REPUBLIC OF SOUTH AFRICA

REPUBLIEK VAN SUID AFRIKA

PATENTS ACT, 1978

CERTIFICATE

In accordance with section 44 (1) of the Patents Act, No. 57 of 1978, it is hereby certified that:

YANBIAN UNIVERSITY

Has been granted a patent in respect of an invention described and claimed in complete specification deposited at the Patent Office under the number

2022/08904

A copy of the complete specification is annexed, together with the relevant Form P2.

In te[illegible]y thereof, the seal of the Patent Office has been affixed at Pretoria with effect from the 30th day of **November 2022**

.....

Registrar of Patents

REPUBLIC OF SOUTH AFRICA FORM P2
PATENTS ACT, 1978
REGISTER OF PATENTS

Official application No.			Lodging date: Provisional		Acceptance date	
21	01	2022/08904	22		47	17 October 2022
International classification			Lodging date: National phase		Granted date	
51	C07C; C11C		23	10 August 2022		30 November 2022
71	Full name(s) of applicant(s)/Patentee(s):					
Yanbian University						
71	Applicant(s) substituted:				Date registrered	
71	Assignee(s):				Date registrered	
72	Full name(s) of inventor(s):					
(1) WANG, Juan; (2) NAN, Jingxi						

Priority claimed:	Country	Number	Date

54	Title of invention
METHOD FOR SIMULTANEOUSLY DERIVATIZING, EXTRACTING AND DETECTING PHENOL COMPOUNDS AND ORGANIC ACID COMPOUNDS IN MEAT	
Address of applicant(s)/patentee(s):	
No. 977 Gongyuan Road, Yanji City, Jilin Province, 133002, China	
74	Address for service
Sibanda and Zantwijk, Oaktree Corner, 9 Kruger Street, Oaklands (PO Box 1615 Houghton 2041), Johannesburg, 2192, SOUTH AFRICA Reference no.: PT_CP_ZA00005399 ([InsID:])	

61	Patent of addition No.	Date of any change
Fresh application based on.		Date of any change

FORM P7

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978
COMPLETE SPECIFICATION
[Section 30(1) – Regulation 28]

OFFICIAL APPLICATION NO.			LODGING DATE	
21	01	2022/08904	22	10 August 2022

INTERNATIONAL CLASSIFICATION	
51	C07C; C11C

FULL NAME(S) OF APPLICANT(S)

71	Yanbian University

FULL NAME(S) OF INVENTORS(S)

72	WANG, Juan NAN, Jingxi

TITLE OF INVENTION

54	METHOD FOR SIMULTANEOUSLY DERIVATIZING, EXTRACTING AND DETECTING PHENOL COMPOUNDS AND ORGANIC ACID COMPOUNDS IN MEAT

METHOD FOR SIMULTANEOUSLY DERIVATIZING, EXTRACTING AND DETECTING PHENOL COMPOUNDS AND ORGANIC ACID COMPOUNDS IN MEAT

TECHNICAL FIELD

[01] The present invention relates to the technical field of analysis of organic matters in food, particularly to a method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat.

BACKGROUND ART

[02] Gas chromatography separation and detection can be performed only after polar compounds in a meat sample, including phenols such as octylphenol (an environmental pollutant) and pentachlorophenol (a residual metabolite of a veterinary drug) and organic acids such as fatty acids (nutritional ingredients), are extracted, purified, concentrated and derivatized. Particularly, the derivatizing process increases a step of sample pre-treatment, and certain reaction conditions, such as solvent, temperature and catalyst, are required during reaction. Integration of multiple steps and integration of multiple functions are the developing direction of the analytical technology. Therefore, it is quite necessary to develop a technical method that integrates extracting, purifying, concentrating and derivatizing processes of phenol compounds and organic acid compounds in a meat sample.

SUMMARY

[03] The present invention provides a method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat. To overcome the extracting, purifying, concentrating and derivatizing processes step by step in the prior art, the present invention provides an integrated method for simultaneously derivatizing and extracting phenol compounds and organic acid compounds in a meat sample, wherein derivatization is simultaneous with extraction, and derivatives are directly subjected to gas chromatography separation and detection, so that the analytical steps are simplified and the analytical time is saved. The whole technology is simple to operate, rapid, integrated, liable to use with a gas chromatography system, and practical in qualitative and quantitative analysis.

[04] The technical scheme of the present invention is as follows:

[05] The phenol compounds and organic acid compounds in a sample are derivatized while subjected to thermal desorption, and driven by air flow to enter an absorption liquid. Thus, derivatives of the phenol compounds and organic acid compounds are obtained by extraction. The phenol compounds and organic acid compounds are qualitatively or quantitatively analyzed after the derivatives are subjected to gas chromatography-mass spectrometer (GC-MS) detection.

[06] The derivatizing reagent is a silylating reagent, preferably *N,O*-bis(trimethyl silicyl) trifluoroacetamide (BSTFA: 99%).

[07] The to-be-detected substance is phenol and organic acids with volatile and semi volatile organic compounds containing reactive hydrogen.

[08] The derivatizing process is performed rapidly by using a thermal desorption temperature condition of the sample.

[09] The thermal desorption temperature of the sample ranges from 100℃ to 300℃, preferably 250℃.

[10] The air flow is inert gas such as nitrogen and helium, preferably nitrogen.

[11] A flow rate of the air flow ranges from 1 mL/min to 5 mL/min, preferably 2 mL/min.

[12] The finally obtained extract is derivatives of the to-be-detected substance.

[13] The absorption liquid is a solvent suitable for gas chromatography, such as normal hexane, cyclohexane, dichloromethane and acetone, preferably normal hexane.

[14] The detection method is a GC-MS technology.

[15] The phenol compounds and organic acid compounds are volatile and semi-volatile. The phenol compounds and organic acid compounds in meat are desorbed to form gaseous molecules when heated. As containing reactive hydrogen, the gaseous molecules can be combined immediately with the silylating reagent in a gaseous form to obtain derivatives. The thermal stability of the phenol compounds and organic acid compounds is improved, the detection requirement on GC-MS technology is satisfied, the chromatographic behavior is good; moreover, the derivatives also play a certain role of protecting a chromatographic column. Meanwhile, the present invention relates to a highly integrated method that integrates extracting, purifying, concentrating and derivatizing processes.

BRIEF DESCRIPTION OF DRAWINGS

[16] FIG. 1 is a schematic diagram of a scheme of a method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat of the present invention.

[17] 1 - carrier-gas cylinder; 2 - pressure reducing valve; 3 - stainless steel tube; 4 - flow controller; 5 - polytetrafluoroethylene tube; 6 - stainless steel tube; 7 - gasket for gas sample inlet; 8 - injector; 9 - derivatizing reagent; 10 - glass sample pool; 11 - sample; 12 - heating body; 13 - gasket for gas sample inlet; 14 - glass tube; 15 - polytetrafluoroethylene tube; 16 - glass absorption pool; 17 - absorption liquid; 18 - condenser.

[18] FIG. 2 is a total ion gas chromatogram of detected components in two beef samples. 92 ingredients are derivatized among 158 ingredients with a peak area of greater than 10^6, and 21 ingredients are derivatized among 26 ingredients with a peak area of greater than 10^7, wherein representative fatty acid ingredients (derivative form) include: a derivative of Tetradecanoic acid: Tetradecanoic acid; a derivative of trimethylsilyl ester and Hexadecanoic acid: Hexadecanoic acid; derivatives of trimethylsilyl ester and (z,z)-9,12-octadecadienoic acid: 9,12-Octadecadienoic acid (Z,Z)-; and derivatives of trimethylsilyl ester and Octadecanoic acid: Octadecanoic acid and trimethylsilyl ester.

BRIEF DESCRIPTION OF DRAWINGS

[19] The present invention provides a method for simultaneously derivatizing,

extracting and detecting phenol compounds and organic acid compounds in meat, including the following steps: a powdery dry to-be-detected substance is weighed and put into a sample tube, and the sample tube is closed at two ends with glass fiber filter membranes. Inert gas is continuously introduced into the sample tube at a certain flow rate, the sample tube is heated, and a derivatizing reagent is continuously injected into the sample tube as well, wherein the phenol compounds and organic acid compounds in meat are desorbed to form a gaseous form when heated, and combined immediately with the derivatizing reagent heated to form a gaseous state to form derivatives. The derivatives are driven by the air flow into an absorption liquid through a glass tube for extraction, so that the derivatives of the phenol compounds and organic acid compounds are extracted. The phenol compounds and organic acid compounds are qualitatively or quantitatively analyzed after the derivatives are subjected to GC-MS detection.

[20] Example 1

[21] 0.0100 g of dry powdery chicken sample and 0.0100 g of chicken sample spiked with 1 μg/g octylphenol and pentachlorophenol were weighed respectively and added into a sample tube, and the sample tube was closed at two ends with glass fiber filter membranes and sealing gaskets successively. Nitrogen was introduced into the sample tube at a flow rate of 2 mL/min, a gas outflowing end was inserted into 50 μL of normal hexane absorption liquid containing an internal standard substance. A temperature of the absorption liquid was controlled at about 0℃. A BSTFA derivatizing reagent was continuously injected into the sample tube at a rate of about 0.4 μL/s when the sample tube was heated to 100℃. This was kept for 1 min after the temperature of the sample tube reached 250℃, and 2 μL of absorption liquid was taken and injected into a gas chromatography-mass spectrometer. The contents of octylphenol and pentachlorophenol in chicken were obtained by an internal standard method, and a spike recovery rate was calculated; the recovery rates of octylphenol and pentachlorophenol were respectively 96.32% and 69.83%.

[22] Example 2

[23] 0.0100 g of dry powdery beef sample was weighed and added into a sample tube, the sample tube was closed at two ends with glass fiber filter membranes and sealing gaskets successively. Nitrogen was introduced into the sample tube at a flow rate of 2 mL/min, a gas outflowing end was inserted into 50 μL of normal hexane absorption liquid. A temperature of the absorption liquid was controlled at about 0℃. A BSTFA derivatizing reagent was continuously injected into the sample tube at a rate of about 0.4 μL/s when the sample tube was heated to 100℃. This was kept for 1 min after the temperature of the sample tube reaches 250℃, and 2 μL of absorption liquid was taken and injected into a gas chromatography-mass spectrometer. The effluent ingredients were qualitatively analyzed through mass spectral databases NIST08S and NIST08 library, as shown in FIG. 2. According to the detection results, 92 ingredients were derivatized among 158 ingredients with a peak area of greater than 10^6, and 21 ingredients were derivatized among 26 ingredients with a peak area of greater than 10^7, wherein representative fatty acid components included: Tetradecanoic acid, Hexadecanoic acid, (z,z)-9,12-octadecadienoic acid and Octadecanoic acid and the like.

[24] Those described above are only the preferred embodiments of the present

invention. It should be noted that improvements and modifications may be made by those of ordinary skill in the art without departing from the principle of the present invention, and these improvements and modifications should also be regarded to fall into the protection scope of the present invention.

WHAT IS CLAIMED IS:

1. A method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat, comprising the following steps:

(1) weighing a powdery dry to-be-detected substance, putting the substance into a sample tube, and closing the sample tube at both ends;

(2) introducing air flow continuously into the sample tube at a certain flow rate, heating the sample tube, and injecting a derivatizing reagent into the sample tube as well to obtain derivatives;

(3) driving the derivatives by the air flow into an absorption liquid through a glass tube for extraction; and

(4) injecting the absorption liquid into a gas chromatography-mass spectrometer, and performing qualitative or quantitative analysis.

2. The method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat of claim 1, wherein the derivatizing reagent is a silylating reagent.

3. The method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat of claim 1, wherein the to-be-detected substance is phenol and organic acids with volatile and semi volatile organic compounds containing reactive hydrogen.

4. The method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat of claim 1, wherein a heating temperature ranges from 100℃ to 300℃.

5. The method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat of claim 1, wherein gas in the air flow is inert gas.

6. The method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat of claim 1, wherein a flow rate of the air flow ranges from 1 mL/min to 5 mL/min.

7. The method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat of claim 1, wherein an extract in the absorption liquid is derivatives of phenol and organic acids.

8. The method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat of claim 1, wherein the absorption liquid is a solvent suitable for gas chromatography.

9. The method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat of claim 1, wherein the detection method is a GC-MS technology.

Drawings

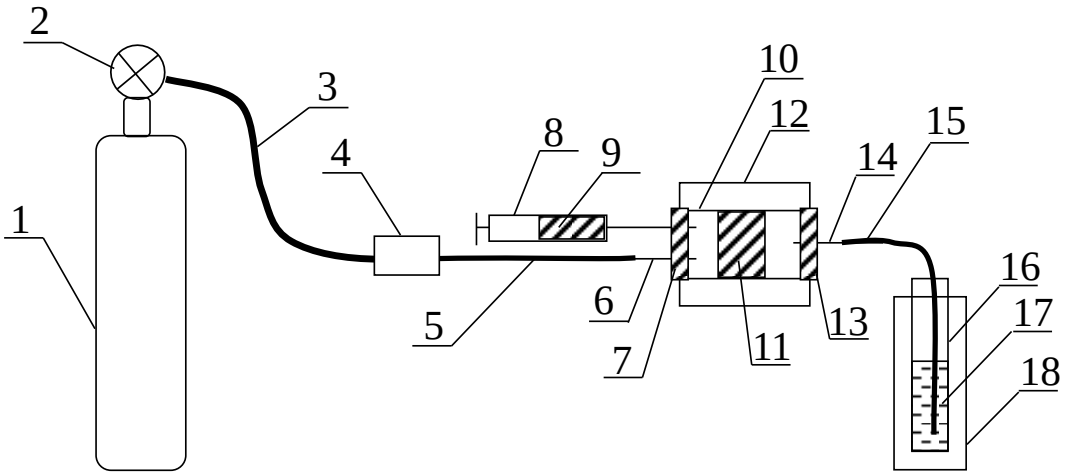

FIG. 1

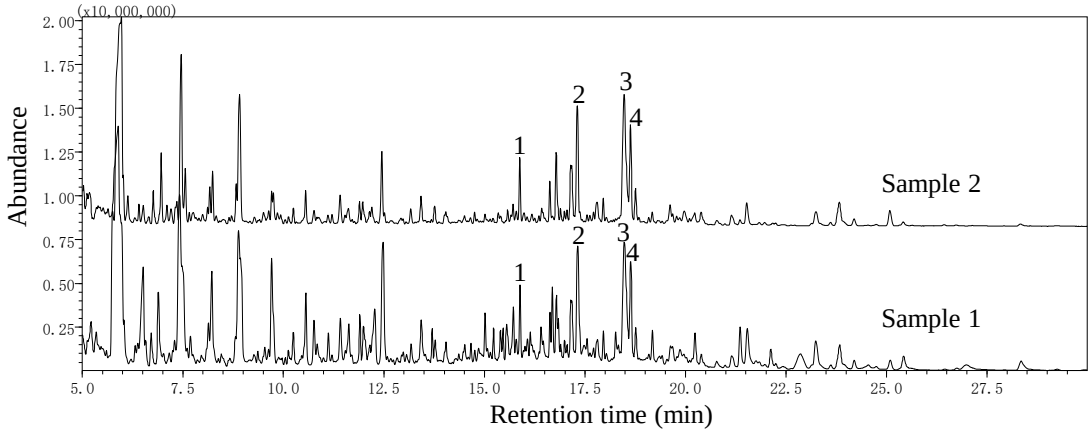

FIG. 2

ABSTRACT OF THE DISCLOSURE

The present invention relates to the technical field of analysis of organic matters in food, particularly to a method for detecting phenol compounds and organic acid compounds in meat. The present invention discloses a method for simultaneously derivatizing, extracting and detecting phenol compounds and organic acid compounds in meat. The phenol compounds and organic acid compounds in a sample are derivatized while subjected to thermal desorption, and driven by air flow to enter an absorption liquid. Thus, derivatives of the phenol compounds and organic acid compounds are obtained by extraction. The phenol compounds and organic acid compounds are qualitatively or quantitatively analyzed after the derivatives are subjected to GC-MS detection. The whole technology is simple to operate, rapid, integrated, liable to use with a gas chromatography system, and practical in qualitative and quantitative analysis.

ABSTRACT DRAWING

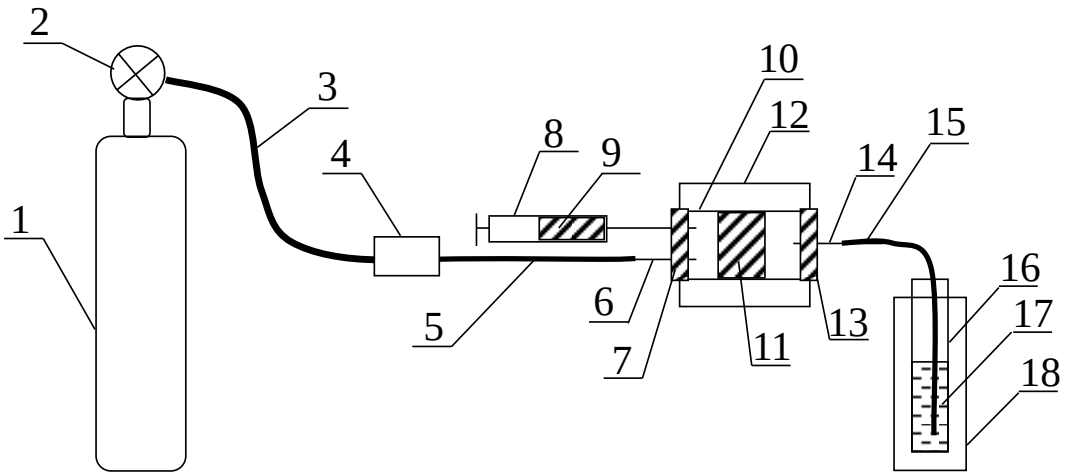

FIG. 1