in certain high alumina clays in Missouri and Pennsylvania. It is a major constituent of Greek, Rumanian, and Russian bauxites.

Pseudoboehmite is gelatinous boehmite. Alumina gels are generally prepared from solutions of Al₂(SO₄)₃ and NH₃, or from NaAlO₂ and an acid, or from NaAlO₂ and Al₂(SO₄)₃ and produce corresponding by-product salts. The precipitate, after being washed, is thoroughly drained to form a cake which embodies 8-20% Al₂O₃. The x-ray diffraction pattern of the alumina at this point is pseudoboehmite [see B. R. Baker and R. M. Pearson, J. Catal. 33, 265 (1974)]. This cake may be dried directly and broken up, or it can be extruded in the form of

cylinders. An alternative is to redurry and spray-dry the washed cake, which produces spherical particles of about 5 mm in diameter.

Alumina suitable for chromatographic adsorption is typically prepared from carefully screened aluminum hydroxide having a particle size which will pack easily in a column without obstructing the seepage of liquids (typical sieve analysis: all through 150 mesh, 40% on 200 mesh, 40% on 325 mesh). It is believed that the average particle size is probably about 7 microns. The aluminum hydroxide is dehydrated and calcined at about 900° C. in a CO₂ stream which tends to coat the individual Al₂O₃ particles with a thin layer of aluminum oxycarbonate approximating the formula [Al₂(OH)₃]_xCO₃·H₂O. Water content and alkalinity are then adjusted by washing with dilute acids.

Silica gel is precipitated silicic acid in the form of hatron granules. It is a colloidal silica resembling coarse white sand in appearance but possessing many fine pores and is therefore extremely adsorbent.

The novel use of these various minerals (and others) to selectively bind and immobilize specific mycotoxins in solution, thereby permitting their ready detection, can best be understood by reference to the following examples. The fabrication and use of various representative test devices ("detector tubes") which embody the present invention are disclosed in the examples below. However, it should be appreciated by those skilled in this art that these examples are merely illustrative of the invention and a great variety of embodiments are possible which employ various combinations of these selective adsorbents and others depending on the various mycotoxins or combinations of mycotoxins for which analysis is desired.

EXAMPLE 1

The packed portion of a detector tube for the resolution and detection of aflatoxins and ochratoxin A which embodies the present invention is depicted in cross section in FIG. 1. The positions of the selective adsorbents (reactive interfaces) having structure-selective affinities to mycotoxins are shown along with the locations of various packing materials used to separate and/or support the adsorbents and to precondition the sample solution and transporting solvent(s). Also shown in FIG. 1 are the locations of the analytes after the detector tube has been developed. The selective adsorption capabilities of the detector tube packings permit the resolution and subsequent fluorescent detection of aflatoxins and ochratoxin A extracted from samples of grain, oilseeds, and the like.

The tube containing the selective adsorbents and associated packings is preferably about 4 mm OD and has the other approximate dimensions shown in the figure. To permit fluorescent detection of the mycotoxins bound to the adsorbents in the detector tube, the tube itself must be substantially transparent to both the exciting uv radiation and the emitted fluorescence in the visible portion of the spectrum and non-fluorescent. Quartz is especially preferred but ordinary borosilicate or sodalime glass will suffice as will polycarbonate, acrylic, and other transparent plastics.

In FIG. 1 detector tube 1 is shown with a number of additional, optional packings which improve the separation and detection of mycotoxins. Layer 2, the top layer in the detector tube shown, is sand. Its purpose is to minimize column loading disturbances when the sample solution is applied to the detector tube. Layer 4 is anhy-

drous sodium sulfate. As the sample solution or transporting solvent passes through this layer any water in the solvent is absorbed by the sodium sulfate via hydration. It is not necessary, however, that the solvents be dried in this manner or even dried at all.

Layer 6 is activated charcoal. It acts to remove contaminants from the sample solution, particularly pigments which may have been extracted along with the analytes. Layer 8 is Celins® diatomaceous earth (infusorial earth). It contains the charcoal layer and separates it from the ochratoxin A selective layer.

Layer 10 preferably comprises bayerite. As discussed above, bayerite is a crystallized aluminum trihydroxide. Bayerite selectively adsorbs ochratoxin A from the sample solution and binds it, immobilizing this mycotoxin at position 22 in FIG. 1. If ochratoxin A is present in the sample in sufficient quantity to permit visual observation of its fluorescence, a bright blue band at position 22 will be seen when the detector tube is exposed to longwave uv radiation. Aflatoxins are not bound by the bayerite layer and they are transported to the downstream layers in the detector tube. Any other selective adsorbent which binds ochratoxin A but not aflatoxins could be substituted for bayerite in this layer. Layer 12 is Celite® diatomaceous earth or sand. It separates the mycotoxin selective layers in the detector tube.

Layer 14 preferably comprises Florisil® activated magnesium silicate. Aflatoxins in the sample are bound by the Florisil® and immobilized. They can subsequently be detected as a bright blue fluorescent band at position 24 in the detector tube when the tube is exposed to longwave uv light (emission maximum at approximately 365 nm). Other selective adsorbents which bind aflatoxins can be employed in this layer. In particular, silica gel can be used for this purpose (see, e.g., Holaday, JAOCS 45:480 (1968)).

It is important that the bayerite layer (or other ochratoxin A selective adsorbent) be upstream from the layer used to bind aflatoxins if the aflatoxin adsorbent also binds ochratoxin A (as is the case with Florisil®). An "upstream" packing in the detector tube is contacted by the sample solution prior to a "downstream" packing as the sample solution passes through the detector tube in the intended direction. If the positions of the selective adsorbents in the detector tube were reversed with respect to the flow of sample solution or transporting solvent, no separation would obtain inasmuch as both the ochratoxin A and the aflatoxins would be bound on the Florisil® layer and the sample solution would be essentially free of any ochratoxin A by the time it contacted the bayerite layer.

Layer 16 supports the Florisil® layer and forms an "optical barrier" which prevents the diffusion of the emitted fluorescence from the excited aflatoxins into layer 16 thereby enhancing the visible detection of that analyte. Any material which satisfies this criterion and is porous but insoluble in the sample solution and transporting solvents could be used. Celite has been found to work well in this application. Other materials considered suitable include glass wool, silicates, salts (if insoluble in the solvents employed), carbon, sulfur, iron filings, various plastics, aluminosilicates, cellulose fiber, sand, and the like, and mixtures of such materials.

The packings of the detector tube shown are supported on a layer of alumina, 18. Glass wool plug or frit 20 holds the packings in the detector tube. It should be noted that the only layers which are essential to the

practice of the invention are the adsorbents which are selective for ochratoxin A and aflatoxins—in the illustrated embodiment, bayerite (layer 10) and Florisil® (layer 14), respectively. The other layers employed in this embodiment serve to support and isolate the active layers (selective adsorbents) or to precondition the sample solution and/or transporting solvent(s).

The detector tube for the selective adsorption of mycotoxins disclosed above allows extracts from grain samples and the like to be analyzed in the field for aflatoxins and ochratoxin A. The analysis is accomplished by concentrating the extract from the sample and dissolving the residue in a known volume of an appropriate solvent such as methanol. Suitable extraction procedures are well-known in the art. In general, any of the AOAC-sanctioned procedures for extractions of such samples may be employed. An aliquot of the extract is loaded on the top of the detector tube and transported with a developing solvent (e.g., methanol). Positive pressure may be applied to the top of the detector tube to decrease the elution period.

After development, the detector tube is exposed to longwave uv radiation. Longwave uv radiation is ultraviolet energy in the wavelength region from about 320 to 400 nanometers. This region is also known as the near ultraviolet or black light. Fluorescent and mercury lamps can be filtered so that visible energy is absorbed and their emission is primarily in this portion of the spectrum. Such sources typically have an emission maximum at about 365 nm. A mercury arc lamp has a prominent line at 365.4 nm. If present in the extract above the detection limit, ochratoxin A will be detected as a bright blue fluorescent band near the upper end (upstream end) of the ochratoxin-selective layer (the bayerite layer in the illustrated embodiment). Similarly, aflatoxins will be detected as a blue fluorescent band near the upstream limit of the aflatoxin-selective layer (Florisil® in the illustrated embodiment).

A semi-quantitative determination may be made by constructing a "mycotoxin standard control card" having fluorescent bands of different intensities corresponding to a range of mycotoxin concentrations in the sample. A side-by-side comparison under the same uv light source of the developed detector tube and the control card would enable an estimation to be made of the ochratoxin A and aflatoxin concentrations in the sample. This technique has the particular advantage of obviating the need for actual mycotoxin standards which would be hazardous to ship and use in the field.

More quantitative results can be obtained by instrumental fluorometry, the intensity of fluorescence from the analytes in the developed detector tube being measured in a fluorometer. The detector tubes of the invention are stable, even after developing. Fluorescence from the mycotoxin analytes has been observed in detector tubes developed more than one year prior to the observation.

Samples of both "spiked" and naturally contaminated grain sorghum, peanuts, corn and wheat were homogenized and partitioned by polarity with aqueous acetone (60% v/v acetone) or acetonitrile and hexane followed by extraction of the aqueous layer with chloroform. The chloroform extracts were concentrated under dry nitrogen and the resulting residue redissolved in a known volume of ethanol prior to analysis. Aliquots ranging from 20 to 100 microliters of this extract were loaded on the top of the detector tube and transported (i.e., moved sequentially through the various packing

layers) with a 1:1 mixture of chloroform and methanol. Positive pressure was applied to the top of the detector tube using a pipette bulb attached to the top of the tube during the development period (about 15–60 seconds).

The developed detector tube was visualized under a uv lamp (365 nm). Ochratoxin A was detected as a bright blue fluorescent band in the bayerite layer. The aflatoxins were concentrated as a tight band at the interface of the Celite® and Florisil® layers and were detected by their bright blue fluorescence.

After use the detector tubes can be decontaminated by packing an oxidizing agent through them. Household bleach (aqueous sodium hypochlorite) can be used for this purpose. Alternatively, if the housing of the detector tube is acrylic or polycarbonate plastic, the device can be disposed of by incineration.

The detector tube and method described above will prove useful as a regulatory tool to monitor aflatoxin contamination in agricultural commodities, prepared foods and mined feeds at buying locations, field installations, processing lines, grain elevators, feed lots, and the like. It can facilitate the rapid differential diagnosis of mycotoxicoses in animals (by testing body fluids or tissue extracts, particularly those of the liver and kidney) and perform presumptive field analyses for aflatoxins and ochratoxin A.

The above-disclosed embodiment of the invention has the unique features of the ability to detect both aflatoxin and ochratoxin A in one step using the same detector tube and the ability to separate aflatoxin from ochratoxin A so that this mycotoxin (when present in combination with aflatoxin) will not generate false positive results for aflatoxin.

Among the advantages which may be realized by the use of detector tubes which embody the present invention are: speed of analysis (15 to 20 minutes for semi-quantitative results); ease of use (technical expertise and a source of vacuum are not required, the force of gravity being sufficient to develop the detector); sensitivity (part-per-billion level detections possible); economy (minimal production costs and no antibodies or enzymes required as they are for many competing techniques such as ELISA); stability (no refrigeration required, detector tube packings are non-thermolabile); and flexibility (the detector tube and associated method can provide a go/no-go field test or be used as a semi-quantitative, pre-screening test for more quantitative instrumental methods such as HPLC).

EXAMPLE 2

A detector tube and assay method for ochratoxin A, zearalenone, and aflatoxins which embodies the present invention utilizes pseudoboechmite, alumina, and Florisil® in combination to separate, immobilize and thereby fluorescently detect ochratoxin A, zearalenone, and aflatoxins, respectively.

The packed portion of the detector tube is shown in cross section in FIG. 2. The tube which houses and contains the selective adsorbents is preferably about 5 mm ID. The preferred approximate thicknesses of the various packings of the detector tube are indicated in the figure. Preferred materials for the detector tube housing are the same as those discussed in Example 1, above.

In the detector tube shown in FIG. 2, nine layers of various packings have been used to accomplish the specific resolution of the three analytes (ochratoxin A, zearalenone, and aflatoxins). Materials in addition to the

selective chemisorbents are employed to improve the separation and detection. In the detector tube shown, layers 1 and 9 are cotton which act to maintain the physical integrity of the other layers during use, handling and storage. Other materials considered suitable for this purpose include glass wool, filter paper and other cellulosic materials, plastic frits, and the like. Layer 1 functions additionally to filter out particulates in the sample solution.

Layers 2, 4, 6, and 8 are preferably sand. They function to physically separate the selective chemisorbents thereby enhancing the differentiation of the fluorescent emission of the mycotoxins immobilized in the chemisorbent layers. Layer 3 performs the additional function of filtering out or adsorbing interfering materials from the sample solution as it passes through the column before contacting the selective chemisorbents. Any material which accomplishes these functions and is porous but insoluble in the sample solution and solvent(s) used to transport the sample through the detector tube could be used.

Layer 3 preferably comprises pseudoboehmite. Pseudoboehmite selectively adsorbs ochratoxin from the liquid sample solution and binds them, immobilizing ochratoxin A at or near the upstream end of layer 3. If ochratoxin A is present in the sample in sufficient quantity to permit visual detection of its fluorescence, a bright blue band in layer 3 will be seen when the detector tube is exposed to longwave uv radiation (365 nm). Zearalenone and aflatoxins are not bound by this chemisorbent and thus these mycotoxins pass through to the downstream layers in the detector tube. Any other chemisorbent which binds ochratoxin A and not zearalenone or aflatoxins could be substituted for pseudoboehmite in this layer.

Layer 5 comprises neutral alumina (Brockmann and Schodder). This chemisorbent selectively adsorbs zearalenone from the sample solution while allowing aflatoxins to pass through to downstream layers. If zearalenone is present in the sample in sufficient quantity to permit visual observations of its fluorescence, a bright blue band in layer 5 will be seen when the detector tube is examined under longwave uv radiation. Any other adsorbent which binds zearalenone and not aflatoxins could be substituted for neutral alumina in this layer.

Layer 7 preferably comprises Florisil® brand activated magnesium silicate. It selectively adsorbs aflatoxins in the sample solution but allows other classes of organic compounds which might be present in the sample solution and constitute an interfering substance to pass through. If aflatoxin is present in the sample in sufficient quantity to permit visual observation of its fluorescence, a bright blue band at layer 7 will be seen when the detector tube is exposed to longwave uv radiation.

It is important that the pseudoboehmite-selective adsorbent be upstream from the chemisorbents used to bind zearalenone and aflatoxin if the ochratoxin A is capable of binding where zearalenone or aflatoxin bind (as is the case where ochratoxin binds to neutral alumina and to Florisil®). Likewise, if zearalenone binds to the aflatoxin adsorbent (which it does if Florisil® is used), the zearalenone adsorbent must be upstream from the aflatoxin adsorbent. Therefore, if the selective adsorbents were arranged in any different relative order, separation of the analytes would not obtain upon development of the detector tube with a sample solution containing these three analytes.

It should be appreciated that the only layers which are essential to the analysis are the adsorbents which are selective for ochratoxin, zearalenone and aflatoxins—in the illustrated case, pseudoboehmite, neutral alumina, and Florisil®, respectively. The other layers employed in the detector tube illustrated serve to support and isolate the selective adsorbents or to precondition the sample solution and/or the transporting solvent (e.g., by removing particulates).

The detector tube described above permits the analysis of the chemicals indicated which are mold-elaborated toxins (mycotoxins) often present in grains, nuts and other feed and food commodities. The analysis may be accomplished by dissolving the mycotoxins in a known volume of an organic solvent having the property of being able to simultaneously dissolve ochratoxin A, zearalenone and aflatoxins as possessing the appropriate characteristics which allow the mycotoxins to bind to the appropriate adsorbents as the sample solution is passed through the detector tube. Examples of appropriate solvents are benzene or toluene saturated with a solution of 40% methanol in water (v/v), or acetone/water mixtures.

The sample is applied to the top of the detector tube (e.g., one mL or less) and is allowed to flow through the device. The detector tube is further developed by adding a volume (e.g., one mL) of a wash or "transporting" solvent which is also allowed to flow through the detector tube. Differential pressure may be applied to the detector tube to increase the rate of flow. Solvents with the same general characteristics as the solvents used to dissolve the sample as well as those solvents themselves may be used as wash solvents. Other appropriate wash solvents are: toluene/acetone (9:1); toluene/chloroform/acetone (95:20:5); chloroform/acetone (9:1); and, ethyl acetate/methanol (4:1).

Following development (i.e., passage of the sample solution through the detector tube), the device is examined under an ultraviolet lamp preferably having an emission maximum at approximately 365 nm. If present in the extract above the detection limit, ochratoxin A will be detected as a bright blue fluorescent band in the ochratoxin selective layer (pseudoboehmite in the illustrated device), aflatoxins will appear as a blue fluorescent band in the aflatoxin-selective layer (here, Florisil®), and zearalenone will similarly be apparent as a blue fluorescent band in the layer which selectively adsorbs resorcinolic acid derivatives (here, alumina).

The intensity of the fluorescence emission in any of the mycotoxin-selective layers in the sample detector tube may be compared to the fluorescence of known quantities of the various mycotoxins applied to similar detector tubes in the same manner and thus representing "reference" devices. In this way, an emission intensity comparison can be used to gauge the amount of each mycotoxin in the sample detector tube, and, by applying appropriate calculations, the absolute amount of each mycotoxin in the original sample may be determined.

Alternatively, the fluorescence of the bands of the sample-developed detector tube may be compared to the fluorescence of a "reference guide" comprising fluorescent materials other than mycotoxins to gauge the level of each mycotoxin. Such a reference guide may conveniently be a card or other support on which a fluorescent compound is contained at varying levels corresponding to known quantities of the mycotoxins of interest. Thus, the fluorescence of each band of the detector tube could be matched to a level of fluores-

cence on the reference guide. A value would be assigned to each level allowing the calculation of the amount of mycotoxin in the original sample.

The developed detector tube could also be used in conjunction with an instrument capable of electronically detecting the fluorescent emission of each band of mycotoxin immobilized in the detector tube thereby providing a more exact quantitation of the fluorescence. Such an instrument would allow the calculation of the level of each such mycotoxin in the original sample. Such an instrument could also employ specific optical filters, monochromators, or the like to discriminate the wavelengths of the fluorescence detected. As will be appreciated by those skilled in the art, such discrimination would offer advantages in the specificity, sensitivity, and reproducibility of the assay.

EXAMPLE 3

This example illustrates how the invention may be employed to separate and detect zearalenone and aflatoxins in a sample potentially comprising a mixture of those mycotoxins as well as ochratoxin A. The particular detector tube illustrated employs pseudoboehmite to adsorb out ochratoxin A and other interfering compounds. A housing having sections of differing diameters is used to enhance effectiveness.

The packed portions of the detector tube is shown in cross section in FIG. 3. This device is designed to assay zearalenone and aflatoxins in a mixture of organic compounds extracted from seeds and foodstuffs. The design uses the same array of materials as that of Example 2, but the dimensions and preferred procedure for use are different.

The tube and the layers of packing material contained therein have the approximate dimensions shown in FIG. 3. The housing tube should have the properties described in Example 1. A Pasteur pipette may conveniently be used to provide the sections of differing diameters.

The nine layers of packing materials serve the same function as in Example 2 with the exception of layer 3 which is enlarged relative to its counterpart in the device of Example 2. The function of layer 3, which preferably comprises pseudoboehmite, is to immobilize ochratoxin A and other fluorescent compounds extracted from the sample which would otherwise bind downstream in the alumina layer (layer 5) or the Florisil® layer (layer 7) and thereby interfere with the proper measurement of zearalenone or aflatoxins, respectively. Thus, this detector tube configuration allows for the specific detection/measurement of zearalenone and aflatoxins but not of ochratoxin A. The positioning of layer 3 in the larger diameter portion of the detector tube provides for a relatively greater quantity of adsorbent in that layer for the sample solution to contact—sufficient to immobilize substantially all ochratoxin A and other potential interfering organic compounds which may be present in the sample solution. A larger diameter layer of adsorbent is preferred to a thicker layer (as a way of providing a greater quantity of adsorbent in the detector tube) inasmuch as a thicker layer would reduce the flow rate of sample solution (or increase the pressure differential needed to maintain an acceptable flow rate) and is more susceptible to channeling and other flow-disturbing phenomena. The locating of layers 5 and 7 in the more narrow portion of the tube effects a greater sensitivity of detection of any

zearalenone and/or aflatoxins, respectively, which become immobilized in the device upon development.

A preferred analytical procedure utilizing the detector tube described above is as follows:

1. 40 g of sample (e.g., corn, sorghum, peanuts, wheat, rice, soybeans) and 200 mL of 80% methanol in water (v/v) are placed in a blender and blended for 3 minutes at low speed.
2. Particulates are allowed to settle out and 5.5 mL of the clear supernatant liquid is transferred into a 20-mL glass screwcap vial through a coffee filter.
3. 6 mL of water are then added to the filter and collected in the same 20-mL vial.
4. 3 mL of toluene are then added to the solution in the vial; the cap is firmly affixed; and the vial inverted 20 times.
5. The vial is placed upright to allow the solvent phases to separate. The cap is then removed and 0.5 mL of extract is transferred to the top of the detector tube and allowed to drain into the packing material.
6. One mL of toluene/chloroform/acetone (95:20:5) is added to the top of the detector tube and allowed to flow through the packings. Positive pressure may be applied to the top of the device to increase the flow rate.
7. Using the procedure described in Example 2, the presence of zearalenone (about 100 ng) and/or aflatoxins (about 1 ng) in the sample is determined by examining for bright blue fluorescence in the appropriate layers.

EXAMPLE 4

This example illustrates the use of the selective adsorbents of the present invention to construct a detector tube for the detection and measurement of sterigmatocystin and aflatoxin M₁ in milk and milk products. Pseudoboehmite is employed to remove interfering compounds and Florisil® and alumina are used to immobilize aflatoxins and sterigmatocystin. A tube housing having sections of differing internal diameters is used to enhance the sensitivity of the analysis.

The packed portion of the detector tube is illustrated in cross section in FIG. 4. The device is designed for the detection and semiquantitative determination of sterigmatocystin and aflatoxin M₁ in a mixture of organic compounds extracted from milk or milk products. The tube housing preferable has the properties discussed in Example 1, above. Preferred approximate thicknesses for the various packing materials of the detector tube are indicated in FIG. 4.

Nine layers of materials are used to accomplish the specific resolution of sterigmatocystin and aflatoxin M₁. Materials other than the mycotoxin-selective adsorbents are employed to improve separation and detection. Layers 1 and 9 comprise cotton which layers act to maintain the integrity of the other layers during use, storage, and handling. Layers 2, 4, 6 and 8 are preferably used. They function to physically separate the selective chemisorbents thereby enhancing discrimination of the fluorescent signals emanating from such layers when the analytes are immobilized therein. Other suitable materials for this purpose are discussed in Example 2.

The detector tube should have the properties described in Example 3. The purpose of layer 3, which preferably comprises pseudoboehmite, is to immobilize ochratoxin A and other fluorescent compounds ex-

tracted from the sample along with the analytes, which compounds would otherwise bind downstream to alumina (layer 5) or Florisil® (layer 7) thereby interfering with the proper measurement of sterigmatocystin or aflatoxin M₁, respectively. Thus, this particular detector tube configuration permits the specific detection/

measurement of sterigmatocystin and aflatoxins but not of ochratoxin A. The locating of layer 3 in the large-diameter portion of the detector tube allows the sample to contact a larger amount of pseudoboehmite than would otherwise be possible. The actual amount should be sufficient to immobilize any and all ochratoxin A and other potentially interfering organic compounds which may be present in the sample.

The positioning of layers 5 and 7 in the more narrow portion of the tube effects a greater sensitivity of detection of any sterigmatocystin and/or aflatoxins, respectively, which are immobilized in these layers.

Layer 5 preferably comprises neutral alumina. Neutral alumina selectively adsorbs sterigmatocystin from the sample solution immobilizing it in the detector tube. Neutral alumina, however, allows other organic compounds including aflatoxin M₁ which might be present in the sample to pass through this layer to downstream layers. If sterigmatocystin is present in sufficient quantity to permit visual observation of its fluorescence, a bright yellow band in layer 5 will be seen when the detector tube is exposed to longwave uv radiation.

Layer 7 preferably comprises Florisil® activated magnesium silicate. It selectively adsorbs aflatoxin M₁ from the sample solution, immobilizing it in that particular layer while allowing other organic compounds which might be in the sample to pass through. If aflatoxins are present in the sample in sufficient quantities to permit visual observation of their fluorescence, a bright blue band in layer 7 will be seen when the detector tube is exposed to longwave uv radiation.

The mycotoxin-selective detector tube described above allows samples of milk and milk products to be assayed for sterigmatocystin and aflatoxin M₁. The analysis is performed by dissolving the mycotoxins in a known volume of a solvent which will dissolve the mycotoxins and which also has the necessary properties to allow both mycotoxins and other potentially interfering compounds to bind to appropriate adsorbents as the sample solution contacts them.

For the analysis of mycotoxins in milk, the following procedure is preferred.

1. A fresh milk sample is mixed with an equal volume of hexane in a container suitable for the thorough mixing of the liquids. A syringe may conveniently be used for this purpose (e.g., 5 mL of milk plus 5 mL of hexane in a 20-mL syringe).
2. After mixing, the two liquid phases are allowed to separate. The aqueous phase (milk) will be the lower of the two.
3. The aqueous phase (milk) is drawn off (e.g., pushed out when the syringe is held with the opening pointed in the downward direction) and transferred to a screwcap vial.
4. Ethyl acetate is then added to the milk in the vial in a ratio of 2-to-1 (e.g., 10 mL of ethyl acetate for 5 mL milk). The vial is then capped and inverted ten times.
5. The phases are allowed to separate and a 2-mL aliquot of the upper phase is withdrawn and applied to the top of the detector tube and allowed to

flow into the packings. One-half mL of ethyl acetate is added to the detector tube as a wash solvent to further develop the detector. Positive pressure may be applied to the top of the tube to increase the flow rate of sample through the tube.

6. The presence of sterigmatocystin (about 1 ng) is determined by examining for a bright yellow fluorescence in layer 5 using a longwave ultraviolet light source. The presence of aflatoxin M₁ (about 1 ng) is similarly determined by examining for a bright blue fluorescence in layer 7. The procedures described in Example 2 above are appropriate for this assay.

EXAMPLE 5

In this example, the novel adsorbents of the present invention are employed to construct a detector tube for the analysis of aflatoxins and deoxynivalenol (DON). Pseudoboehmite is used to remove ochratoxin A and other potentially interfering compounds. Alumina is used to immobilize DON and heat is used to provide enhanced levels of fluorescence, thereby lowering the detection limit of the determination. Florisil® is used to selectively bind aflatoxins. The combined use of Florisil® and alumina permits the resolution and separate detection of aflatoxins and DON.

The packed portion of a detector tube for the determination of aflatoxins and DON in a sample comprising a mixture of mycotoxins and other organic compounds is shown in cross section in FIG. 4. The tube itself preferably has the properties discussed in Example 1, above. The approximate preferred thicknesses of the various layers of packing materials within the detector tube are indicated in the figure.

Ten layers of materials are used to accomplish the specific resolution of the two mycotoxins of interest. Materials other than the chemisorbents selective for the two mycotoxins are included to improve the separation and detection. Layers 1 and 9 preferably comprise cotton. These layers function to maintain the physical integrity of the other layers during use, storage and handling. Layer 1 acts additionally to filter out particulates.

Layers 3, 5, 7, and 9 are sand. They are employed to physically separate the selective adsorbents thereby enhancing differentiation of the fluorescence of the mycotoxins immobilized in those chemisorptive layers. Other suitable materials for this purpose are discussed in Example 2, above.

Layer 2 is a preferably a mixture comprising sepiolite and halloysite. Sepiolite (or "meerschaum") is a mineral $Mg_3Si_4O_{10}(OH)_2 \cdot 4H_2O$ consisting of a hydrous magnesium silicate that is an extremely light fine soft white clay dug chiefly in Asia Minor. Halloysite is a clay mineral $Al_2Si_2O_5(OH)_4 \cdot nH_2O$ occurring in soft white or light-colored masses and in at least 2 states of hydration— $n=2$ and $n=4$. The purpose of this layer is to immobilize fluorescent compounds other than aflatoxins and DON which may be in the sample which would otherwise bind downstream in the Florisil® layer (layer 4) or the alumina layer (layer 8), thus interfering with the detection/measurement of aflatoxins and DON, respectively. Any other materials which bind the interfering compounds and not aflatoxins or DON could be substituted for or used in addition to sepiolite and halloysite in this layer.

Layer 4 preferably comprises Florisil®. It selectively adsorbs aflatoxins from the mixture while allowing DON to pass through to downstream layers in the

detector tube. If aflatoxins are present in the sample in sufficient quantity to permit visual observation of their fluorescence (at least about 2-5 ng), a bright blue band in layer 4 will be observed when the detector tube is exposed to longwave uv radiation.

Layer 6 preferably comprises charcoal. Its purpose is to immobilize fluorescent compounds other than DON which might bind in the downstream alumina layer (layer 8), thus interfering with the detection/measurement of DON. Any other materials which bind the interfering compounds and not DON could be substituted for or used in conjunction with charcoal in this layer.

Layer 8 preferably comprises neutral alumina. It selectively adsorbs DON from the sample solution but allows other classes of organic compounds which might be in the sample solution to pass through the layer. If DON is present in the sample in sufficient quantity (about 300 ng), upon heating the detector tube (e.g., 120° C. for 5 min.), a bright blue band in layer 8 will be observed when the device is exposed to longwave uv radiation. Any other material which binds DON (e.g., pseudoboehmite) could be substituted for neutral alumina in this layer. The adsorbent may be treated with an acidic substance such as aluminum chloride or oxalic acid to enhance the fluorescence of bound DON.

It is important that the Florisil® layer be upstream from the layers used to bind DON and interfering compounds if aflatoxins are capable of binding to those layers (as is the case where aflatoxins bind to charcoal). Therefore, if the relative positions of the selective adsorbents were in any different order, separation of the mycotoxins from each other and from interfering compounds would not occur.

The detector tube described in this example allows the analysis of the mycotoxins indicated. These mycotoxins often contaminate grains, nuts and other feeds and food commodities.

The analysis may be accomplished by dissolving the mycotoxins in a known volume of an organic solvent having the properties of being able to dissolve aflatoxins and DON and possessing the appropriate characteristics to allow the mycotoxins to bind to the selective adsorbents when the sample solution contacts them in the detector tube. Examples of appropriate solvents are ethyl acetate, acetone, toluene and various combinations of these.

The sample solution is applied to the top of the detector tube (e.g., one mL or less) and is allowed to flow into the packing material. The device is further developed by adding a volume (e.g., one mL) of the same solvent as a wash solvent which is also allowed to flow through the various packings. Differential pressure may be applied to the tube to increase the rate of flow.

After such development, the detector tube containing immobilized mycotoxins is heated to effect an enhancement of fluorescence; for example, in an oven at 120° C. for 5 minutes. The device is then examined under longwave uv radiation. If present in the extract above the detection limit (above about 5 ng), aflatoxins will be observed as a bright blue fluorescent band in layer 4. Similarly, deoxynivalenol (if present in an amount greater than about 300 ng) will appear as a bright blue fluorescent band in layer 8. Additional methods for determining the quantity of the mycotoxins at this stage of the procedure are described in Example 2.

EXAMPLE 6

Described in this example are a detector tube and method for the assay of feeds and other foodstuffs for sterigmatocystin. In the device described, alumina or pseudoboehmite is used to immobilize the mycotoxin of interest (sterigmatocystin) thereby permitting its separation and detection in a mixture of organic compounds including other mycotoxins.

The packed portion of the detector tube for the assay of sterigmatocystin is shown in cross section in FIG. 5. The tube itself preferably has the properties discussed in Example 1. Preferred approximate dimensions for the various packing materials are indicated in the figure.

In the detector tube shown, six layers of packing materials are employed to accomplish the specific resolution of sterigmatocystin. Materials other than the selective adsorbents are included to improve the separation and detection. Layers 1 and 6 are preferably cotton. These layers function to maintain the integrity of the other layers during use, storage and handling. Layers 3 and 5 are preferably sand. These layers physically separate the selective adsorbent layers thereby enhancing discrimination of the fluorescent emission of sterigmatocystin immobilized in the selective adsorbent. Other suitable packing materials for these layers are discussed in Example 2, above.

Layer 2 preferably comprises halloysite. It immobilizes chemicals in the sample solution which would otherwise bind in the downstream alumina layer (layer 4) and interfere with the accurate measurement of sterigmatocystin. Any other packing materials which bind interfering chemicals and not sterigmatocystin could be substituted for, or used in addition to halloysite in this layer of the device.

Layer 4 preferably comprises neutral alumina. Neutral alumina (as does acidic and basic alumina) selectively binds sterigmatocystin in the sample solution while allowing other organic chemicals to be transported through this layer. If sterigmatocystin is present in the sample solution in sufficient quantity to permit visualization of its fluorescence (approximately 2-5 ng), a bright yellow band in layer 4 will be observed when the detector tube is examined under longwave uv light. Any other material which binds sterigmatocystin and permits its fluorescence to be observed could be substituted for the neutral alumina in this layer. Specifically, pseudoboehmite or hayerite could be substituted as the selective adsorbent in this layer of the detector tube.

The detector tube disclosed in this example permits one to analyze for sterigmatocystin in feed and foods. The analysis is accomplished by dissolving the mycotoxin in a known volume of an organic solvent having the properties of being able to dissolve sterigmatocystin while allowing that mycotoxin to bind to the selective adsorbent in the detector tube when the sample solution is transported through that layer. Examples of appropriate solvents are acetone, ethyl acetate, methanol and toluene.

The sample solution is applied to the top of the detector tube (e.g., one mL or less) and allowed to flow through the device. The column is preferably further developed by adding a volume (e.g., one mL) of the same solvent as a wash solvent which is also allowed to flow through the tube. Differential pressure may be applied to the detector tube to increase the flow rate of sample solution and wash solvent. This may be conve-

sicently accomplished by simply attaching a pipette bulb to the top of the tube and then squeezing the bulb.

Following development, the detector tube is examined under a longwave uv lamp preferably having an emission maximum at around 365 nm. If present in the sample in sufficient quantity, sterigmatocystin will be detected as a bright yellow fluorescence in layer 4. Suitable methods for determining or estimating the quantity of the mycotoxin are described in Example 2.

EXAMPLE 7

Described in this example are a detector tube and associated method for the assay of patulin in various fruits, fruit juices, and other commodities. Alumina is employed in the device described to selectively immobilize patulin thereby permitting its detection and/or measurement.

The packed portion of the detector tube for the assay of patulin is shown in cross section in FIG. 8. The tube itself preferably has the properties discussed in Example 1. Preferred approximate dimensions for the various packing materials are indicated in the figure.

In this detector tube, six layers of packing material are used to accomplish the specific resolution of patulin. Materials other than the selective adsorbents are included to enhance the separation and detection. Layers 1 and 6 are preferably cotton. These layers function to maintain the integrity of the other layers in the device during use, storage and handling. Layers 3 and 5 are preferably sand. They are used to physically separate the selective chemisorbent layer thereby enhancing discrimination of the fluorescence of the immobilized mycotoxin. Other suitable materials which may be employed in these layers are discussed in Example 2, above.

Layer 2 preferably comprises a mixture of neutral alumina and pseudoboehmite (1:1 by volume). The purpose of this layer is to immobilize chemicals in the sample solution which would otherwise bind downstream in the alumina layer (layer 4) and interfere with the accurate measurement of patulin. Any other packing materials which bind the interfering chemicals and not patulin could be substituted for, or used in addition to alumina and pseudoboehmite in this layer of the device.

Layer 4 preferably comprises neutral alumina coated with oxalic acid. This material may be prepared by dissolving a quantity of oxalic acid equal to about 0.05 to about 0.5% of the weight of neutral alumina to be used in diethyl ether and mixing this solution with a slurry of neutral alumina. The ether is subsequently removed by evaporation under vacuum. The alumina so treated is then activated by heating in a vacuum oven at about 30° to 100° C. for about 4 to 6 hours. It is contemplated that any other nonvolatile acids having pKa's less than about 3 may be used in place of oxalic acid (e.g., trichloroacetic acid). Patulin, which binds to the acid-treated alumina, will fluoresce blue when exposed to longwave uv radiation after being heated at about 120° C. for 5 minutes. Silica gel may be used in place of neutral alumina in this procedure.

The detector tube described above can be used for the analysis of patulin in samples of fruits, fruit juices and other commodities. The analysis is performed by dissolving the mycotoxin in a known volume of an organic solvent which both dissolves the patulin and permits the patulin and any co-dissolved interfering compounds to bind to the appropriate adsorbents as the sample solution is transported through the detector

tube. Effective solvents include ethyl acetate and toluene.

The sample solution is applied to the top of the detector tube (for example, one mL or less) and is allowed to flow into the packings of the device. Further development is accomplished by adding a volume (e.g., one mL) of the same solvent used to dissolve the mycotoxin to the tube as a wash or "transporting" solvent. This is allowed to flow through the tube. The flow rate may be increased by applying differential pressure to the tube.

After development, the column is heated to produce a fluorescent compound from any patulin immobilized in layer 4. The heating may be accomplished by placing the detector tube in an oven at about 120° C. for about 5 minutes. The tube is then examined under longwave uv radiation. If present in the sample solution above the detection limit, patulin (or its fluorescent product) will appear as a blue fluorescent band in layer 4. Additional methods for determining or quantitating the patulin at this point are discussed in Example 2, above.

EXAMPLE 8

A detector tube and associated method for the analysis of citrinin are described in this method. The device utilizes bayerite or pseudoboehmite to selectively adsorb and immobilize citrinin from a solution comprising citrinin and other organic compounds including other mycotoxins. The immobilization of citrinin permits its detection and/or measurement by observation of its fluorescence.

The packed portion of the detector tube for the assay of patulin is shown in cross section in FIG. 8. The tube itself preferably has the properties discussed in Example 1. Preferred approximate dimensions for the various packing materials are indicated in the figure.

In this detector tube, six layers of packing material are used to accomplish the specific resolution of citrinin. Materials other than the selective adsorbents are included to enhance the separation and detection. Layers 1 and 6 are preferably cotton. These layers function to maintain the integrity of the other layers in the device during use, storage and handling. Layers 3 and 5 are preferably sand. They are used to physically separate the selective chemisorbent layer thereby enhancing discrimination of the fluorescence of the immobilized mycotoxin. Other suitable materials which may be employed in these layers are discussed in Example 2, above.

Layer 2 preferably comprises sepiolite. It functions immobilize chemicals in the sample solution which would otherwise bind downstream in the pseudoboehmite layer (layer 4) and interfere with the measurement of citrinin. Any other packing material(s) which would bind interfering compounds and not citrinin could be substituted for or used in conjunction with the sepiolite in this layer of the device.

Layer 4 preferably comprises pseudoboehmite. It selectively adsorbs citrinin in the sample solution while allowing other dissolved compounds in the sample solution to be transported through this layer. If citrinin is present in the sample solution in sufficient quantity to permit observation of its fluorescence, a yellow-green band in layer 4 will be observed when the detector tube is exposed to longwave uv radiation. Any other material which selectively binds citrinin and allows observation of its fluorescence (e.g., bayerite or alumina) could be substituted for pseudoboehmite in this layer.

The detector tube described above allows one to conveniently analyze for citrinin in feeds and foodstuffs. The analysis may be performed by dissolving the mycotoxin in a known volume of an organic solvent which both effectively solubilizes the mycotoxin and allows it and other co-dissolved compounds to bind to the appropriate chemisorbents in the detector tube. Examples of appropriate solvents for this purpose are chloroform, acetone, toluene and ethyl acetate.

The sample solution is applied to the top of the detector tube (for example, one mL or less) and is allowed to flow into the packings of the device. Further development is accomplished by adding a volume (e.g., one mL) of the same solvent used to dissolve the mycotoxin to the top of the tube as a wash or "transporting" solvent. This is allowed to flow through the tube. The flow rate may be increased by applying positive pressure to the top of the column.

After such development, the detector tube is examined under a uv light source having an emission maximum at about 365 nm. If present in the sample solution in sufficient quantity, citrinin will be detected as a bright yellow-green fluorescence in layer 4. Methods for determining the quantity of the mycotoxin in the feed or food sample are discussed in Example 2, above.

EXAMPLE 9

A detector tube and associated method for the analysis of ergots are described in this method. The device utilizes alumina or silica gel to selectively adsorb and immobilize ergots from a solution comprising ergots and other organic compounds including other mycotoxins. The immobilization of ergots permits their detection and/or measurement by observation of their fluorescence.

The packed portion of the detector tube for the assay of ergots is shown in cross section in FIG. 3. The tube itself preferably has the properties discussed in Example 1. Preferred approximate dimensions for the various packing materials are indicated in the figure.

In this detector tube, six layers of packing material are used to accomplish the specific resolution of ergots. Materials other than the selective adsorbents are included to enhance the separation and detection. Layers 1 and 6 are preferably cotton. These layers function to maintain the integrity of the other layers in the device during use, storage and handling. Layers 3 and 5 are preferably sand. They are used to physically separate the selective chemisorbent layer thereby enhancing discrimination of the fluorescence of the immobilized mycotoxin. Other suitable materials which may be employed in these layers are discussed in Example 2, above.

Layer 2 preferably comprises the natural zeolite mordenite [Chemical Abstracts Service Registry numbers 12445-20-4 and 12173-98-7]. It functions to immobilize chemicals in the sample solution which would otherwise bind downstream in the alumina layer (layer 4) and interfere with the measurement of ergots. Any other packing material(s) which would bind interfering compounds and not ergots could be substituted for or used in conjunction with the zeolite in this layer of the device.

Layer 4 preferably comprises acidic alumina. It selectively adsorbs ergots in the sample solution while allowing other co-extracted organic compounds to pass through the layer. If ergots are present in the sample solution in sufficient quantities to permit observation of

their fluorescence, a blue band will be observed when the detector tube is exposed to longwave uv radiation. Any other material which selectively binds ergots and permits observation of their fluorescence could be substituted in this layer of the device.

The detector tube described above allows one to conveniently and rapidly analyze feeds and foodstuffs for ergot contamination. The analysis is performed by dissolving the mycotoxins (e.g., ergotamine) in a known volume of an organic solvent which both effectively solubilizes the mycotoxin(s) and allows it and other co-dissolved compounds to bind to the appropriate chemisorbents in the detector tube. Examples of appropriate solvents for this purpose are chloroform, acetone, and ethyl acetate.

The sample solution is applied to the top of the detector tube (for example, one mL or less) and is allowed to flow into the packings of the device. Further development is accomplished by adding a volume (e.g., one mL) of the same solvent used to dissolve the mycotoxin to the top of the tube as a wash or "transporting" solvent. This is allowed to flow through the tube. The flow rate may be increased by applying positive pressure to the top of the column.

After such development, the detector tube is examined under a uv light source having an emission maximum at about 365 nm. If present in the sample solution in sufficient quantity, the ergot(s) will be detected as a blue fluorescence in layer 4. Methods for determining the quantity of the mycotoxin in the feed or food sample are discussed in Example 2, above.

The foregoing description has been directed to particular embodiments of the invention in accordance with the requirements of the United States patent statutes for the purposes of illustration and explanation. It will be apparent to those skilled in this art, however, that many modifications and changes in the compositions, devices, and methods set forth will be possible without departing from the scope and spirit of the invention. It is intended that the following claims be interpreted to embrace all such modifications and changes.

What is claimed is:

1. An apparatus for selective separation and detection of a mycotoxin from a solution which may contain interfering materials comprising:

a transparent tube containing a sequence of discrete layers including:

(1) A first adsorbent layer which is capable of selectively binding interfering materials from the solution as it passes through the tube;

(2) A second adsorbent layer having a structure-selective affinity for a mycotoxin said second adsorbent layer being capable of immobilizing said mycotoxin in the presence of an appropriate solvent; and

(3) A support layer between the first and second adsorbent layers;

said support layer being made of material other than those of said first and second adsorbent layers.

2. The apparatus of claim 1 wherein said first adsorbent layer comprises nepholite, halloysite, mordenite, activated magnesium silicate, silica gel, or mixtures thereof; said support layer comprises diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; said second adsorbent layer comprises pseudobrookite, bayerite, gibbsite, boehmite, bauxite, alumina, or mixtures thereof and is selective for a mycotoxin

selected from the group consisting of zearalenone, ochratoxin A, and citrinin.

3. The apparatus of claim 1 wherein said first adsorbent layer comprises pseudoboehmite, bayerite, gibbsite, boehmite, bentonite, alumina, or mixtures thereof; said support layer comprises diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; said second adsorbent layer comprises activated magnesium silicate and is selective for a mycotoxin selected from the group consisting of aflatoxin and aflatoxin-M1.

4. The apparatus of claim 1 wherein said second adsorbent layer is selective for a mycotoxin selected from the group consisting of deoxynivalenol, sterigmatocystin, patulin and ergot.

5. Apparatus for the separation and detection of aflatoxins and ochratoxin A from a solution which comprises:

a transparent tube containing a sequence of discrete layers including:

- (1) A first adsorbent layer having a structure-selective affinity for ochratoxin A, but not aflatoxin;
- (2) A second adsorbent layer having a structure-selective affinity for aflatoxin; and
- (3) A support layer between the first and second adsorbent layers;

said support layer being made of material other than those of said first and second adsorbent layers.

6. The apparatus of claim 5 wherein said first adsorbent layer comprises pseudoboehmite, bayerite, gibbsite, boehmite, bentonite, alumina having activity grade IV or V, or mixtures thereof; said support layer comprises diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; and said second adsorbent layer comprises activated magnesium silicate.

7. The apparatus of claim 5 wherein said first adsorbent layer comprises bayerite, said support layer comprises diatomaceous earth, and said second adsorbent layer comprises activated magnesium silicate.

8. Apparatus for the separation and detection of ochratoxin A, zearealenone, and aflatoxin from a solution which comprises:

a transparent tube containing a sequence of discrete layers including:

- (1) A first adsorbent layer having a structure-selective affinity for ochratoxin A, but not zearealenone or aflatoxin;
- (2) A second adsorbent layer having a structure-selective affinity for zearealenone, but not aflatoxin;
- (3) A third adsorbent layer having a structure-selective affinity for aflatoxin;
- (4) A first support layer between the first and second adsorbent layers;
- said first support layer being made of material other than those of said first, second and third adsorbent layers; and
- (5) A second support layer between the second and third adsorbent layers;
- said second support layer being made of material other than those of said first, second and third adsorbent layers.

9. The apparatus of claim 8 wherein said first adsorbent layer comprises pseudoboehmite; said first and second support layers comprise diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an

aluminosilicate, cellulose fiber, sand, or mixtures thereof; said second adsorbent layer comprises neutral alumina; and said third adsorbent layer comprises activated magnesium silicate.

10. An apparatus for the separation and detection of zearealenone and aflatoxin from a solution which comprises:

a transparent tube containing a sequence of discrete layers including:

- (1) A first adsorbent layer having a structure-selective affinity for zearealenone, but not aflatoxin, said first adsorbent being capable of immobilizing said zearealenone in the presence of an appropriate solvent, but not aflatoxin;
- (2) A second adsorbent layer having a structure-selective affinity for aflatoxin, said second adsorbent layer being capable of immobilizing said aflatoxin in the presence of an appropriate solvent;
- (3) A first support layer between the first and second adsorbent layers;

said first support layer being made of material other than those of said first and second adsorbent layers.

11. The apparatus of claim 10 wherein said solution may contain interfering materials which further comprises:

- (1) A third adsorbent layer which is capable of selectively binding interfering materials from the solution as it passes through the tube located prior to said first adsorbent layer; and
- (2) A second support layer between the third and first adsorbent layers;

said second support layer being made of material other than those of said first, second and third adsorbent layers.

12. The apparatus of claim 11 wherein said third adsorbent layer comprises pseudoboehmite; said first and second support layers comprise diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; said first adsorbent layer comprises neutral alumina; and said second adsorbent layer comprises activated magnesium silicate.

13. Apparatus for the separation and detection of sterigmatocystin and aflatoxin-M1 which comprises:

a transparent tube containing a sequence of discrete layers including:

- (1) A first adsorbent layer having a structure-selective affinity for sterigmatocystin, but not aflatoxin-M1;
- (2) A second adsorbent layer having a structure-selective affinity for aflatoxin-M1; and
- (3) A first support layer between the first and second adsorbent layers;

said first support layer being made of material other than those of said first and second adsorbent layers.

14. The apparatus of claim 13 wherein said solution may contain interfering materials which further comprises:

- (1) A third adsorbent layer which is capable of selectively binding interfering materials from the solution as it passes through the tube located prior to said first adsorbent layer; and
- (2) A second support layer between said third and first adsorbent layers;

said second support layer being made of material other than those of said first, second and third adsorbent layers;

15. The apparatus of claim 14 wherein said third adsorbent layer comprises pseudoboehmite; said first and second support layers comprise diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; said first adsorbent layer comprises neutral alumina; and said second adsorbent layer comprises activated magnesium silicate.

16. Apparatus for the separation and detection of aflatoxin and deoxyaivalenol from a solution which comprises:

a transparent tube containing a sequence of discrete layers including:

(1) A first adsorbent layer having a structure-selective affinity for aflatoxin, but not deoxyaivalenol;

(2) A second adsorbent layer having a structure-selective affinity for deoxyaivalenol; and

(3) A first support layer between the first and second adsorbent layers;

said first support layer being made of material other than those of said first and second adsorbent layers.

17. The apparatus of claim 16 wherein said solution may contain interfering materials which further comprises:

(1) A third adsorbent layer which is capable of selectively binding interfering materials from the solution as it passes through the tube located prior to said first adsorbent layer; and

(2) A second support layer between the third and first adsorbent layers;

said second support layer being made of material other than those of said first, second and third adsorbent layers.

18. The apparatus of claim 17 wherein said third adsorbent layer comprises halloysite, sepiolite, or mixtures thereof; said first and second support layers comprise diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; said first adsorbent layer comprises activated magnesium silicate; and said second adsorbent layer comprises neutral alumina.

19. Apparatus for the separation and detection of strigamycin from a solution which may contain interfering materials comprising:

a transparent tube containing a sequence of discrete layers including:

(1) A first adsorbent layer which is capable of selectively binding interfering materials from the solution as it passes through the tube;

(2) A second adsorbent layer having a structure-selective affinity for strigamycin; and

(3) A support layer between said first and second adsorbent layers;

said support layer being made of material other than those of said first and second adsorbent layers.

20. The apparatus of claim 19 wherein said first adsorbent layer comprises halloysite, said support layer comprises diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; and said second adsorbent layer comprises neutral alumina, pseudoboehmite, or bayerite.

21. Apparatus for separation and detection of patulin from a solution which may contain interfering materials comprising:

a transparent tube containing a sequence of discrete layers including:

(1) A first adsorbent layer which is capable of selectively binding interfering materials from the solution as it passes through the tube;

(2) A second adsorbent layer having a structure-selective affinity for patulin; and

(3) A support layer between the first and second adsorbent layers;

said support layer being made of material other than those of said first and second adsorbent layers.

22. The apparatus of claim 21 wherein said first adsorbent layer comprises a mixture of neutral alumina and pseudoboehmite; said support layer comprises diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; and said second adsorbent layer comprises neutral alumina coated with oxalic acid or silica gel coated with oxalic acid.

23. Apparatus for separation and detection of citrinin from a solution which may contain interfering materials comprising:

a transparent tube containing a sequence of discrete layers including:

(1) A first adsorbent layer which is capable of selectively binding interfering materials from the solution as it passes through the tube;

(2) A second adsorbent layer having a structure-selective affinity for citrinin; and

(3) A support layer between the first and second adsorbent layers;

said support layer being made of material other than those of said first and second adsorbent layers.

24. The apparatus of claim 23 wherein said first adsorbent layer comprises sepiolite; said support layer comprises diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; and said second adsorbent layer comprises pseudoboehmite.

25. Apparatus for separation and detection of ergots from a solution which may contain interfering materials comprising:

a transparent tube containing a sequence of discrete layers including:

(1) A first adsorbent layer which is capable of selectively binding interfering materials from the solution as it passes through the tube;

(2) A second adsorbent layer having a structure-selective affinity for ergots; and

(3) A support layer between the first and second adsorbent layers;

said support layer being made of material other than those of said first and second adsorbent layers.

26. The apparatus of claim 25 wherein said first adsorbent layer comprises mordantite; said support layer comprises diatomaceous earth, glass wool, a silicate, carbon, sulfur, iron filings, an aluminosilicate, cellulose fiber, sand, or mixtures thereof; and said second adsorbent layer comprises acidic alumina.

27. A kit for selective separation and detection of a mycotoxin from a solution which may contain interfering materials comprising:

a transparent tube containing a sequence of discrete layers including:

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- (1) A first adsorbent layer which is capable of selectively binding interfering materials from the solution as it passes through the tube;
 - (2) A second adsorbent layer having a structure-selective affinity for a mycotoxin said second adsorbent layer being capable of immobilizing said mycotoxin in the presence of an appropriate solvent;
 - (3) A support layer between the first and second adsorbent layers; said support layer being made of material other than those of said first and second adsorbent layers; and an appropriate solvent.
28. The kit of claim 27 wherein the second adsorbent layer comprises activated magnesium silicate.
29. The kit of claim 27 wherein the appropriate solvent comprises toluene.
30. A kit for the separation and detection of zearalenone and aflatoxin from a solution comprising: a transparent tube containing a sequence of discrete layers including:

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- (1) A first adsorbent layer having a structure-selective affinity for zearalenone, but not aflatoxin, said first adsorbent being capable of immobilizing said zearalenone in the presence of an appropriate solvent, but not aflatoxin;
 - (2) A second adsorbent layer having a structure-selective affinity for aflatoxin, said second adsorbent layer being capable of immobilizing said aflatoxin in the presence of an appropriate solvent;
 - (3) A first support layer between the first and second adsorbent layers; said first support layer being made of material other than those of said first and second adsorbent layers; and an appropriate solvent.
31. The kit of claim 30 wherein the first adsorbent layer comprises neutral alumina and the second adsorbent layer comprises magnesium silicate.
32. The kit of claim 30 wherein the appropriate solvent comprises toluene.
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**UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION**

PATENT NO. : 5,178,832

DATED : Jan. 12, 1993

INVENTOR(S) : Timothy D. Phillips, et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On title page, item [75] the addresses of "William C. Gordon and Linda J. Gordon" should read as follows:

—both of Stillwater, Minnesota—

In column 25, line 48, please delete "ochralayer" and insert therefor —ochratoxin—.

In column 27, line 35, please delete "adsorent" and insert therefor —adsorbent—.

Signed and Sealed this
Twenty-fourth Day of May, 1994

Attest:



BRUCE LEHMAN

Attending Officer

Commissioner of Patents and Trademarks

PRODUCTOS DE CEREZA DE CAFÉ CON

BAJA MICOTOXINA

CAMPO DE LA INVENCION

El campo de la invención son los productos alimenticios, y especialmente los productos alimenticios preparados de cerezas de café sub-maduras con secado rápido completo, o porciones/fragmentos de estas.

ANTECEDENTES DE LA INVENCION

Varias partes del árbol del café han sido utilizadas con propósitos nutricionales durante un tiempo relativamente largo (ver por ejemplo, Pendergrast, M. *Uncommon Grounds*. Basic Books: Nueva York, 1999). Por ejemplo, las hojas del árbol del café y las cerezas de café frescas, maduras, fueron hervidas para hacer infusión. En otros ejemplos, la pulpa de la cereza del café se puede fermentar para producir vino como se describió en la Patente China CN 1021949. En aún un ejemplo adicional bien conocido, las semillas (es decir, los granos) del árbol del café son extraídas de la cereza, secadas, tostadas, molidas, y extraídas con agua caliente

para suministrar la bebida que disfrutaban muchos usuarios como café.

Desafortunadamente, las cerezas de café, y especialmente la pulpa y la cáscara tienden a arruinarse rápidamente en la presencia de mohos, hongos, y otros microorganismos, y por lo tanto contienen casi siempre niveles significativos de micotoxinas (ver por ejemplo, Pittet, A., Tornare, D., Huggett, A., Viani, R. Liquid Chromatographic Determination of Ochratoxin A in Pure and Adulterated Soluble Coffee Using an Immunoaffinity Column Cleanup Procedure. *J. Agric. Food Chem.* 1996, 44, 3564-3569; o Bucheli, P., Kanchanomai, C., Meyer I., Pittet, A. Development of Ochratoxin A during Robusta (*Coffea canephora*) Coffee Cherry Drying. *J. agric. Food Chem.* 2000, 48, 1358-1362). Así, las bebidas producidas de la pulpa del café, cáscara, mucílago, y/o cereza de café completa generalmente no encuentran aceptación como ingrediente para bebidas. (Aunque un producto sea anunciado como "infusión de cereza de café" [<http://www.paradiserelocation.com/paradisetogo/foodproducts.htm>], el producto es de hecho elaborado de pulpa de cereza de café y se determinó recientemente que tenía cantidades sustanciales de micotoxinas).

Aún en situaciones en donde la pulpa, mucílago, y la cascarilla es removida, las micotoxinas pueden aún estar presentes sobre y/o en el grano de café. Consecuentemente, se han hecho esfuerzos considerables para desintoxicar los granos de café y otros productos alimenticios. Por ejemplo, cuando la micotoxina ya está presente en el producto alimenticio, las micotoxinas seleccionadas se pueden extraer del producto alimenticio utilizando varios solventes y procedimientos como se describió en la Patente Estadounidense No. 4,436,756 de Canella et al. De otra parte, varias micotoxinas pueden ser adsorbidas del producto alimenticio sobre un mineral portador como se describió en la Patente Estadounidense No. 5,935,623 de Alonso-Debolt.

Aún en otros métodos, las micotoxinas seleccionadas se pueden degradar utilizando enzimas como se describió en la Patente Estadounidense No. 5,716,820 de Duvick et al. Los inventores de la referencia '820 aún contemplan que los genes que codifican para tales enzimas puedan ser clonados para producir plantas transgénicas que se piensa pueden estar menos contaminadas con micotoxinas. Alternativamente, los microorganismos se pueden emplear para destruir micotoxinas enzimáticamente encontradas en los productos alimenticios como se describió en la Patente Estadounidense No. 6,025,188 de Duvick et al.

Donde las micotoxinas aún no son producidas mediante un microorganismo presente en una planta u otro material alimenticio, se pueden emplear los pesticidas u otras composiciones que controlan el crecimiento microbiano o la producción de micotoxinas en los microorganismos. Por ejemplo, Emerson et al. describe en la Patente Estadounidense No. 5,639,794 el uso de una saponina como un sinérgico para controlar la colonización y/o el crecimiento de patógenos de plantas y animales. Alternativamente, como se describió en la Patente Estadounidense No. 4,199,606 de Bland, se puede emplear ácido propiónico sobre un portador como un inhibidor de crecimiento difundible para varios microorganismos. Composiciones conocidas adicionales (ver por ejemplo, Patente Estadounidense No. 5,698,599 de Subía o Patente Estadounidense No. 3,798,323 de Leary) se pueden emplear para suprimir o al menos reducir la síntesis de micotoxinas en un microorganismo.

Alternativamente, los productos alimenticios que contienen micotoxinas pueden ser mezclados con productos alimenticios no contaminados a una concentración que es aceptable y/o por debajo de la cantidad máxima permisible de micotoxinas en productos alimenticios (ver por ejemplo, Herrman, T. y Trigo-Stockli, D.; *Mycotoxins in Feed Grains and Ingredients*;

Kansas State University, Mayo 2002), o (al menos potencialmente) productos de cereza de café que contienen micotoxina se pueden emplear en un producto no alimenticio. Aún en otros usos, el contenido de micotoxina puede no ser considerado relevante en la medida en que el producto de cereza de café es incinerado y así las micotoxinas son al menos parcialmente distribuidas como se describió en la Patente Estadounidense No. 4,165,752, GB 2026839, o CA 1104410. Aquí, el inventor enseña que las cerezas de café pueden ser comprimidas, deshidratadas, molidas, y tostadas para producir un producto ahumado.

Sin embargo, aunque la mayoría de los métodos conocidos reducen la concentración de micotoxinas al menos en algún grado, subsisten numerosas desventajas. Entre otras cosas, las etapas de procesamiento adicional requerirán equipo dedicado, incrementando de esta manera el tiempo y los costos de procesamiento. Más aún, y especialmente cuando son utilizados pesticidas y/o fungicidas, pueden surgir nuevos problemas con químicos tóxicos residuales.

Así, a pesar de numerosas propiedades benéficas de las cerezas de café y de sus componentes, las cerezas de café completas no son generalmente utilizadas como productos alimenticios en la medida en que las micotoxinas están

típicamente presentes en cantidades sustanciales en la fruta madura y sobre madura. Por lo tanto, subsiste la necesidad de suministrar métodos y composiciones mejoradas para las cerezas de café, y especialmente para los productos que comprenden cerezas de café con bajo o ningún contenido de micotoxina para el consumo humano y veterinario.

RESUMEN DE LA INVENCION

La presente invención está dirigida a composiciones y métodos que incluyen cerezas de café o porciones de estas con secado rápido (preferiblemente sub-maduras), en donde las cerezas de café son sustancialmente desprovistas de, o tienen muy bajo contenido de micotoxinas.

En un aspecto de la materia objeto de la invención, un producto alimenticio comprende una preparación de una cereza de café que es de secado rápido de tal forma que el nivel micotoxina de la cereza de café es menor de 20 ppb para las aflatoxinas, menos de 10 ppb para las ocratoxinas totales, y menos de 5 ppm para las fumonisinas totales. Las preparaciones preferidas en tales productos alimenticios incluyen el grano, pulpa, mucílago, y/o cascarilla de la cereza de café de secado rápido, o fragmentos de molido de la cereza de café, o un extracto de ésta. Se prefiere

adicionalmente que la cereza de café sea una cereza de café sub-madura. Los productos alimenticios preferidos incluyen una infusión mezclada de cerezas de café de secado rápido (preferiblemente sub-maduras), o una bebida que comprende un extracto de la cereza de café. Alternativamente, los productos alimenticios adecuados también incluyen suplementos nutricionales en forma líquida y sólida que comprenden un extracto de la cereza de café.

Las cerezas de café sub-maduras contempladas tienen un color primariamente verde con menos del 25% de color rojo, más preferiblemente un color rojo primariamente con menos del 25% de color verde, y aún más preferiblemente un color rojo primariamente con menos del 5% de área manchada. Las cerezas de café (sub-maduras) pueden ser secadas rápidamente utilizando varios métodos, sin embargo, se prefiere generalmente que las cerezas de café sean secadas rápidamente utilizando aire calentado o expuestas al sol y/o el aire ambiente.

En otro aspecto de la materia objeto de la invención, una infusión es mezclada de cereza de café (preferiblemente sub-madura) de secado rápido desmenuzada o molida o porciones de ésta, en donde la cereza de café tiene un nivel de micotoxina de menos de 20 ppb para las aflatoxinas totales, menos de 10

ppb de ocratoxinas totales, y menos de 5 ppm de fumonisinas totales, y preferiblemente tiene una concentración de polifenol de al menos 10 mg/oz (más preferiblemente en una proporción de ácido clorogénico a cafeína de al menos 2,7).

Así, visto desde otra perspectiva, se contempla que la cereza de café de secado rápido o la porción de ésta tenga un nivel de micotoxina de menos de 20 ppb para aflatoxinas totales, menos de 10 ppb de ocratoxinas totales, y menos de 5 ppm de fumonisinas totales, preferiblemente que tenga un contenido de ácido clorogénico de al menos 2% (p/p) y contenido de polifenol de al menos 3,2% (p/p).

Varios objetos, características, aspectos y ventajas de la presente invención serán más evidentes de la siguiente descripción detallada de las modalidades de la invención.

DESCRIPCIÓN DETALLADA

Los inventores han descubierto que se pueden producir infusiones y otros productos alimenticios con micotoxinas bajas o aún libres de micotoxinas a partir de cerezas de café completas, sustancialmente no dañadas que sean preferiblemente recogidas en etapa sub-madura, y en donde las cerezas de café sean secadas rápidamente después de la

cosecha. Entre otras ventajas, los inventores descubrieron que tales cerezas de café reducen significativamente la probabilidad de infestación de la cereza de café con moho y hongos conocidos por producir micotoxinas. Aún más, los inventores descubrieron que las cerezas de café sub-maduras (como contraste a las cerezas de café completamente no maduras) suministran un nivel deseable de sabor y perfil de aroma en los productos de cereza de café (por ejemplo infusión de cereza de café) así como también niveles relativamente altos de polifenoles, polisacáridos, y otros nutrientes.

El término "producto alimenticio" como se utiliza aquí se refiere a cualquier producto que sea ingerido por un humano y/o animal para propósito nutricional, de mantenimiento de la salud, de mejora de la salud, y/o recreacional. Los productos alimenticios particularmente preferidos incluyen aquellos consumidos por humanos, en donde tales productos alimenticios pueden ser productos sólidos (por ejemplo, suplemento de dieta, pasabocas, infusiones en bolsa, etc) o productos líquidos (por ejemplo, bebidas de infusión u otras, melasas o elixires, etc.).

Como también se utiliza aquí, el término "cereza de café" se refiere a la fruta del árbol de café (*Coffea spec.*, Familia

Rubiaceae) en la cual el exocarpio y el mesocarpio exterior (es decir la pulpa) circundan el mesocarpio interior (es decir el mucílago) y endocarpio (es decir, la cascarilla), que a su vez circunda las semillas (es decir, los granos). Así, el término cereza de café se refiere específicamente a la cereza de café completa, que puede estar o no incluida en el tallo de la cereza.

El término "cereza de café sub-madura" se refiere a una cereza de café que no ha alcanzado el estado maduro, que se caracteriza generalmente por susceptibilidad a o presencia de infección fungosa y/o presencia de micotoxinas. Así, una cereza de café sub-madura está en una etapa de maduración en la cual la cereza de café, cuando se seca rápidamente, exhibirá niveles de micotoxina que estén por debajo de 20 ppb para aflatoxinas totales, por debajo de 5 ppm para fumonisinas totales, por debajo de 5 ppm para vomitoxinas totales, y por debajo de 5 ppb para ocratoxinas. Consecuentemente, las cerezas de café de secado rápido son típicamente secadas dentro de 0-48 horas (y más preferiblemente entre 6-24 horas) o cosechadas de tal forma que el contenido de agua residual no es mayor del 20% (p/p), y más típicamente no mayor de 6-12% (p/p).

Vistas desde otra perspectiva, las cerezas de café sub-maduras tienen generalmente un color rojo completo o casi completo (al menos el 95% de la cereza) (o en algunos casos color amarillo), y típicamente incluyen varios defectos superficiales (por ejemplo, manchas, cortes, y/o huecos que cubren un área de más de 5% de la cereza). Así, la cereza de café sub-madura típicamente exhibirá al menos algún color verde (al menos 5%, más típicamente al menos 10%) y estará típicamente libre de cualquier defecto superficial (por ejemplo, manchas, cortes, y/o huecos que cubren un área de menos del 5% de la cereza). Las cerezas de café sub-maduras pueden también estar caracterizadas porque ellas permanecerán sobre el árbol del café durante una ronda subsecuente de la recogida cuando las cerezas de café son recogidas a mano y utilizadas para la producción de los granos de café. Alternativamente, una máquina separadora por color con equipo CCD puede ser empleada para identificar y seleccionar cerezas de café sub-maduras sobre una base de color cuantitativa donde las cerezas de café son cosechadas en masa y separadas automáticamente.

Se debe además apreciar que aunque muchos de los siguientes aspectos y ejemplos emplean cerezas de café en estado sub-maduro, las cerezas de café completamente maduras también son contempladas como adecuadas aquí, especialmente cuando tales

cerezas de café maduras están sustancialmente libres de daño superficial (es decir, no más del 5% del área de superficie) o de infección microbiana (es decir, infestación que da como resultado niveles de micotoxina de menos de 20 ppb de aflatoxinas totales, menos de 5 ppm para fumonisinas totales, menos de 5 ppm de vomitoxinas totales, y menos de 5 ppb de ocratoxinas sobre una base de peso seco). Así, todos los productos alimenticios contemplados y/o las cerezas de café pueden comprender cerezas de café completamente maduras así como también sub-maduras en proporciones varias. Por ejemplo, las proporciones adecuadas incluyen 100% de maduras: 0% sub-maduras, preferiblemente 90% maduras: 10% sub-maduras, más preferiblemente 75% maduras: 25% sub-maduras, aún más preferiblemente 50% maduras: 50% sub-maduras, y más preferiblemente menos del 25% maduras: más del 75% de sub-maduras.

Como adicionalmente se utiliza aquí, el término cereza de café de "secado rápido" significa que la cereza de café completa se seca bajo un protocolo que limita el crecimiento de mohos, hongos, y/o levaduras en una proporción tal que la cereza de café seca exhibirá niveles de micotoxina que están por debajo de 20 ppb de aflatoxinas totales, menos de 5 ppm de fumonisinas totales, menos de 5 ppm de vomitoxinas totales, y por debajo de 5 ppb de ocratoxinas.

Consecuentemente, las cerezas de café de secado rápido son típicamente secadas dentro de las 0-48 horas (y más preferiblemente entre 6-24 horas) de la cosecha de tal forma que el contenido de agua residual no es mayor del 20% (p/p), y más típicamente no mayor de 6-12% (p/p).

Adicionalmente, como se utiliza aquí, el término "micotoxina" se refiere a cualquier producto tóxico formado en un moho, hongo, y/o levadura que exhibe toxicidad significativa a un humano o animal cuando se ingiere. Así, las micotoxinas específicamente contempladas incluyen aflatoxinas (y particularmente B1, B2, G1, y G2), fumonisinas (y particularmente B1, B2 y B3), ocratoxinas, desoxinivalenol (DON, vomitoxina), T-2 toxina, y zearalenona. El término "aflatoxinas totales" por lo tanto se refiere a la suma de todas las variantes de aflatoxina, el término "fumonisinas totales" se refiere a la suma de todas las variantes de fumonisina, y el término "ocratoxinas totales" por lo tanto se refiere a la suma de todas las variantes de ocratoxina.

A manera de ejemplo de la materia objeto de la invención, las cerezas de café sub-maduras no dañadas (por ejemplo, semi-maduras o casi maduras) son recogidas a mano en aproximadamente una hora de secado rápido utilizando un secador de aire seco a aproximadamente 140° F hasta que se

obtiene un peso constante. Las cerezas de café así obtenidas típicamente poseen una estabilidad de almacenamiento significativa, alta resistencia a la infección por hongos y peso más bajo de embarque que la cereza húmeda.

Se contempla generalmente que las cerezas de café sub-maduras se pueden derivar de varias fuentes, y el uso particular de las cerezas de café sub-maduras determinará al menos en parte la o las fuentes particulares. Sin embargo, se prefiere que las cerezas de café sub-maduras se deriven de especies de café simples (por ejemplo, *coffea arabica*), que son cultivadas bajo condiciones de crecimiento similares (por ejemplo, crecen bajo sombra). Entre otras ventajas, se contempla que la fuente simple de cerezas de café facilitará el secado rápido de las cerezas de café sub-maduras. Se debe reconocer, sin embargo, que una vez que las cerezas de café sub-maduras sean secadas rápidamente, varias especies de café y/o cerezas de café de varias condiciones de crecimiento pueden ser mezcladas para lograr una mezcla con características particularmente preferidas.

Adicionalmente, se debe apreciar que dependiendo del producto particular o el uso para la cereza de café, el grado de madurez de la cereza de café puede variar considerablemente. Por ejemplo, cuando se desea la extracción de polifenoles y/o

ácido clorogénico de la cereza de café completa, se pueden utilizar cerezas de café semi-maduras (etapa 1 o etapa 2). De otra parte, cuando se utiliza la cereza de café para la producción de una infusión de cereza de café y el sabor y aroma son preeminentes, las cerezas de café casi maduras pueden ser recogidas. Aún en aspectos adicionales, las cerezas de café no maduras, o cualquier mezcla razonable de grados de madurez variante puede ser utilizada. Especialmente cuando la cereza de café es una cereza de café madura, se contempla que la cereza completa esté preferiblemente libre de defectos de superficie, que incluyen fisuras, rajaduras, hueco, u otras aberturas. Sin embargo, aunque no se prefiere, también se pueden utilizar las cerezas de café con defectos de superficie. Aunque no limita la materia objeto de la invención, se prefiere generalmente que las cerezas de café sub-maduras (o las mezclas de cereza de café) sean lavadas con agua u otra solución acuosa (por ejemplo, solución de hipoclorito diluida) para remover las partículas de suciedad u otros desechos antes del secado.

El secado rápido se debe desarrollar preferiblemente después de recoger, hasta aproximadamente dos días después de recogida hasta que se obtenga un peso constante (o hasta que la pulpa exterior de la cereza se halla secado). Así, y dependiendo de la fuente de calor particular disponible, se

prefiere generalmente que el secado rápido se desarrolle a una temperatura entre aproximadamente 100° a 180° F durante un periodo de 6-48 horas. Por ejemplo, cuando la energía eléctrica está fácilmente disponible (u otra), las cerezas de café sub-maduras pueden ser secadas en un secador de aire caliente, en un tambor estacionario o rotatorio, o en un proceso de secado de ventana de refractancia. Alternativamente, las cerezas de café sub-maduras también se pueden secar por congelamiento. De otra parte, y especialmente cuando las fuentes de energía no estén rápidamente disponibles, las cerezas de café sub-maduras pueden ser secadas al sol. Sin embargo, sin importar el método de secado particular, se debe reconocer que las cerezas de café sub-maduras son secadas rápidamente para evitar la producción de micotoxinas provenientes de hongos, mohos, y/o levaduras que ya están presentes y/o colonizar (por ejemplo, por vía de infección o esporulación) la cereza de café. Así, las cerezas de café sub-maduras son ventajosamente secadas sobre una superficie que esté limpia y libre de fuentes de contaminación de micotoxina. Como aspectos alternativos adicionales de la materia objeto de la invención, las cerezas de café sub-maduras también pueden ser congeladas y almacenadas/transportadas hasta que el secado rápido pueda ser implementado.

Las cerezas de café sub-maduras de secado rápido obtenidas pueden entonces ser empleadas sin destoxificación de micotoxina adicional para varios usos y numerosos productos alimenticios. Por ejemplo, cuando la cereza de café sub-madura secada rápidamente completa se utiliza en productos alimenticios, la cereza de café se puede mezclar con otros consumibles (por ejemplo, mezclada con grano para alimento animal, o recubrimiento con chocolate para consumo humano). En otro ejemplo especialmente preferido, la cereza de café sub-madura secada rápidamente es molida y utilizada como aditivo alimenticio o como una base para mezclar infusión de cereza de café (por ejemplo, para uso como infusión suelta, se prefiere molida a un tamaño de 500-3000 μm , o para infusión en bolsa, se prefiere molida a un tamaño de 200-1000 μm).

Alternativamente, se debe reconocer que solamente las partes de la cereza de café sub-madura secada rápidamente se pueden emplear en un producto alimenticio. Por ejemplo, cuando la cereza de café sub-madura está en un estado casi maduro, se contempla que la pulpa, mucílago, y/o cascarilla se pueda separar de las semillas, las cuales son entonces (opcionalmente mezcladas con otras semillas) tostadas para granos de café con grado comercial. El resto de la pulpa, mucílago, y/o cascarilla proveniente de la cereza de café

sub-madura secada rápidamente puede entonces ser empleada como aditivo alimenticio o base para la extracción de uno o más componentes deseados (por ejemplo, polifenoles).

Se contemplan aún adicionalmente los usos de cerezas de café sub-maduras secadas rápidamente así obtenidas, se contempla que las cerezas de café (o porciones de estas) se puedan emplear como material de partida para la extracción de varios componentes benéficos. Por ejemplo, las cerezas de café sub-maduras secadas rápidamente se pueden extraer con un solvente acuoso (por ejemplo agua, mezcla de agua-etanol) o solvente no acuoso (por ejemplo, CO₂ de punto crítico, dimetilformamida) para aislar uno o más componentes que puedan ser utilizados en un producto alimenticio. Por ejemplo, las cerezas de café sub-maduras de secado rápido pueden suministrar una fuente excelente de polifenoles, ácido clorogénico, y/o cafeína.

El término "polifenoles" como se utiliza aquí se refiere a un grupo diverso de compuestos producidos por una planta, en donde los compuestos incluyen un anillo de fenol en el cual al menos un grupo OH, y más típicamente al menos dos grupos OH están covalentemente unidos. Por ejemplo, los fenoles representativos incluyen ácido elágico, ácido tánico, vanilina, ácido cafeico, ácido clorogénico, ácido ferúlico,

catequinas (por ejemplo, epicatequina galato, epigalocatequina), flavonoles (por ejemplo, antocianidinas, quercetina, campferol), y diversos otros flavonoides, y sus glicósidos y despidos. Más aún, los polifenoles contemplados pueden también estar en forma oligomérica o polimérica (por ejemplo proantocianidinas oligoméricas o taninos condensados).

En otro aspecto preferido de la materia objeto de la invención, los inventores contemplan el uso de cerezas de café submaduras secadas rápidamente en la producción de diversas bebidas. Por ejemplo, se observó que los té producidos en el estado 1 no maduro (verde) y semimaduro de las cerezas de café secadas rápido poseen relativamente un bajo aroma y bajas características de sabor. Así, los extractos o té por lo menos parcialmente condensados de cerezas de café secadas completamente rápido en la etapa 1 no madura (verde) y semimadura pueden agregar tan poca adición de sabor a una bebida comercialmente disponible para promover las propiedades nutricionales.

A medida que la madurez se incrementa, más aroma y sabor es evidente en las cerezas de café submaduras secadas rápidamente. Consecuentemente, el té producido de las cerezas de café casi completamente maduras en la etapa 2 semimaduro

poseen un alto aroma y un sabor agradable, y los inventores contemplan que las cerezas de café casi maduras y semimaduras podrían ser usadas directamente después de un secado rápido y un molido para un "té de frutas de café completas", por si mismo o después de colocarlo en bolsas de té. Como se ilustra más adelante, tal té suministraría altos niveles de polifenoles típicamente 60-70 mg por copa de 6 onzas. Más aún, los té preparados a partir de cerezas de café submaduro secado rápidamente generalmente poseen proporciones relativamente altas de ácido clorogénico con respecto a cafeína (típicamente 2-4,5) en comparación con las bebidas hechas de café tostado (típicamente cerca de 0,4). Por lo tanto, debería apreciarse que el té preparado a partir de cerezas de café submaduras secadas rápidamente es mucho más nutricional (con base en los polifenoles y el ácido clorogénico) del café tostado.

Por lo tanto, los inventores contemplan un producto alimenticio que incluye una preparación de una cereza de café (preferiblemente submadura) que es secada rápidamente de modo que un nivel de micotoxina de la cereza de café sea menor de 20 ppb para el total de aflatoxinas, menos de 10 ppb para el total de ocratoxinas, y menos de 5 ppm para el total de fumonisinas. Alternativamente, los niveles de micotoxinas contemplados también pueden estar en el rango entre 20 a 50

ppb, pero más preferiblemente menos de 15 ppb, y aún mucho más preferible menos de 10 ppb, y mucho más preferible menos de 5 ppb para el total de aflatoxinas. Similarmente, los niveles de micotoxinas contemplados también incluyen un rango entre 10 a 30 ppb, pero más preferiblemente menos 5 ppb, aún más preferiblemente menos de 3 ppb, y aún más preferiblemente menos de 2 ppb para el total de ocratoxinas. Similarmente, los niveles de micotoxinas contemplados también incluyen un rango entre 5 a 20 ppm, pero más preferiblemente menos 15 ppb, aún más preferiblemente menos de 10 ppm, y mucho más preferiblemente menos de 5 ppm para el total de fumonisinas y/o vomitoxinas.

Como ya se discutió, la preparación de la cereza de café submadura pueden incluir la cereza de café completa, comprende un fragmento molido de la cereza de café completa, o incluye el grano, la pulpa, el mucílago, y/o la cascara de la cereza de café submadura secada rápidamente. Alternativamente, se puede reconocer que la preparación también comprende un extracto de la cereza de café submadura secada rápidamente (o fragmento o porción de ella).

Los productos alimenticios contemplados especialmente incluyen bebidas preparadas a partir de la cerezas de café submaduras de secado rápido contempladas (o fragmentos o

porciones de ella), o bebidas a las cuales los extractos o piezas de las cerezas de café submaduro de secado rápido contempladas (o fragmentos o porciones de ella) han sido agregadas. Similarmente, los productos alimenticios contemplados adicionalmente incluyen bienes orneados (por ejemplo, pan, galletas, etc.), pasabocas (por ejemplo dulces o barras de energía) cereales, y otros nutrientes sólidos a los cuales los extractos o piezas de las cerezas de café submaduro de secado rápido contempladas (o fragmentos o porciones de ellas) se han agregado. Alternativamente, los productos alimenticios contemplados también incluyen un suplemento nutricional en forma líquida o sólida que comprende un extracto de cereza de café submadura secada rápidamente.

Dependiendo del propósito particular, debería reconocerse que tales productos alimenticios pueden ser preparados a partir de cerezas de café submaduros secadas rápidamente que tienen un color principal verde con menos de 25% de color rojo, más preferiblemente con menos de 25% de color verde y más preferiblemente a partir de cerezas de café submaduras secadas rápidamente que tienen principalmente (no menos de 90%, mucho más típicamente no menos de 95%) de color rojo con menos de 5% de área manchada. Adicionalmente, las composiciones contempladas y los métodos contemplados que se

describen en nuestra solicitud de Patente Estadounidense copediente con el titulo "Métodos para Productos de Cereza de Café", presentada el 16 de Abril, 2003, la cual se incorpora aquí como referencia.

Ejemplos

Los siguientes ejemplos son suministrados para permitir que una persona versada en la técnica haga y use las composiciones de acuerdo con la materia objeto de la invención y para ilustrar como ejemplos composiciones y métodos generalmente descritos aquí.

Cosecha de Cerezas de Café Completas

La madurez de las cerezas de café fue determinada por medio de estimar visualmente la cantidad de color verde y rojo (o amarillo, en donde sea aplicable) de las cerezas completas. A medida que las cerezas maduran, las cerezas verde típicamente incrementan en tamaño y subsecuentemente desarrollan cantidades incrementales de color rojo. Para los ejemplos presentes, las cerezas de café fueron recolectadas en cuatro etapas de madurez:

Completamente, o casi completamente verde (no maduras; típicamente menos de 5% de rojo o amarillo en la cereza de café), principalmente verde con algún rojo (semimadura, etapa 1; típicamente menos de 25% de rojo o amarillo en la cereza de café), principalmente roja con algún verde (semimadura, etapa 2; típicamente menos de 25% de verde en la cereza de café), y no rota, roja sin manchar (casi madura; típicamente menos de 10% de verde en la cereza de café; las áreas de manchado, corte, o de otra manera superficies rotas en menos de 5%). Tanto como sea posible, las cerezas completas, no rotas y no cortadas fueron recolectadas.

Secado Rápido de las Cereza de Café Completas

Las cerezas de café completas para la extracción de la muestra fueron preparadas por medio de secar las cerezas en un periodo de 1 a 12 horas después de ser cosechadas en bandejas separadas o en un secador de aire de acuerdo con el siguiente procedimiento. Las cerezas de café (400 a 600 g) fueron pesadas en recipientes de boca anchas y fueron lavadas dos veces con agua corriente, seguido por un lavado único con agua destilada. Las cerezas de café así lavadas fueron colocadas en una bandeja de un secador de aire para drenarlas, y luego se secaron a 150-160° F durante 16 a 18 horas a peso constante. El secado se detuvo cuando el peso en

dos intervalos consecutivos de una hora difería en menos de 1 g. La producción típica de cereza completa secada fue de 160-220 g.

Un análisis adicional indicó un 6 a 12% de contenido de agua residual en las cerezas secadas.

Análisis de Micotoxinas

Para poder determinar la viabilidad de la cereza de café completa en estados: no maduro, semimaduro, y casi maduro (ver antes) para el uso en un producto nutricional (y especialmente para el uso en té), el nivel de micotoxinas seleccionado fue medido y comparado contra productos comparativos y productos intermedios de cereza de café madura y roja de producción de café. Como se puede ver claramente en la tabla 1 enseguida, las cerezas de café secadas rápidamente de todas las etapas cosechadas submaduras tenían un nivel de micotoxina inferior al límite de detección de un 1 ppb (como se midió para la aflatoxina y la ocratoxina)

La concentración de micotoxina fue determinada en un laboratorio independiente tanto por análisis ELISA y HPLC. Con base en los resultados que se muestran adelante, los inventores concluyeron que todas las muestras de las diferentes etapas de cosechas submadura son adecuadas para el

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uso directo en un producto nutricional tanto para consumo humano como veterinario. En contraste, los productos intermedios de la producción de café (predominantemente, consistentes de pulpa, mucílago, y cáscaras de las cerezas de café) a partir de cerezas maduras de color rojo con manchas (típicamente mayor a 20% de la superficie de la cereza) tenían un contenido sustancial tanto en aflatoxinas como ocratoxinas. Similarmente, el producto comparativo "Té para Ir al Paraíso" (hecho de pulpa de cereza de café) exhibió micotoxinas en concentraciones de dos dígitos.

Tabla 1

MADUREZ	COLOR	AFLATOXINA	OCRATOXINA
Secada rápidamente no madura	Verde	< 1 ppb	< 1 ppb
Secada rápidamente, Etapa 1 Semimadura	La mayor parte verde con algún rojo	< 1 ppb	< 1 ppb
Secada rápidamente, Etapa 2 semimadura	La mayor parte roja con algún verde	< 1 ppb	< 1 ppb
Casi madura secada rápidamente	Roja, con áreas manchadas <5%	< 1 ppb	< 1 ppb
Madura (productos intermedios de producción de café)	Roja, área manchada >20%	> 200 ppb	> 500 ppb
Te para Ir al Paraíso (materia seca)	N/A	> 25 ppb	> 40 ppb

Análisis de Polifenol (PP), Ácido Clorogénico (CG), y Cafeína (CF) para Cerezas de Café Completas Submaduras Secadas Rápidamente

En una serie adicional de experimentos, los niveles de polifenoles, ácido clorogénico, y cafeína totales de la cereza de café completa secada rápidamente en diversas etapas submaduras fueron medidas y comparadas contra granos de café verdes y tostados. La Tabla 2 resume los resultados de este análisis.

De manera muy interesante, mientras el nivel de polifenol (PP) de las cerezas de café secadas rápidamente en todas las etapas de cosecha submaduras era algo menos que el nivel en los granos verdes o tostados de café, cantidades significantes de polifenoles en las cerezas de café submaduras secadas rápidamente todavía permanece. Similarmente, el contenido de ácido clorogénico (CG) de la cereza de café completa secada rápidamente en diversas etapas de submadurez permaneció sustancialmente en altos niveles en comparación con el café tostado, pero fue algo menor en comparación con granos verdes. El nivel de cafeína (CF) de las cerezas de café secadas rápidamente en todas las etapas de cosecha submadura estuvo sustancialmente dentro del nivel de cafeína de los granos de café verde y tostado (debe

anotarse que todos los datos dados se toman sobre la base de materia seca y no son normalizados en cuanto a peso seco del grano).

Análisis de de polifenol: la cereza de café completa secada (o granos verdes o granos tostados) (1,00 g) fueron molidos en un molino de café de cuchillas de acero giratorias durante 30 segundos para producir una muestras molida. La muestra molida fue agregada a 100 ml de agua destilada y la mezcla resultante se calentó hasta la ebullición en un frasco Erlenmeyer durante 30 minutos. Se retiró el calor y la mezcla se le permitió enfriarse a temperatura ambiente. La suspensión resultante fue transferida a un cilindro graduado de 100 ml y se agregó agua hasta llenar un volumen de 100 mL. La mezcla fue entonces transferida de nuevo al frasco Erlenmeyer, agitada brevemente y se permitió que los sólidos se asentaran. Una alícuota (aproximadamente 3 ml) de la solución supernadante fue filtrada a través de un filtro Acrodisc de 0,45 μm y la solución clara resultante fue diluida 1:10 con agua destilada usando un frasco volumétrico (1,00 mL diluido con 9,00 mL de agua destilada).

El método Folin-Ciocalteu fue usado para medir el contenido de polifenol de la solución diluida como sigue. Un mL de la solución diluida fue agregado a un tubo de ensayo, mezclado

con 1 mL de reactivo de fenol al 0,2N de Folin-Ciocalteu (solución Sigma, 2N, diluido 1:10 con agua) y se le permitió permanecer 5 minutos a temperatura ambiente. Un mL de NaHCO_3 al 1N se agregó y la mezcla de reacción se dejó a temperatura ambiente durante 2 horas. El nivel de polifenol fue determinado usando un espectrofotómetro de UV visible estandarizado contra catecol, a un $\lambda_{\text{max}}=750$ nm contra agua destilada como blanco.

Ácido clorogénico: la determinación del ácido clorogénico fue hecha usando separación HPLC de la solución clara filtrada preparada anteriormente usando protocolos analíticos y de separación estándar bien conocidas en la técnica. Similarmente, la determinación de cafeína fue hecha usando separación HPLC de la solución clara filtrada preparada anteriormente usando protocolos analíticos y de separación estándar bien conocidos en la técnica (por ejemplo) para protocolos ilustrativos ver por ejemplo, Bispo M.S., et al en J. Chromatogr. Sci.; 2002, enero; 40(1):45-8, o Nakakuki, H. et al en J. Chromatogr. A.; 1999, Jul 2;848(1-2):523-7).

Tabla 2

MADUREZ	COLOR	% PP	%CG	%CF	CG/CF
No madura, secado rápido	Verde	3,80	2,64	1,03	2,56
Etapa 1 semimadura, secado rápido	La mayor parte verde con algún rojo	3,28	2,70	1,00	2,70
Etapa 2 semimadura, secado rápido	la mayor parte roja con algún verde	3,54	2,00	0,70	2,86
Casi madura, secado rápido	Roja, con áreas manchadas <5%	3,35	N/D	N/D	N/D
Granos de café verde	Verde	4,58	3,31	0,95	3,48
Granos de café tostado	Café	3,93	0,50	1,20	0,42

Análisis de Polifenol (PP), Ácido Clorogénico (CG), y Cafeína (CF) para un Té Fabricado a partir de Cerezas de Café Completas Submaduras Secadas Rápidamente

La cereza de café submadura secada rápidamente fueron molidas en un molino para café de cuchillas de acero giratorias durante 10 a 30 segundos para producir una muestra molida. A la muestra molida (1,00 g) se agregaron 90 ml (3 onzas fluidas aproximadamente) de agua destilada hirviendo y la mezcla resultante se le permitió decantarse en un frasco Erlenmeyer durante 10 minutos para producir un té de cereza de café una alícuota (aproximadamente 3 mL) de la solución

supernadante fue filtrada a través de un filtro Acrodisc de 0,45 μ m y la solución clara resultante fue diluida 1:10 con agua destilada usando un frasco volumétrico (1,00 mL diluido con 9,00 mL de agua destilada). El método Folin Ciocalteu como se describió anteriormente fue usado para medir el contenido de polifenol (sobre base de materia seca, equivalentes de catecol) del té de cereza de café así preparado. La Tabla 3 resume los resultados.

Tabla 3

MADUREZ	DISOLVENTE	% PP	%CG	%CF	CG/CF
No madura, secado rápido	Agua	10,93	8,61	3,04	2,83
Etapa 1 semimadura, secado rápido	Agua	9,38	7,58	2,72	2,78
Etapa 2 semimadura, secado rápido	Agua	8,51	6,74	1,71	3,95
Casi madura, secado rápido	Agua	6,92	1,34	0,29	4,61

Fabricación de té a Partir de Cereza de Café Completa de Diferente Maduración

El siguiente procedimiento fue usado para preparar té a partir de cerezas de café completas de diferente maduración para ensayos de aroma y gusto. La cereza de café secada completa (10-20 g) fue molida en un molino para café de

cuchillas de acero giratorias durante 10 a 30 segundos para producir una muestra molida. Las cerezas de café molida (2,0 g) fueron colocadas en una copa de cerámica y se agregó agua cercana a ebullición (6 onzas, 190-200° F) la suspensión fue agitada y se le permitió decantarse durante 3 minutos, después de cuyo tiempo el aroma y el gusto del líquido supernadante se notaron. Los resultados son como se muestran en la Tabla 4.

Tabla 4

MADUREZ	COLOR	AROMA	GUSTO	TÉ PP/6 ONZAS
No madura, secado rápido	Verde	Ninguno	Casi no hay sabor	76 mg
Etapas 1 semimadura, secado rápido	La mayor parte verde con algún rojo	Muy suave olor a fruta	Ligero sabor a fruta	66 mg
Etapas 2 semimadura, secado rápido	La mayor parte rojo con algún verde	Suave olor a fruta	Sabor a fruta suave	71 mg
Casi madura, secado rápido	Roja, con áreas manchadas <5%	Fruta	Rico sabor a fruta	67 mg

Así, las modalidades y aplicaciones específicas de los productos de cereza de café bajos en micotoxinas han sido descritas. Debe ser evidente, sin embargo, para aquellos versados en la técnica que muchas más modificaciones aparte de aquella ya descritas son posibles sin salirse del concepto

inventivo dado aquí. La materia objeto de la invención, por lo tanto, no debe estar restringida excepto en el espíritu de las reivindicaciones adjuntas. Más aún, interpretando tanto la especificación como las reivindicaciones, todos los términos deben ser interpretados de la manera más amplia posible en consistencia con el contexto. En particular, los términos "comprenden" y "que comprende" deben ser interpretados en cuanto a que se refieren a elementos, componentes, o etapas de una manera no exclusiva, indicando que los elementos referenciados, componentes, o etapas pueden estar presentes, o ser utilizados, o combinados con otros elementos, componentes, o etapas que no son referenciados de manera expresa.

REIVINDICACIONES

Lo que se reivindica es:

1. Un producto alimenticio que comprende una preparación de una cereza de café que es secada de manera rápida tal que un nivel de micotoxina de la cereza de café es menor a 20 ppb para el total de aflatoxinas, menos de 10 ppb para el total de ocratoxinas, y menos de 5 ppm para el total de fumonisinas.
2. El producto alimenticio de la reivindicación 1 en donde la preparación de la cereza de café comprende un fragmento molido de la cereza de café.
3. El producto alimenticio de la reivindicación 2 en donde la preparación de la cereza de café comprende un extracto a partir de un fragmento molido de la cereza de café.
4. El producto alimenticio de la reivindicación 1 en donde la preparación de la cereza de café comprende por lo menos uno entre un grano de la cereza de café, una pulpa de la cereza de café, un mucílago de la cereza de café y una cáscara de la cereza de café

5. El producto alimenticio de la reivindicación 1 en donde la preparación de la cereza de café comprende un extracto de por lo menos uno entre un grano de la cereza de café, una pulpa de la cereza de café, un mucílago de la cereza de café y un cáscara de la cereza de café.
6. El producto alimenticio de la reivindicación 1 en donde la cereza de café es una cereza de café submadurada y tienen principalmente un color verde con menos de 25% de color rojo.
7. El producto alimenticio de la reivindicación 1 en donde la cereza de café es una cereza de café submadurada y tienen principalmente un color rojo con menos de 25% de color verde.
8. El producto alimenticio de la reivindicación 1 en donde la cereza de café es una cereza de café submadurada y tienen principalmente color rojo con menos de 5% de áreas manchadas.
9. El producto alimenticio de la reivindicación 1 en donde la cereza de café es secada de manera rápida en un secador usando aire caliente.

10. El producto alimenticio de la reivindicación 1 en donde la cereza de café es secada de manera rápida en un secador usando radiación solar.
11. El producto alimenticio de la reivindicación 1 en donde la cereza de café es secada rápidamente por medio de exponer la cereza de café a por lo menos uno entre aire ambiente y luz solar.
12. El producto alimenticio de la reivindicación 1 en donde el producto alimenticio es un té manufacturado a partir de cerezas de café.
13. El producto alimenticio de la reivindicación 1 en donde el producto alimenticio es una bebida que comprende un extracto de cereza de café.
14. El producto alimenticio de la reivindicación 1 en donde el producto alimenticio es un suplemento nutricional en forma sólida o líquida y comprende un extracto de cereza de café.
15. Un té manufacturado a partir de una cereza de café secada de manera rápida molida con un porción de ella.

16. El té de la reivindicación 15 en donde la cereza de café tienen un nivel de micotoxina menor de 20 ppb para el total de aflatoxina menor de 10 ppb para el total de ocratoxinas y menor de 5 ppm para el total de fumonisinas.
17. El té de la reivindicación 16 que tienen una concentración de polifenol de por lo menos 10 mg/onza.
18. El té de la reivindicación 15 en donde la cereza de café es una cereza de café submadurada.
19. Una cereza de café secada de manera rápida o porción de ella que tiene un nivel de micotoxinas de menos de 20 ppb para el total de aflatoxinas, menos de 10 ppb para el total de ocratoxinas, y menos de 5 ppm para el total de fumonisina.
20. La cereza de café secada de manera rápida de la reivindicación 19 en donde la cereza de café es una cereza de café submadurada.

RESUMEN

Una cereza de café es cosechada, preferiblemente en un estado submadurado, y es secada rápidamente para suministrar una base para numerosos productos nutricionales. Tales cerezas de café o porciones de ellas pueden ser caracterizadas particularmente por su concentración extremadamente baja de micotoxinas, incluyendo varias aflotoxinas, fumonisinas, ocratoxinas, y/o vomitoxinas (DON, deoxinivalenol).

LOW-MYCOTOXIN COFFEE CHERRY PRODUCTS

Field of The Invention

The field of the invention is food products, and especially food products prepared from whole quick-dried sub-ripe coffee cherries, or fragments/portions thereof.

5 Background of The Invention

Various parts of the coffee tree have been used for nutritional purposes for a relatively long time (see *e.g.*, Pendergrast, M. *Uncommon Grounds*. Basic Books: New York, 1999). For example, coffee tree leaves and fresh, ripe coffee cherries were boiled to make tea. In other examples, the pulp of the coffee cherry can be fermented to produce wine as described in
10 Chinese Patent CN 1021949. In a still further well known example, the seeds (*i.e.*, the beans) of the coffee tree are extracted from the cherry, dried, roasted, ground, and extracted with hot water to provide the beverage that many users enjoy as coffee.

Unfortunately, coffee cherries, and especially the pulp and husk tend to rapidly spoil in the presence of molds, fungi, and other microorganisms, and therefore contain almost always
15 significant levels of mycotoxins (see *e.g.*, Pittet, A., Tornare, D., Huggett, A., Viani, R. Liquid Chromatographic Determination of Ochratoxin A in Pure and Adulterated Soluble Coffee Using an Immunoaffinity Column Cleanup Procedure. *J. Agric. Food Chem.* 1996, 44, 3564-3569; or Bucheli, P., Kanchanomai, C., Meyer I., Pittet, A. Development of Ochratoxin A during Robusta (*Coffea canephora*) Coffee Cherry Drying. *J. Agric. Food Chem.* 2000, 48, 1358-1362). Thus,
20 beverages produced from the coffee pulp, husk, mucilage, and/or whole coffee cherry generally failed to find acceptance as beverage ingredients (Although one product is advertised as "coffee cherry tea" [<http://www.paradiserelocation.com/paradisetogo/foodproducts.htm>], the product is actually made from coffee cherry pulp and was recently determined to have substantial quantities of mycotoxins).

25 Even in situations where the pulp, mucilage, and hull is removed, mycotoxins may still be present on and/or in the coffee bean. Consequently, considerable efforts have been undertaken to detoxify coffee beans and other food products. For example, where the mycotoxin is already present in the food product, selected mycotoxins can be extracted from the food product using

various solvents and procedures as described in U.S. Pat. No. 4,436,756 to Canella et al. On the other hand, various mycotoxins can be adsorbed from the food product onto a mineral carrier as described in U.S. Pat. No. 5,935,623 to Alonso-Debolt.

In still other methods, selected mycotoxins can be degraded using enzymes as described in U.S. Pat. No. 5,716,820 to Duvick et al. The inventors in the '820 reference even contemplate that the genes encoding for such enzymes may be cloned to produce transgenic plants that are then thought to be less contaminated with mycotoxins. Alternatively, microorganisms may be employed to destroy enzymatically mycotoxins found in food products as described in U.S. Pat. No. 6,025,188 to Duvick et al.

Where mycotoxins are not yet produced by a microorganism present on a plant or other food stuff, pesticides or other compositions that control microbial growth or production of mycotoxins in microorganisms may be employed. For example, Emerson et al. describe in U.S. Pat. No. 5,639,794 use of a saponin as a synergist to control colonization and/or growth of plant and animal pathogens. Alternatively, as described in U.S. Pat. No. 4,199,606 to Bland, propionic acid on a carrier may be employed as a diffusible growth inhibitor for various microorganisms. Further known compositions (see e.g., U.S. Pat. No. 5,698,599 to Subbiah or U.S. Pat. No. 3,798,323 to Leary) may be employed to suppress or at least reduce synthesis of mycotoxins in a microorganism.

Alternatively, mycotoxin-containing food products may be blended with uncontaminated food products to a concentration that is acceptable and/or below the maximum allowable amount of mycotoxins in food products (see e.g., Herrman, T. and Trigo-Stockli, D.; Mycotoxins in Feed Grains and Ingredients; Kansas State University, May 2002), or (at least potentially) mycotoxin-containing coffee cherry products may be employed in a non-food product. In still other uses, the mycotoxin content may not be considered relevant as the coffee cherry product is incinerated and thus the mycotoxins are at least partially destroyed as described in U.S. Pat. No. 4,165,752, GB 2026839, or CA 1104410. Here, the inventor teaches that the coffee cherries may be compressed, dehydrated, ground, and roasted to yield a smokable product.

However, while most of the known methods reduce the concentration of mycotoxins to at least some degree, numerous disadvantages remain. Among other things, additional processing

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steps will require dedicated equipment, thereby increasing processing time and costs. Moreover, and especially where pesticides and/or fungicides are used, new problems with residual toxic chemicals may arise.

Thus, despite numerous beneficial properties of coffee cherries and its components, whole
5 coffee cherries are generally not used as food products as mycotoxins are typically present in substantial quantities in the ripe and overripe fruit. Therefore, there is still a need to provide improved methods and compositions for coffee cherries, and especially for products comprising coffee cherries with low or no mycotoxin content for human and veterinary consumption.

Summary of the Invention

10 The present invention is directed to compositions and methods that include quick-dried (preferably sub-ripe) coffee cherries or portions thereof, wherein the coffee cherries are substantially devoid of, or have a very low content of mycotoxins.

In one aspect of the inventive subject matter, a food product comprises a preparation of a coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20
15 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins. Preferred preparations in such food products include the bean, pulp, mucilage, and/or hull of the quick-dried coffee cherry, or ground fragments of the coffee cherry, or an extract thereof. It is further preferred that the coffee cherry is a sub-ripe coffee cherry. Preferred food products include a tea brewed from the quick-dried (preferably sub-ripe) coffee cherries, or
20 a beverage comprising an extract of the coffee cherry. Alternatively, suitable food products also include nutritional supplements in liquid or solid form comprising an extract of the coffee cherry.

Contemplated sub-ripe coffee cherries have a primarily green color with less than 25% red color, more preferably a primarily red color with less than 25% green color, and even more preferably a primarily red color with less than 5% blemished area. The (sub-ripe) coffee cherries
25 may be quick-dried using various methods, however, it is generally preferred that the coffee cherries are quick dried using heated air or exposure to sun and/or ambient air.

In another aspect of the inventive subject matter, a tea is brewed from a comminuted or ground quick-dried (preferably sub-ripe) coffee cherry or portion thereof, wherein the coffee

cherry has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins, and preferably has a polyphenol concentration of at least 10 mg/oz (most preferably at a chlorogenic acid to caffeine ratio of at least 2.7).

5 Thus, viewed from another perspective, it is contemplated that a quick-dried coffee cherry or portion thereof has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins, preferably having a chlorogenic acid content of at least 2% (wt/wt) and a polyphenol content of at least 3.2% (wt/wt).

10 Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention.

Detailed Description

15 The inventors have discovered that low-mycotoxin or even mycotoxin-free tea and other food products may be produced from whole, substantially non-damaged coffee cherries that are preferably picked at a sub-ripe stage, and wherein the coffee cherry is quick-dried after harvest. Among other advantages, the inventors discovered that such coffee cherries significantly reduce the likelihood of infestation of the coffee cherry with mold and fungi known to produce mycotoxins. Still further, the inventors discovered that sub-ripe coffee cherries (in contrast to completely unripe coffee cherries) provide a desirable level of flavor and an aroma profile in the coffee cherry product (e.g., coffee cherry tea) as well as relatively high levels of polyphenols, polysaccharides, and other nutrients.

20 The term "food product" as used herein refers to any product that is ingested by a human and/or animal for nutritional, health-maintenance, health-improvement, and/or recreational purpose. Particularly preferred food products include those consumed by human, wherein such food products may be solid products (e.g., dietary supplement, snack bar, bagged tea, etc.) or liquid products (e.g., tea or other beverage, syrup or elixir, etc.).

 As also used herein, the term "coffee cherry" refers to the fruit of the coffee tree (*Coffea spec.*, Family *Rubiaceae*) in which exocarp and outer mesocarp (i.e., the pulp) surround the inner

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mesocarp (*i.e.* the mucilage) and endocarp (*i.e.*, the hull), which in turn surround the seeds (*i.e.*, the beans). Thus, the term coffee cherry specifically refers to a whole coffee cherry, which may or may not include the stem of the cherry.

The term "sub-ripe coffee cherry" refers to a coffee cherry that has not yet reached the ripe stage, which is generally characterized by susceptibility to or presence of a fungal infection and/or presence of mycotoxins. Thus, a sub-ripe coffee cherry is at a ripeness stage in which the coffee cherry - when quick-dried - will exhibit mycotoxin levels that are below 20 ppb for total aflatoxins, below 5 ppm for total fumonisins, below 5 ppm for total vomitoxins, and below 5 ppb for ochratoxins. Consequently, quick-dried coffee cherries are typically dried within 0-48 hours (and more preferably between 6-24 hours) of the harvest such that the residual water content is no higher than 20% (wt/wt), and more typically no higher than 6-12% (wt/wt).

Viewed from an other perspective, sub-ripe coffee cherries generally have a complete or almost complete (at least 95% of the cherry) red color (or in some cases yellow color), and typically include various surface defects (*e.g.*, blemishes, cuts, and/or holes covering an area of more than 5% of the cherry). Thus, a sub-ripe coffee cherry will typically exhibit at least some green color (at least 5%, more typically at least 10%) and will typically be free of any surface defects (*e.g.*, blemishes, cuts, and/or holes covering an area of less than 5% of the cherry). Sub-ripe coffee cherries may also be characterized in that they will remain on the coffee tree for a subsequent round of picking where the coffee cherries are hand picked and used for the production of coffee beans. Alternatively, a color sorting machine with CCD equipment may be employed to identify and select sub-ripe coffee cherries on a quantitative color basis where the coffee cherries are mass-harvested and automatically sorted.

It should further be appreciated that while many of the following aspects and examples employ coffee cherries in a sub-ripe state, completely ripe coffee cherries are also contemplated suitable herein, especially where such ripe coffee cherries are substantially devoid of surface damage (*i.e.*, no more than 5% of surface area) or microbial infection (*i.e.*, infestation that results in mycotoxin levels of less than 20 ppb for total aflatoxins, less than 5 ppm for total fumonisins, less than 5 ppm for total vomitoxins, and less than 5 ppb for ochratoxins on a dry weight basis). Thus, all contemplated food products and/or coffee cherries may comprise completely ripe as

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well as sub-ripe coffee cherries in varying proportions. For example, suitable proportions include 100% ripe : 0% sub-ripe, preferably 90% ripe : 10% sub-ripe, more preferably 75% ripe : 25% sub-ripe, even more preferably 50% ripe : 50% sub-ripe, and most preferably less than 25% ripe : more than 75% sub-ripe.

5 As further used herein, the term "quick-dried" coffee cherry means that the whole coffee cherry is dried under a protocol that limits growth of molds, fungi, and/or yeast to an extent such that the dried coffee cherry will exhibit mycotoxin levels that are below 20 ppb for total aflatoxins, below 5 ppm for total fumonisins, below 5 ppm for total vomitoxins, and below 5 ppb for ochratoxins. Consequently, quick-dried coffee cherries are typically dried within 0-48 hours
10 (and more preferably between 6-24 hours) of the harvest such that the residual water content is no higher than 20% (wt/wt), and more typically no higher than 6-12% (wt/wt).

As still further used herein, the term "mycotoxin" refers to any toxic product formed in a mold, fungus, and/or yeast that exhibits significant toxicity to a human or animal when ingested. Thus, specifically contemplated mycotoxins include aflatoxins (and particularly B1, B2, G1, and
15 G2), fumonisins (and particularly B1, B2, and B3), ochratoxin, deoxynivalenol (DON, vomitoxin), T-2 toxin, and zearalenone. The term "total aflatoxins" therefore refers to the sum of all aflatoxin variants, the term "total fumonisins" refers to the sum of all fumonisin variants, and the term "total ochratoxins" therefore refers to the sum of all ochratoxin variants.

In one exemplary aspect of the inventive subject matter, whole undamaged sub-ripe (e.g.,
20 semi ripe or almost ripe) coffee cherries are hand picked and within about one hour quick-dried using a dry air drier at about 140°F until constant weight is obtained. The so obtained coffee cherries typically possess significant storage stability, high resistance to infection by fungi, and lower shipping weight than wet cherry.

It is generally contemplated that the sub-ripe coffee cherries may be derived from various
25 sources, and the particular use of the sub-ripe coffee cherries will at least in part determine the particular source(s). However, it is preferred that the sub-ripe coffee cherries are derived from a single coffee species (e.g., *coffea arabica*), which is cultivated under similar growth conditions (e.g., shade-grown). Among other advantages, it is contemplated that a single source of coffee cherries will facilitate quick-drying the sub-ripe coffee cherries. It should be recognized,

however, that once the sub-ripe coffee cherries are quick-dried, various coffee species and/or coffee cherries from various growth conditions may be blended to achieve a mixture with particularly preferred characteristics.

Furthermore, it should be appreciated that depending on the particular product or use for the coffee cherry, the degree of ripeness of the coffee cherry may vary considerably. For example, where extraction of polyphenols and/or chlorogenic acid from the whole coffee cherry is desired, semi-ripe (stage 1 or stage 2) coffee cherries may be used. On the other hand, where the coffee cherry is used for the production of a coffee cherry tea and flavor and aroma are paramount, almost ripe coffee cherries may be picked. In still further contemplated aspects, unripe coffee cherries, or any reasonable mixtures of varying ripeness degrees may be used. Especially where the coffee cherry is a ripe coffee cherry, it is contemplated that the whole cherry is preferably free of surface defects, including cracks, splits, holes, or other openings. However, while not preferred, coffee cherries with surface defects may also be used. While not limiting to the inventive subject matter, it is generally preferred that the sub-ripe coffee cherries (or coffee cherry mixtures) are washed with water or other aqueous solution (e.g., diluted hypochlorite solution) to remove soil particles and other debris before drying.

Quick-drying is preferably performed immediately after picking to up to about two days after picking until a constant weight is obtained (or until the outer pulp of the cherry has dried). Thus, and depending on the particular heat source available, it is generally preferred that quick-drying is performed at a temperature of about 100° to about 180°F for a period of about 6-48 hours. For example, where electrical (or other) energy is readily available, the sub-ripe coffee cherries may be dried in a warm air drier in a stationary or rotating drum, or in a refractance window drying process. Alternatively, the sub-ripe coffee cherries may also be freeze dried. On the other hand, and especially where energy sources are not readily available, the sub-ripe coffee cherries may be sun dried. However, regardless of the particular drying method, it should be recognized that the sub-ripe coffee cherries are quick-dried to prevent production of mycotoxins from fungi, molds, and/or yeast that are already present and/or colonize (e.g., via infection or sporulation) the coffee cherry. Thus, sub-ripe coffee cherries are advantageously dried on a surface that is clean and free of sources of mycotoxin contamination. In further alternative

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aspects of the inventive subject matter, the sub-ripe coffee cherries may also be frozen and stored/transported until quick-drying can be implemented.

The so obtained quick-dried sub-ripe coffee cherries may then without further mycotoxin detoxification be employed for various uses in numerous food products. For example, where the whole quick-dried sub-ripe coffee cherry is used in a food product, the coffee cherry may be admixed with another consumable (*e.g.*, admixture with grain for animal feed, or coating with chocolate for human consumption). In another especially preferred example, the quick-dried sub-ripe coffee cherry is ground and used as a food additive or as a basis for brewing coffee cherry tea (*e.g.*, for use as loose tea, grinding to a size of 500-3000 μm is preferred, or for bagged teas, grinding to a size of 200-1000 μm is preferred).

Alternatively, it should be recognized that only parts of the quick-dried sub-ripe coffee cherry may be employed in a food product. For example, where the sub-ripe coffee cherry is in an almost ripe state, it is contemplated that the pulp, mucilage, and/or hull may be separated from the seeds, which are then (optionally admixed with other seeds) roasted to commercial grade coffee beans. The remaining pulp, mucilage, and/or hull from the quick-dried sub-ripe coffee cherry may then be employed as food additive or basis for extraction of one or more desired components (*e.g.*, polyphenols).

In still further contemplated uses of so obtained quick-dried sub-ripe coffee cherries, it is contemplated that the coffee cherries (or portions thereof) may be employed as starting material for extraction of various beneficial components. For example, the quick-dried sub-ripe coffee cherries may be extracted with an aqueous (*e.g.*, water, water-ethanol mixture) or non-aqueous solvent (*e.g.*, critical point CO_2 , dimethylformamide) to isolate one or more components that can be used in a food product. For example, quick-dried sub-ripe coffee cherries may provide an excellent source of polyphenols, chlorogenic acid, and/or caffeine.

The term "polyphenol" as used herein refers to a diverse group of compounds produced by a plant, wherein the compounds include a phenol ring to which at least one OH group, and more typically at least two OH groups are covalently attached. For example, representative polyphenols include ellagic acid, tannic acid, vanillin, caffeic acid, chlorogenic acid, ferulic acid, catechins (*e.g.*, epicatechin gallate, epigallocatechin), flavonols (*e.g.*, anthocyanidins, quercetin,

kaempferol), and various other flavonoids, and their glycosides and depsides. Furthermore, contemplated polyphenols may also be in oligomeric or polymeric form (e.g., oligomeric proanthocyanidins or condensed tannins).

5 In another preferred aspect of the inventive subject matter, the inventors contemplate use of whole quick-dried sub-ripe coffee cherries in the production of various beverages. For example, it was observed that teas produced from unripe (green) and semi-ripe stage 1 whole quick-dried coffee cherries possess relatively low aroma and flavor characteristics. Thus, extracts or at least partially condensed teas from unripe (green) and semi-ripe stage 1 whole quick-dried coffee cherries may be added as low-flavor additive to a commercially available beverage to
10 enhance the nutritional properties.

As ripeness increases, more aroma and flavor is evident in the quick-dried sub-ripe coffee cherries. Consequently, tea produced from semi-ripe stage 2 and almost ripe whole coffee cherries will possess high aroma and fruity flavor, and the inventors contemplate that semi-ripe and almost ripe coffee cherries could be used directly after quick-drying and grinding for a
15 "Whole Coffee Fruit Tea", either by itself or after placing into tea bags. As illustrated below, such a tea would provide high levels of polyphenols, typically 60-70 mg per 6 oz. cup. Furthermore, teas prepared from whole quick-dried sub-ripe coffee cherries generally possess relatively high ratios of chlorogenic acid to caffeine (typically about 2-4.5) as compared to beverages made from roasted coffee (typically about 0.4). Therefore, it should be appreciated
20 that tea prepared from quick-dried sub-ripe coffee cherries is much more nutritional (based upon polyphenols and chlorogenic acid) than roasted coffee.

Therefore, the inventors contemplate a food product that includes a preparation of a (preferably sub-ripe) coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than
25 5 ppm for total fumonisins. Alternatively, contemplated mycotoxin levels may also be in the range of 20-50 ppb, but more preferably less than 15 ppb, even more preferably less than 10 ppb, and most preferably less than 5 ppb for total aflatoxins. Similarly contemplated mycotoxin levels also include a range of 10-30 ppb, but more preferably less than 5 ppb, even more preferably less than 3 ppb, and most preferably less than 2 ppb for total ochratoxins. Likewise, contemplated

mycotoxin levels also include a range of 5-20 ppm, but more preferably less than 15 ppm, even more preferably less than 10 ppm, and most preferably less than 5 ppm for total fumonisins and/or vomitoxins.

As already discussed above, the preparation of the sub-ripe coffee cherry may include the whole coffee cherry, comprise a ground fragment of the whole coffee cherry, or include the bean, the pulp, the mucilage, and/or the hull of the quick-dried sub-ripe coffee cherry. Alternatively, it should be recognized that the preparation may also comprise an extract from the whole quick-dried sub-ripe coffee cherry (or fragment or portion thereof).

Contemplated food products especially include beverages prepared from contemplated quick-dried sub-ripe coffee cherries (or fragments or portions thereof), or beverages to which extracts or pieces from contemplated quick-dried sub-ripe coffee cherries (or fragments or portions thereof) have been added. Similarly, further contemplated food products include baked goods (e.g., bread, crackers, etc.), snacks (e.g., candy or energy bars), cereals, and other solid nutrients to which extracts or pieces from contemplated quick-dried sub-ripe coffee cherries (or fragments or portions thereof) have been added. Alternatively, contemplated food products also include a nutritional supplement in liquid or solid form that comprises an extract of the quick-dried sub-ripe coffee cherry.

Depending on the particular purpose, it should be recognized that such food products may be prepared from quick-dried sub-ripe coffee cherries having a primarily green color with less than 25% red color, more preferably with less than 25% green color, and most preferably from quick-dried sub-ripe coffee cherries having primarily (no less than 90%, most typically no less than 95%) red color with less than 5% blemished area. Still further contemplated compositions and methods are disclosed in our co-pending U.S. Patent application with the title "Methods for Coffee Cherry Products", filed on or about April 16, 2003, which is incorporated by reference herein.

Examples

The following examples are provided to enable a person of ordinary skill in the art to make and use compositions according to the inventive subject matter and to illustrate exemplary compositions and methods generally described herein.

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Harvest Of Whole Coffee Cherries

The ripeness of the coffee cherries was determined by visually estimating the amount of green and red color (or yellow, where applicable) of the whole cherries. As the cherries ripen, the green cherries will typically increase in size and subsequently develop increasing amounts of red color. For the present examples, the coffee cherries were collected at four stages of ripeness: Completely, or almost completely green (unripe; typically less than 5% of the coffee cherry red or yellow), primarily green with some red (semi-ripe, stage 1; typically less than 25% of the coffee cherry red or yellow), primarily red with some green (semi-ripe, stage 2; typically less than 25% of the coffee cherry green), and unbroken, unblemished red (almost ripe; typically less than 10% of the coffee cherry green; area of blemishes, cuts, or otherwise broken surface less than 5%). As much as possible, whole, unbroken and uncut cherries were collected.

Quick-Drying Of The Whole Coffee Cherries

Whole coffee cherries for sample extraction were prepared by drying the cherries within 1-12 hours after harvest on separate trays of an air dryer according to the following procedure. Coffee cherries (400-600 g) were weighed into beakers and washed two times with tap water, followed by a single wash with distilled water. The so washed coffee cherries were placed on a tray of an air dryer to drain, and then dried at 150-160°F for 16-18 hours to constant weight. Drying was stopped when the weight at two consecutive one-hour intervals differed by less than 1 g. Typical yields of dried whole cherry were 160-220 g. Further analysis indicated 6-12% residual water content in the dried cherry.

Mycotoxin Analysis

In order to determine the viability of the whole coffee cherry at the unripe, semi-ripe, and almost ripe stages (see above) for use in a nutritional product (and especially for use in tea), the level of selected mycotoxins was measured and compared against comparative products and red, ripe coffee cherry by-product from coffee production. As can be clearly seen in Table 1 below, quick-dried coffee cherries of all sub-ripe harvest stages had a mycotoxin level below the detection limit of 1 ppb (as measured for aflatoxin and ochratoxin).

The mycotoxin concentration was determined in an independent laboratory by both ELISA and HPLC analysis. Based on the below results, the inventors conclude that all samples from the different sub-ripe harvest stages are suitable for direct use in a nutritional product for both human and veterinary consumption. In contrast, the by-product of coffee production (predominantly consisting of pulp, mucilage, and hull from coffee cherries) from ripe cherries of red color with blemishes (typically greater than 20% of the cherry surface) had a substantial content in both aflatoxins and ochratoxins. Similarly, the comparative product "Paradise to Go Tea" (made from coffee cherry pulp) exhibited mycotoxins in double-digit concentrations.

RIPENESS	COLOR	AFLATOXIN	OCHRATOXIN
Unripe, quick-dried	Green	< 1ppb	< 1ppb
Semi-ripe Stage 1, quick-dried	Mostly green with some red	< 1ppb	< 1ppb
Semi-ripe Stage 2, quick-dried	Mostly red with some green	< 1ppb	< 1ppb
Almost ripe, quick-dried	Red, Blemished Area <5%	< 1ppb	< 1ppb
Ripe (by-product of Coffee Production)	Red, Blemished Area >20%	> 200 ppb	> 500 ppb
Paradise to Go Tea (dry matter)	N/A	> 25 ppb	> 40 ppb

Table 1

Polyphenol(PP), Chlorogenic Acid (CG), and Caffeine (CF) Analysis for Quick-dried Sub-ripe Whole Coffee Cherries

In a further series of experiments, the levels of total polyphenols, chlorogenic acid, and caffeine from quick-dried whole coffee cherry at various sub-ripe stages were measured and compared against green and roasted coffee beans. Table 2 summarizes the results of this analysis.

Interestingly, while the polyphenol (PP) level of quick-dried coffee cherries of all sub-ripe harvest stages was somewhat less than the level of green or roasted coffee beans, significant quantities of polyphenols in quick-dried sub-ripe coffee cherries still remain. Similarly, the chlorogenic acid (CG) content of quick-dried whole coffee cherry at various sub-ripe stages remained at substantial high levels as compared to roasted coffee, but was somewhat lower as

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compared to green beans. The caffeine (CF) level of quick-dried coffee cherries of all sub-ripe harvest stages was substantially within the caffeine level of green and roasted coffee beans (It should be pointed out that all data given are on a dry matter basis and are not normalized to the dry weight of the bean).

5 Polyphenol analysis: Dried whole coffee cherry (or green beans or roasted beans) (1.00 g) were ground in a rotating steel knife coffee grinder for 30 seconds to produce a ground sample. The ground sample was added to 100 mL distilled water and the resulting mixture heated to boiling in an Erlenmeyer flask for 30 minutes. The heat was removed and the mixture allowed to cool to room temperature. The resulting suspension was transferred to a 100 mL graduated
10 cylinder and water added to bring the volume to 100 mL. The mixture was then transferred back to the Erlenmeyer flask, stirred briefly, and the solids allowed to settle. An aliquot (~3 mL) of the supernatant solution was filtered through an 0.45 µm Acrodisc filter, and the resulting clear solution was diluted 1:10 with distilled water using a volumetric flask (1.00 mL diluted with 9.00 mL distilled water).

15 The Folin-Ciocalteu method was used to measure the polyphenol content of the diluted solution as follows. One mL of the diluted solution was added to a test tube, mixed with 1 mL of 0.2N Folin-Ciocalteu's Phenol reagent (Sigma solution, 2N, diluted 1:10 with water), and allowed to stand 5 minutes at room temperature. One mL of 1N NaHCO₃ was added and the reaction mixture left at room temperature for 2 hours. The polyphenol level was determined
20 using a UV-visible spectrophotometer standardized against catechin, at λ_{max}=750 nm against distilled water as blank.

Chlorogenic acid: Determination of chlorogenic acid was done using HPLC separation of the filtered clear solution prepared above using standard analytical and separation protocols well known in the art. Similarly, determination of caffeine was done using HPLC separation of the
25 filtered clear solution prepared above using standard analytical and separation protocols well known in the art (for exemplary protocols see e.g., Bispo M.S., et al. in J. Chromatogr. Sci.; 2002, Jan;40(1):45-8, or Nakakuki, H. et al. in J. Chromatogr. A.;1999, Jul 2;848(1-2):523-7).

RIPENESS	COLOR	% PP	%CG	% CF	CG/CF
Unripe, quick-dried	Green	3.80	2.64	1.03	2.56

Semi-ripe stage 1, quick-dried	Mostly green with some red	3.28	2.70	1.00	2.70
Semi-ripe stage 2, quick-dried	Mostly red with some green	3.54	2.00	0.70	2.86
Almost ripe, quick-dried	Red, Blemished Area <5%	3.35	N/D	N/D	N/D
Green Coffee beans	Green	4.58	3.31	0.95	3.48
Roasted Coffee beans	Brown	3.93	0.50	1.20	0.42

Table 2

Polyphenol(PP), Chlorogenic Acid (CG), and Caffeine (CF) Analysis for a Tea brewed from Quick-dried Sub-ripe Whole Coffee Cherries

Quick-dried sub-ripe coffee cherry were ground in a rotating steel knife coffee grinder for 10-30 seconds to produce a ground sample. To the ground sample (1.00 g) was added 90 mL (approx. 3 fluid oz.) boiling distilled water and the resulting mixture allowed to stand in an Erlenmeyer flask for 10 minutes to produce a coffee cherry tea. An aliquot (~3 mL) of the supernatant solution was filtered through a 0.45 μ m Acrodisc filter, and the resulting clear solution was diluted 1:10 with distilled water using a volumetric flask (1.00 mL diluted with 9.00 mL distilled water). The Folin-Ciocalteu method as described above was used to measure the polyphenol content (on dry matter basis, catechin equivalents) of the so prepared coffee cherry tea. Table 3 summarizes the results.

RIPENESS	SOLVENT	% PP	%CG	% CF	CG/CF
Unripe, quick-dried	Water	10.93	8.61	3.04	2.83
Semi-ripe stage 1, quick-dried	Water	9.38	7.58	2.72	2.78
Semi-ripe stage 2, quick-dried	Water	8.51	6.74	1.71	3.95
Almost ripe, quick-dried	Water	6.92	1.34	0.29	4.61

Table 3

Brewing Of Teas From Whole Coffee Cherry Of Different Ripeness

The following procedure was used to prepare teas from whole coffee cherry of different ripeness for aroma and taste testing. Whole dried coffee cherry (10-20 g) was ground in a rotating steel knife coffee grinder for 10-30 seconds to produce a ground sample. The ground

coffee cherry (2.0 g) was placed in a ceramic cup and of nearly boiling water (6 oz., 190-200°F) added. The slurry was stirred and allowed to stand for 3 minutes, at which time the aroma and taste of the supernatant liquid were noted. Results are shown in Table 4.

RIPENESS	COLOR	AROMA	TASTE	PP/6 OZ. TEA
Unripe, quick-dried	Green	None	Almost no taste	76 mg
Semi-ripe stage 1, quick-dried	Mostly green with some red	Very mild fruity	Slight fruit flavor	66 mg
Semi-ripe stage 2, quick-dried	Mostly red with some green	Mild fruity	Mild fruit flavor	71 mg
Almost ripe, quick-dried	Red, Blemished Area <5%	Fruity	Rich fruit flavor	67 mg

Table 4

Thus, specific embodiments and applications of low-mycotoxin coffee cherry products have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

CLAIMS

What is claimed is:

1. A food product comprising a preparation of a coffee cherry that is quick-dried such that a mycotoxin level of the coffee cherry is less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
2. The food product of claim 1 wherein the preparation of the coffee cherry comprises a ground fragment of the coffee cherry.
3. The food product of claim 2 wherein the preparation of the coffee cherry comprises an extract from a ground fragment of the coffee cherry.
4. The food product of claim 1 wherein the preparation of the coffee cherry comprises at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
5. The food product of claim 1 wherein the preparation of the coffee cherry comprises an extract from at least one of a bean of the coffee cherry, a pulp of the coffee cherry, a mucilage of the coffee cherry, and a hull of the coffee cherry.
6. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily green color with less than 25% red color.
7. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 25% green color.
8. The food product of claim 1 wherein the coffee cherry is a sub-ripe coffee cherry and has a primarily red color with less than 5% blemished area.
9. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using heated air.
10. The food product of claim 1 wherein the coffee cherry is quick-dried in a dryer using solar radiation.

11. The food product of claim 1 wherein the coffee cherry is quick dried by exposing the coffee cherry to at least one of ambient air and sun light.
12. The food product of claim 1 wherein the food product is a tea brewed from the coffee cherry.
- 5 13. The food product of claim 1 wherein the food product is a beverage comprising an extract of the coffee cherry.
14. The food product of claim 1 wherein the food product is nutritional supplement in liquid or solid form and comprising an extract of the coffee cherry.
15. A tea brewed from a comminuted quick-dried coffee cherry or portion thereof.
- 10 16. The tea of claim 15 wherein the coffee cherry has a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
17. The tea of claim 16 having a polyphenol concentration of at least 10 mg/oz.
18. The tea of claim 15 wherein the coffee cherry is a sub-ripe coffee cherry.
- 15 19. A quick-dried coffee cherry or portion thereof having a mycotoxin level of less than 20 ppb for total aflatoxins, less than 10 ppb for total ochratoxins, and less than 5 ppm for total fumonisins.
20. The quick-dried coffee cherry of claim 19 wherein the coffee cherry is a sub-ripe coffee cherry.

20

ABSTRACT

A coffee cherry is harvested, preferably in a sub-ripe state, and quick-dried to provide a basis for numerous nutritional products. Such coffee cherries and portions thereof may be particularly characterized by their extremely low concentration of mycotoxins, including various
5 aflatoxins, fumonisins, ochratoxins, and/or vomitoxin (DON, deoxynivalenol).

Title:
Inventor:
Filing Date:
Serial Number:

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VDF - Dusan Miljkovic, et al.
16 April 2003
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REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

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PATENTE DE INVENCION	()	()
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- Indicación de que se solicita la concesión de una patente	()	()
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	()	()
- Descripción de la invención	()	()
- Dibujos de ser estos pertinentes	()	()
- Comprobante de Pago de las tasas establecidas	()	()

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ESQUEMA DE TRAZADO ()

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Fecha

18/157

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor (a)
CUELLAR TRUJILLO JAIME LUIS

REFERENCIA	Radicación No.	5 116274
	Solicitud internacional	US2003/11950
	Fecha inicio fase nacional	16/11/2005
	Trámite	11
	Evento	379
	Actuación	430
	Oficio No.	13675

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. Si fuere el caso, el solicitante debe adjuntar la traducción de los anexos del reporte de examen preliminar internacional (Art. 36.2.b) del tratado PCT.
2. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.
3. La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.
4. El solicitante deberá diligenciar en su totalidad el formulario de PETITORIO fase nacional.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

Continúa en la página siguiente...

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REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Radicación No.:5 116274

Clara de Jaimes

CLARA DE JAIMES

Jefe de la división de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista de
fecha 23 DIC. 2005
adpall