Notice

DESCRIPTION CN110541015A

A method for simultaneous extraction of indole-3-methanol and 3,3′-diindolemethane

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一种吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取方法

[0001]

Technical Field

技术领域

[0002]

This invention relates to the field of plant extraction technology, and in particular to a method for the simultaneous extraction of indole-3-methanol and 3,3′-diindolemethane.

本发明涉及植物提取技术领域，特别涉及一种吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取方法。

[0003]

Background Technology

背景技术

[0004]

Indole-3-carbinol (I3C) has the molecular formula C₉H₉NO and a molecular weight of 147.18.

吲哚-3-甲醇(indole-3-carbinol，I3C)分子式为C₉H₉NO，分子量147.18。

Studies have found that 3-indolemethylthioglucoside is mainly found in cruciferous vegetables, including cabbage, cauliflower, and Chinese cabbage. Under suitable pH conditions, indolemethylthioglucoside can react with endogenous myrosinase to hydrolyze and generate indole-3-methanol.

研究发现，3-吲哚甲基硫代葡萄糖苷主要存在于十字花科蔬菜中，包括甘蓝、花椰菜、大白菜等，在合适的pH值条件下，吲哚甲基硫代葡萄糖苷能够和内源性的黑芥子酶反应水解生成吲哚-3-甲醇。

Since Wattenberg and Loub first reported in 1978 that indole-3-methanol could inhibit the formation of polycyclic aromatic hydrocarbon-induced rat tumor cells, more and more studies have shown that indole-3-methanol can inhibit the occurrence of breast cancer, cervical cancer, prostate cancer, and colon cancer by inhibiting the binding of carcinogens to DNA, repairing DNA damage, and inducing apoptosis.

自1978年Wattenberg和Loub首次报道了吲哚-3-甲醇可以抑制多环芳烃诱导大鼠肿瘤细胞的形成以来，越来越多的研究表明吲哚-3-甲醇可以通过抑制致癌物质与DNA的结合，修复DNA损伤，造成细胞凋亡等途径来抑制乳腺癌、宫颈癌、前列腺癌、结肠癌的发生。

[0005]

Indole-3-methanol is unstable and readily forms dimer 3,3′-diindolemethane (DIM) or various oligomers in the human intestine, among which DIM has important anti-tumor effects.

吲哚-3甲醇不稳定，很容易形成二聚体3,3′-二吲哚甲烷(DIM)或在人体肠道内形成多种寡聚物，其中DIM具有重要的抗肿瘤功效。

3,3′-Diindolemethane, also known as diindole, is a chemical with the chemical formula C₁₇H₁₄N₂ and a molecular weight of 246.31.

3,3′-二吲哚甲烷，又名二聚吲哚，是一种化学品，化学式是C₁₇H₁₄N₂，分子量246.31。

Studies have found that I3C and its dimer DIM can repair DNA damage, and its mechanism may be related to estrogen receptor-α (ER-α). It can also repair related proteins (RAD51) by

affecting the human breast cancer susceptibility gene BRCA1. I3C and DIM can participate in the regulation of various transcription factors to expel carcinogens, repair DNA damage, and induce apoptosis in cancer cells, thus having significant effects in preventing and reducing various cancers such as breast cancer and uterine cancer. There are no existing reports on methods for the simultaneous extraction of indole-3-methanol and 3,3′-bisindolemethane.

研究发现，I3C及其二聚体DIM可修复DNA损伤，其机制可能与雌激素受体-α(ER-α)有关，同时可以通过影响人类乳腺癌易感基因BRCA1来修复相关蛋白(RAD51)等。 I3C和DIM可参与多种转录因子的调控途径来排出致癌物，进行DNA损伤修复，造成癌细胞细胞凋亡等过程，在预防和降低乳腺癌、子宫癌等多种癌症方面具有明显的功效。 现有技术中还没有吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取方法的相关报道。

[0006]

Summary of the Invention

发明内容

[0007]

The purpose of this invention is to provide a method for the simultaneous extraction of indole-3-methanol and 3,3′-bisindolemethane.

本发明的目的在于提供一种吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取方法。

This invention provides a method for the simultaneous extraction of indole-3-methanol and 3,3′-bisindolemethane, which simplifies the extraction process.

本发明提供了一种吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取方法，简化了提取方法。

[0008]

This invention provides a method for the simultaneous extraction of indole-3-methanol and 3,3′-bisindolemethane, comprising the following steps:

本发明提供了一种吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取方法，包括以下步骤：

[0009]

Fresh samples of the edible organs of cruciferous vegetables were crushed to obtain fresh samples.

将十字花科蔬菜的可食性器官的新鲜样品破碎，得到新鲜样品；

[0010]

The fresh sample was vacuum freeze-dried and then pulverized to obtain a dried sample;

将所述新鲜样品真空冷冻干燥后粉碎，得到干燥样品；

[0011]

The fresh or dried sample was mixed with a citrate-phosphate buffer solution and then subjected to enzymatic hydrolysis to obtain an enzymatic hydrolysate.

将所述新鲜样品或干燥样品分别与柠檬酸-磷酸盐缓冲溶液混合后进行酶解反应，得到酶解液；

[0012]

The enzymatic hydrolysate was extracted with ethyl acetate, and the resulting supernatant was evaporated to dryness to obtain the extract;

将所述酶解液用乙酸乙酯萃取后，将所得上清液进行蒸干，得到提取物；

[0013]

The extract was mixed with a potassium dihydrogen phosphate-dipotassium hydrogen phosphate buffer solution, and then ethanol was added to saturate indole-3-methanol. After standing, a 3,3′-diindolemethane solution and an indole-3-methanol extract were obtained. The 3,3′-diindolemethane solution and the indole-3-methanol extract were evaporated to obtain 3,3′-diindolemethane and indole-3-methanol, respectively.

将所述提取物与磷酸二氢钾-磷酸氢二钾缓冲溶液混合后，再加入乙醇使吲哚-3-甲醇饱和，静置，得到3,3′-二吲哚甲烷溶液和吲哚-3-甲醇提取物，分别将所述3,3′-二吲哚甲烷溶液和吲哚-3-甲醇提取物蒸发，得到3,3′-二吲哚甲烷和吲哚-3-甲醇。

[0014]

Preferably, the cruciferous vegetables include one or more of cabbage, broccoli, kale, and kohlrabi.

优选地，所述十字花科蔬菜包括甘蓝、青花菜、芥蓝和苤蓝中的一种或多种。

[0015]

Preferably, the edible organ includes one or more of the following: root, stem, leaf, flower head, and seed.

优选地，所述可食性器官包括根、茎、叶、花球和种子中的一种或多种。

[0016]

Preferably, the ratio of the fresh sample to the citrate-phosphate buffer solution is 1:20 to 1:30 g/mL, and the ratio of the dried sample to the citrate-phosphate buffer solution is 1:40 to 1:60 g/mL.

优选地，所述新鲜样品与柠檬酸-磷酸盐缓冲溶液的料液比为1:20～1:30g/mL，所述干燥样品与柠檬酸-磷酸盐缓冲溶液的料液比为1:40～1:60g/mL。

[0017]

Preferably, the vacuum freeze-drying pressure is 10-15 Pa, the temperature is -45 to -80°C, and the time is 3-6 days.

优选地，所述真空冷冻干燥的压力为10～15Pa，温度为-45～-80℃，时间为3～6天。

[0018]

Preferably, the enzymatic hydrolysis reaction is carried out at a temperature of 20–25°C for 20–25 minutes.

优选地，所述酶解反应的温度为20～25℃，时间为20～25min。

[0019]

Preferably, the pH value of the citric acid-phosphate buffer solution (potassium dihydrogen phosphate-dipotassium hydrogen phosphate buffer) is 8.0.

优选地，所述柠檬酸-磷酸盐缓冲溶液磷酸二氢钾-磷酸氢二钾缓冲液的pH值为8.0。

[0020]

Preferably, the pH value of the potassium dihydrogen phosphate-dipotassium hydrogen phosphate buffer solution is 6.0.

优选地，所述磷酸二氢钾-磷酸氢二钾缓冲溶液的pH值为6.0。

[0021]

Preferably, the fresh sample is a slice, segment, or block, wherein the segment is 2-3cm × 3-5cm, and the block is 0.3-1.0cm thick.

优选地，所述新鲜样品为片、段或块，所述段为2～3cm×3～5cm，所述块的厚度为0.3～1.0cm。

[0022]

Preferably, the particle size of the dried sample is greater than 70 mesh.

优选地，所述干燥样品的粒径大于70目。

[0023]

This invention provides a method for the simultaneous extraction of indole-3-methanol and 3,3′-diindolemethane, comprising the following steps: crushing fresh samples of edible organs of cruciferous vegetables to obtain fresh samples; vacuum freeze-drying the fresh

samples and then pulverizing them to obtain dried samples; mixing the fresh samples or dried samples separately with a citrate-phosphate buffer solution and performing an enzymatic hydrolysis reaction to obtain an enzymatic hydrolysate; extracting the enzymatic hydrolysate with ethyl acetate and evaporating the resulting supernatant to obtain an extract; mixing the extract with a potassium dihydrogen phosphate-dipotassium hydrogen phosphate buffer solution, then adding ethanol to saturate indole-3-methanol, allowing it to stand to obtain a 3,3′-diindolemethane solution and an indole-3-methanol extract; evaporating the 3,3′-diindolemethane solution and the indole-3-methanol extract separately to obtain 3,3′-diindolemethane and indole-3-methanol.

本发明提供了一种吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取方法，包括以下步骤：将十字花科蔬菜的可食性器官的新鲜样品破碎，得到新鲜样品；将所述新鲜样品真空冷冻干燥后粉碎，得到干燥样品；将所述新鲜样品或干燥样品分别与柠檬酸-磷酸盐缓冲溶液混合后进行酶解反应，得到酶解液；将所述酶解液用乙酸乙酯萃取后，将所得上清液进行蒸干，得到提取物；将所述提取物与磷酸二氢钾-磷酸氢二钾缓冲溶液混合后，再加入乙醇使吲哚-3-甲醇饱和，静置，得到3,3′-二吲哚甲烷溶液和吲哚-3-甲醇提取物，分别将所述3,3′-二吲哚甲烷溶液和吲哚-3-甲醇提取物蒸发，得到3,3′-二吲哚甲烷和吲哚-3-甲醇。

This invention uses the edible organs of cruciferous vegetables as raw materials to achieve

the simultaneous extraction of indole-3-methanol and 3,3′-diindolemethane, which simplifies the extraction method and is easy to apply industrially.

本发明以十字花科蔬菜的可食性器官为原料，实现了吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取，简化了提取方法，易于工业化应用。

Data from the examples show that the extraction method provided by the present invention has a linear range of 2.04–65.00 μg・mL⁻¹, and the relative standard deviation (RSD) of 6 tests is 2.503E⁻⁹, indicating good precision.

实施例的数据表明，本发明提供的提取方法线性范围为2.04～65.00μg・mL⁻¹，6次检测的相对标准偏差RSD＝2.503E⁻⁹%，精密度良好。

[0024]

Attached Figure Description

附图说明

[0025]

Figure 1 is an HPLC chromatogram of the indole-3-methanol extract in Example 1 of the present invention.

图1为本发明实施例1中吲哚-3-甲醇提取物的HPLC谱图。

[0026]

Detailed Implementation

具体实施方式

[0027]

This invention provides a method for the simultaneous extraction of indole-3-methanol and 3,3′-bisindolemethane, comprising the following steps:

本发明提供了一种吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取方法，包括以下步骤：

[0028]

Fresh samples of the edible organs of cruciferous vegetables were crushed to obtain fresh samples.

将十字花科蔬菜的可食性器官的新鲜样品破碎，得到新鲜样品；

[0029]

The fresh sample was vacuum freeze-dried and then pulverized to obtain a dried sample;

将所述新鲜样品真空冷冻干燥后粉碎，得到干燥样品；

[0030]

The fresh or dried sample was mixed with a citrate-phosphate buffer solution and then subjected to enzymatic hydrolysis to obtain an enzymatic hydrolysate.

将所述新鲜样品或干燥样品分别与柠檬酸-磷酸盐缓冲溶液混合后进行酶解反应，得到酶解液；

[0031]

The enzymatic hydrolysate was extracted with ethyl acetate, and the resulting supernatant was evaporated to dryness to obtain the extract;

将所述酶解液用乙酸乙酯萃取后，将所得上清液进行蒸干，得到提取物；

[0032]

The extract was mixed with a potassium dihydrogen phosphate-dipotassium hydrogen phosphate buffer solution, and then ethanol was added to saturate indole-3-methanol. After standing, a 3,3′-diindolemethane solution and an indole-3-methanol extract were obtained.

The 3,3′-diindolemethane solution and the indole-3-methanol extract were evaporated to obtain 3,3′-diindolemethane and indole-3-methanol, respectively.

将所述提取物与磷酸二氢钾-磷酸氢二钾缓冲溶液混合后，再加入乙醇使吲哚-3-甲醇饱和，静置，得到3,3′-二吲哚甲烷溶液和吲哚-3-甲醇提取物，分别将所述3,3′-二吲哚甲烷溶液和吲哚-3-甲醇提取物蒸发，得到3,3′-二吲哚甲烷和吲哚-3-甲醇。

[0033]

This invention involves crushing fresh samples of the edible organs of cruciferous vegetables to obtain fresh samples.

本发明将十字花科蔬菜的可食性器官的新鲜样品破碎，得到新鲜样品。

In this invention, the cruciferous vegetables preferably include one or more of cabbage, broccoli, kale, and kohlrabi.

在本发明中，所述十字花科蔬菜优选包括甘蓝、青花菜、芥蓝和苤蓝中的一种或多种。

[0034]

In this invention, the edible organs preferably include one or more of the following: roots, stems, leaves, flower heads, and seeds.

在本发明中，所述可食性器官优选包括根、茎、叶、花球和种子中的一种或多种。

[0035]

In this invention, the fresh sample is preferably a slice, segment, or block, the segment is preferably 2-3cm × 3-5cm, and the block is preferably 0.3-1.0cm thick.

在本发明中，所述新鲜样品优选为片、段或块，所述段优选为2～3cm×3～5cm，所述块的厚度优选为0.3～1.0cm。

The present invention does not impose any special limitation on the crushing method; any crushing method known to those skilled in the art can be used.

本发明对所述破碎方式没有特殊的限定，采用本领域技术人员熟知的破碎方式即可。

[0036]

After obtaining a fresh sample, the present invention freeze-dries the fresh sample under vacuum and then pulverizes it to obtain a dried sample.

得到新鲜样品后，本发明将所述新鲜样品真空冷冻干燥后粉碎，得到干燥样品。

In this invention, the vacuum freeze-drying pressure is preferably 10-15 Pa, the temperature is preferably -45 to -80°C, and the time is preferably 3-6 days, more preferably 4-5 days.

在本发明中，所述真空冷冻干燥的压力优选为10～15Pa，温度优选为-45～-80℃，时间优选为3～6天，更优选为4～5天。

[0037]

In this invention, the particle size of the dried sample is preferably greater than 70 mesh.

在本发明中，所述干燥样品的粒径优选大于70目。

[0038]

In this invention, the dried sample is preferably stored in a sealed container for later use.

在本发明中，所述干燥样品优选密闭保存以备后用。

[0039]

After obtaining fresh and dried samples, the present invention mixes the fresh or dried samples with a citric acid-phosphate buffer solution and performs enzymatic hydrolysis to obtain an enzymatic hydrolysate.

得到新鲜样品和干燥样品后，本发明将所述新鲜样品或干燥样品分别与柠檬酸-磷酸盐缓冲溶液混合后进行酶解反应，得到酶解液。

In this invention, the preferred material-to-liquid ratio of the fresh sample to the citrate-phosphate buffer solution is 1:20 to 1:30 g/mL, and the preferred material-to-liquid ratio of the dried sample to the citrate-phosphate buffer solution is 1:40 to 1:60 g/mL.

在本发明中，所述新鲜样品与柠檬酸-磷酸盐缓冲溶液的料液比优选为1:20～1:30g/mL，所述干燥样品与柠檬酸-磷酸盐缓冲溶液的料液比优选为1:40～1:60g/mL。

[0040]

In this invention, the temperature of the enzymatic hydrolysis reaction is preferably 20-25°C, and the time is preferably 20-25 min.

在本发明中，所述酶解反应的温度优选为20～25℃，时间优选为20～25min。

In this invention, the enzymatic hydrolysis reaction is preferably carried out under stirring conditions, and there is no special limitation on the stirring speed.

在本发明中，所述酶解反应优选在搅拌的条件下进行，本发明对所述搅拌的转速没有特殊的限定。

The present invention does not impose any special limitation on the mixing method; any mixing method known to those skilled in the art can be used.

本发明对所述混合方式没有特殊的限定，采用本领域技术人员熟知的混合方法即可。

[0041]

In this invention, the pH value of the citric acid-phosphate buffer solution is preferably 8.0.

在本发明中，所述柠檬酸-磷酸盐缓冲溶液的pH值优选为8.0。

[0042]

After obtaining the enzymatic hydrolysate, the present invention extracts the enzymatic hydrolysate with ethyl acetate, and evaporates the resulting supernatant to dryness to obtain the extract.

得到酶解液后，本发明将所述酶解液用乙酸乙酯萃取后，将所得上清液进行蒸干，得到提取物。

In this invention, the extraction is preferably performed three times, and there is no special limitation on the amount of ethyl acetate used in each extraction.

在本发明中，所述萃取的次数优选为3次，本发明对每次萃取时乙酸乙酯的用量没有特殊的限定。

In this invention, the extraction time for each extraction is preferably 20 to 25 minutes, and after each extraction, the supernatant is preferably collected after centrifugation at 5000 rpm for 5 minutes for the next extraction.

在本发明中，所述每次萃取的时间优选为20～25min，每次萃取后优选经5000rpm离心5min后取上清液进行下次萃取。

[0043]

In this invention, the evaporation is preferably carried out by rotary evaporation, and the temperature of the rotary evaporation does not exceed 30°C. This invention does not have a special limitation on the time of rotary evaporation, as long as the moisture is completely removed.

在本发明中，所述蒸干优选为旋蒸，所述旋蒸的温度不超过30℃，本发明对所述旋蒸的时间没有特殊的限定，能够完全除去水分即可。

[0044]

After obtaining the extract, the present invention mixes the extract with a potassium

dihydrogen phosphate-dipotassium hydrogen phosphate buffer solution, then adds ethanol to saturate indole-3-methanol, and allows it to stand to obtain a 3,3′-diindolemethane solution and an indole-3-methanol extract. The 3,3′-diindolemethane solution and the indole-3-methanol extract are then evaporated to obtain 3,3′-diindolemethane and indole-3-methanol, respectively.

得到提取物后，本发明将所述提取物与磷酸二氢钾-磷酸氢二钾缓冲溶液混合后，再加入乙醇使吲哚-3-甲醇饱和，静置，得到3,3′-二吲哚甲烷溶液和吲哚-3-甲醇提取物，分别将所述3,3′-二吲哚甲烷溶液和吲哚-3-甲醇提取物蒸发，得到3,3′-二吲哚甲烷和吲哚-3-甲醇。

[0045]

In this invention, the pH value of the potassium dihydrogen phosphate-dipotassium hydrogen phosphate buffer solution is preferably 6.0.

在本发明中，所述磷酸二氢钾-磷酸氢二钾缓冲溶液的pH值优选为6.0。

[0046]

In this invention, the concentration of the potassium dihydrogen phosphate-dipotassium hydrogen phosphate buffer solution in the mixture after the extract is mixed with the potassium dihydrogen phosphate-dipotassium hydrogen phosphate buffer solution is preferably 0.01M.

在本发明中，所述提取物与磷酸二氢钾-磷酸氢二钾缓冲溶液混合后的混合物中磷酸二氢钾-磷酸氢二钾缓冲溶液的浓度优选为0.01M。

[0047]

In this invention, the settling time is preferably 24 hours, and the settling temperature is preferably room temperature, without the need for additional heating or cooling.

在本发明中，所述静置的时间优选为24h，所述静置的温度优选为室温，不需要额外的加热或降温。

[0048]

In this invention, the evaporation temperature is preferably no more than 60°C.

在本发明中，所述蒸发的温度优选为不超过60°C。

[0049]

After obtaining indole-3-methanol and 3,3′-diindolemethane, the present invention preferably dissolves the indole-3-methanol and 3,3′-diindolemethane in methanol or ethanol respectively and then filters them through a 0.22 μm membrane to obtain I3C and DIM solutions with high purity. After rotary evaporation to dryness, they can be stored in a sealed container at low temperature in the dark, or they can be used directly for backup needs.

得到吲哚-3-甲醇和3,3′-二吲哚甲烷后，本发明优选将所述吲哚-3-甲醇和3,3′-二吲哚甲烷分别采用甲醇或乙醇溶解后过滤膜(0.22μm)，可获得纯度较高的I3C和DIM溶液，旋转蒸干后可以低温避光密闭保存，也可直接用于后备需要。

[0050]

To further illustrate the present invention, the simultaneous extraction method of indole-3-methanol and 3,3′-bisindolemethane provided by the present invention will be described in detail below with reference to the embodiments, but these should not be construed as limiting the scope of protection of the present invention.

为了进一步说明本发明，下面结合实施例对本发明提供的吲哚-3-甲醇和3,3′-二吲哚甲烷的同时提取方法进行详细地描述，但不能将它们理解为对本发明保护范围的限定。

[0051]

Example 1

实施例1

[0052]

1.

1.

Experimental materials and instruments

试验材料与仪器

[0053]

Swiss BüCHI constant temperature water bath, pH meter (HANNA), SHIMADZU LC-20A series high performance liquid chromatograph system, SPD-20AUV/VIS detector, Waters PAH reversed phase C18 (250×4.6mm, 5.0μm).

瑞士BüCHI恒温水浴锅，pH计(HANNA公司)，SHIMADZU LC-20A系列高效液相色谱仪体系，SPD-20AUV/VIS检测器，Waters PAH反相C18(250×4.6mm，5.0μm)。

[0054]

Indole-3-methanol standard was purchased from SIGMA, with a purity greater than 95%; methanol (HPLC); acetonitrile (HPLC); double-distilled water; ethyl acetate (AR); sodium dihydrogen phosphate (AR); citric acid (AR); ultrapure water.

吲哚-3-甲醇标准品购自SIGMA公司，纯度大于95%；甲醇(HPLC)；乙腈(HPLC)；双蒸水；乙酸乙酯(AR)；磷酸二氢钠(AR)；柠檬酸(AR)；超纯水。

[0055]

2.

2.

Extraction method

提取方法

[0056]

2.1 Sample Pretreatment

2.1样品前处理

[0057]

Fresh samples: First, select, remove impurities, and dry the fresh samples. Cut the edible parts of cruciferous vegetables, such as cabbage heads, into small pieces (2-3cm) for later use.

新鲜样品：首先将新鲜样品进行挑选、去杂、擦干，将十字花科蔬菜可食性器官，如甘蓝的叶球，切成小段(2～3cm)备用。

[0058]

Drying the sample: The above fresh sample was dried by vacuum freeze-drying (10 Pa, temperature -45°C) for 3 days according to the machine. After drying, the sample was mechanically crushed (greater than 70 mesh) and the crushed sample was sealed and stored for later use.

干燥样品：将上述鲜样经真空冷冻(10Pa，温度-45°C)干燥处理，根据机器干燥3天，干燥后的样品经机械粉碎后(大于70目)，将粉碎样品密闭保存以备后用。

[0059]

2.2 I3C and DIM extraction

2.2 I3C和DIM提取

[0060]

Take the freeze-dried sample or fresh sample, add a certain volume of buffer solution (pH 8.0, which can be prepared with citrate-phosphate), according to the material-liquid ratio (g/mL), the ratio is 1:40 to 11:60 for dried samples and 1:20 to 1:30 for fresh samples. Carry out the enzymatic hydrolysis reaction at 25°C or room temperature for 1.5 hours, shaking appropriately. Then add ethyl acetate (analytical grade - AR is fine) for extraction for 20

minutes. After centrifugation at 5000 rpm for 5 minutes, take the supernatant. Continue to add ethyl acetate and repeat the above operation twice. Combine the collected (three) supernatants and perform rotary evaporation. The rotary evaporation temperature should not exceed 30°C. Finally, the residue (extract) after evaporation is mainly composed of I3C. It should be sealed and stored at low temperature and protected from light.

取经冷冻干燥后的样品或鲜样，加入一定体积的缓冲液(pH 8.0，可用柠檬酸-磷酸盐配制)，按照料液比(g/mL)，干燥样品为1:40～11:60，鲜样为1:20～1:30的比例加入，25°C条件下或室温下进行酶解反应，反应时间为1.5h，可适当摇动，然后加入乙酸乙酯(分析纯-AR即可)萃取，时间为20min，5000rpm离心5min后取上清，继续加入乙酸乙酯按照上述操作再重复萃取两次，将收集(三次)的上清液合并进行旋转蒸发，旋蒸温度不超过30°C，最后将蒸干后的残留物(提取物)主要成分为I3C，要密封低温避光保存。

[0061]

The residue obtained above (mainly I3C) was dissolved in a buffer solution prepared with potassium dihydrogen phosphate and dipotassium hydrogen phosphate at pH 6.0 and a

concentration of 0.01M. It was stored at room temperature and protected from light. Alcohol with a purity of 99% or higher was added until I3C was saturated. After standing for 24 hours, a light red DIM aqueous solution was obtained. The DIM residue was obtained by heating to evaporate the water (water temperature below 60°C).

将上述获得的残留物(主要成分为I3C)溶解在用磷酸二氢钾和磷酸氢二钾配成的缓冲液中，pH为6.0，浓度为0.01M的条件下，室温下，避光保存，加入纯度99％以上的酒精直至I3C饱和，静止24h后便可得到淡红色的DIM水溶液，加热使得水分蒸发(水温低于60°C)后可得到DIM残留物。

[0062]

3.

3.

HPLC detection system

HPLC检测体系

[0063]

3.1 Chromatographic conditions

3.1色谱条件

[0064]

The system used was a SHIMADZU LC-20A series high-performance liquid chromatograph (HPLC) system from Japan, with an SPD-20AUV/VIS detector, a Waters PAH reversed-phase C18 column (250×4.6mm, 5.0μm); a flow rate of 1.0 mL・min⁻¹; a column

temperature of 30°C; an injection volume of 10 μL; a detection wavelength of 279 nm; and gradient elution with acetonitrile and water as the mobile phase (elution program shown in Table 1).

日本SHIMADZU LC-20A系列高效液相色谱仪体系，SPD-20AUV/VIS检测器，WatersPAH反相C18 (250×4.6mm，5.0μm)；流速1.0mL・min⁻¹；柱温：30°C；进样量10μL；检测波长279nm；梯度洗脱，流动相为乙腈和水(洗脱程序见表1)。

[0065]

The retention time of indole-3-methanol was 4.95 min (see Figure 1).

吲哚-3-甲醇保留时间为4.95min(见图1)。

[0066]

Table 1 Elution Procedure

表1洗脱程序

[0068]

3.2 Standards and Samples

3.2标准品和样品

[0069]

Accurately weigh 50.00 mg of indole-3-methanol standard sample and dilute to a 10.00 mL amber volumetric flask with methanol for later use.

精确称取吲哚-3-甲醇标准样品50.00mg，用甲醇定容至10.00m L棕色容量瓶中，备用。

[0070]

Add an appropriate amount of I3C extract to methanol to make up the volume, then filter it through a 0.22μm nylon filter membrane (D 13mm) before injection for detection.

将I3C提取物物适量加入甲醇进行定容，然后过尼龙滤膜0.22μm(D 13mm)后即可进样检测。

[0071]

3.3 Linear Equations

3.3线性方程

[0072]

The indole-3-methanol standard solution was diluted with methanol to prepare 2.04 μg・mL NER9, 4.07 μg・mL NER10, 8.13 μg・mL NER11, 32.50 μg・mL NER12, and 65.00 μg・mL NER13, and then injected separately. The standard curve was calculated with peak area as the abscissa and concentration as the ordinate.

将吲哚-3-甲醇标准溶液用甲醇稀释成2.04μg・mL⁻¹、4.07μg・mL⁻¹、8.13μg・mL⁻¹、32.50μg・mL⁻¹、65.00μg・mL⁻¹，然后分别进样，以峰面积为横坐标，以浓度为纵坐标计算标准曲线。

[0073]

The regression equation for indole-3-methanol in the range of 2.04–65.00 μg・mL⁻¹ is Y = 3.828 × 10⁻⁵X - 0.730, R² = 0.9999 (n = 5, RSD = 3.09%).

吲哚-3-甲醇在2.04～65.00μg・mL⁻¹范围内的回归方程为Y＝3.828×10⁻⁵X-0.730，R²＝0.9999(n＝5，RSD＝3.09%)。

[0074]

3.4 Precision

3.4精密度

[0075]

A standard solution of indole-3-methanol with a concentration of 50.00 μg・mL⁻¹ was prepared and repeatedly injected and measured 6 times. The precision was calculated based on the measured peak area of the standard. The relative standard deviation of the peak area of the 6 measurements was RSD = 2.503E⁻⁹, indicating good precision.

配制浓度为50.00μg・mL⁻¹的吲哚-3-甲醇标准品溶液，反复进样测定6次，根据测得的标准品峰面积计算精密度，测得6次峰面积的相对标准偏差RSD＝2.503E⁻⁹%，精密度良好。

[0076]

3.5 Stability

3.5稳定性

[0077]

A standard solution of indole-3-methanol with a concentration of 50.00 μg・mL NER19 was prepared and injected at 0.0 h, 2.0 h, 4.0 h, 6.0 h, 8.0 h, and 10.0 h for determination. The stability was calculated based on the peak area. The results showed that the alcoholic solution of indole-3-methanol had good stability (RSD = 0.36%).

配制浓度为50.00μg・mL⁻¹的吲哚-3-甲醇标准品溶液，分别于0.0h、2.0h、4.0h、6.0h、8.0h、10.0h进样测定，根据峰面积计算稳定性，结果表明哚-3-甲醇的醇溶液稳定性良好(RSD＝0.36%)。

[0078]

3.6 Recovery rate

3.6加样回收率

[0079]

Accurately weigh the aqueous solution of indole-3-methanol with known content, add appropriate amounts of indole-3-methanol standard (see Table 2), and determine according to the method described in "3.1".

准确称取已知含量吲哚-3-甲醇水溶液，分别加入适量的吲哚-3-甲醇标准品，见表2，按照“3.1”所述方法进行测定。

The average recovery rate was 99.25% (n=6, RSD=1.00%).

测得平均回收率为99.25%(n＝6，RSD＝1.00%)。

[0080]

Table 2 Recovery rates of indole-3-methanol

表2吲哚-3-甲醇的回收率

[0082]

4.

4.

Sample Analysis

样品分析

[0083]

Taking cabbage as an example, 15 cabbage heads were tested, with each sample tested in triplicate (n=3). The results, obtained using the method described above, are shown in Table 3. Significant differences in indole-3-methanol content were observed among the samples. The indole-3-methanol content ranged from 8.21 to 87.88 mg/kg DW (dry weight), with an average content of 28.85 mg/kg DW. The highest content was 87.88 mg/kg DW in C238, and the lowest was 6.62 mg/kg DW in C575. This indicates a significant difference in indole-3-methanol content among different materials, which can be used for material screening and research.

以甘蓝为例，对15份甘蓝叶球进行测定，每份样品设置3次重复(n＝3)，按照上述方法操作，结果如下表3，样品间吲哚-3-甲醇含量达到了显著差异，DW其变异范围为8.21～87.88mg/kg DW(干重)，平均含量为28.85mg/kg DW，最高含量为C238中87.88mg/kg DW，最低含量为C575的6.62mg/kg DW，表明不同材料间间吲哚-3-甲醇含量差异较大，可以用于材料的筛选和研究。

[0084]

Table 3. HPLC analysis results of indole-3-methanol in cabbage heads

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表3甘蓝叶球中吲哚-3-甲醇HPLC分析结果

[0087]

The above description is merely a preferred embodiment of the present invention and is not intended to limit the present invention in any way.

以上所述仅是本发明的优选实施方式，并非对本发明作任何形式上的限制。

It should be noted that those skilled in the art can make various improvements and modifications without departing from the principles of this invention, and these improvements and modifications should also be considered within the scope of protection of this invention.

应当指出，对于本技术领域的普通技术人员来说，在不脱离本发明原理的前提下，还可以做出若干改进和润饰，这些改进和润饰也应视为本发明的保护范围。