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(54) Title: A PROCESS FOR PRODUCING GARDENIA BLUE PIGMENT FORM GENIPOSIDE

(57) Abstract: A process for producing the gardenia blue pigment comprising treating geniposide with a glycosidase to obtain a hydrolysate, extracting the hydrolysate with a solvent and removing the solvent after the extraction to obtain a product comprising genipin, reacting the product comprising genipin with an amino acid and/or a salt thereof under an atmosphere of inert gas, and introducing oxygen after genipin is consumed to produce the gardenia blue pigment. The process is easy-to-workup and suitable for industry and the obtained gardenia blue pigment is bright and suitable for industrial application.



A PROCESS FOR PRODUCING GARDENIA BLUE PIGMENT FROM GENIPOSIDE

Technical Field

- 5 The present invention is related to an improved process for producing a natural pigment. In particular, the present invention is related to an improved process for producing the gardenia blue pigment.

Background of the Invention

10 The gardenia blue pigment is a water-soluble natural pigment widely used in food, pharmaceutical and cosmetics industries.

Nowadays, the gardenia blue pigment is usually produced from the raw material geniposide contained in *Gardenia Jasminoides Ellis* of Rubiaceae by the process of treating geniposide with a β -glucosidase to obtain genipin which reacts with an amino acid to obtain the gardenia blue pigment. (see Shijing WU, *et al.*, National Food Additive Communications, 1992 (3): 90-93)
15 However, the gardenia blue pigment obtained by this process is dark, it has a low color value and is of low quality. In addition, the color reaction time of the process is long (10-50 hours). Accordingly, it is not suitable for industrial applications. (see e.g. CN103509368A and CN102021210A)

Feng CHEN discloses a process for producing the gardenia blue pigment with a high color value, which comprises ultra-filtering the gardenia blue pigment obtained from the reaction of genipin with an amino acid to remove the residual geniposide and then extracting the filtrate to obtain the gardenia blue pigment of a high color value (see CN103525883A). Lijun SUN *et al.* discloses another process, which comprises passing the raw material geniposide through a large mesh non-polar resin to remove α -crocin before treating it with β -glucosidase. (Lijun SUN *et al.*, Journal of
20 Nanjing Agricultural University, 1994, 17(4): 98-101) However, these processes involve high cost and complicated operations, and are not suitable for being carried out at large scale in industry.

Therefore, there is still the need for an improved process, which is easy-to-workup and suitable for industry, for producing the gardenia blue pigment which is bright, stable and suitable for industrial applications.

30 Summary of the Invention

The present invention provides an improved process for producing a gardenia blue pigment, comprising the following steps:

- a) Treating geniposide with a glycosidase to obtain a hydrolysate; and

- b) Extracting the hydrolysate obtained in step a) with a solvent and removing the solvent after the extraction to obtain a product comprising genipin; and
- c) Reacting the product comprising genipin obtained in step b) with an amino acid and/or a salt thereof under an atmosphere of inert gas, and
- 5 d) Introducing oxygen after genipin is consumed to produce the gardenia blue pigment; and
- e) Optionally, purifying the gardenia blue pigment produced in step d).

The process of the present invention is easy-to-workup and thus suitable for manufacture in the industry. In addition, the obtained gardenia blue pigment is sky blue, brighter than the blue such as ultramarine blue produced by the known processes, and it has a color value of >100, so it is

10 suitable for industrial applications.

Detailed Description of the Invention

The present invention provides a process for producing the gardenia blue pigment which is sky blue and may have a color value of >100. In particular, the process of the present invention comprises the following steps:

- 15 a) Treating geniposide with a glycosidase to obtain a hydrolysate; and
- b) Extracting the hydrolysate obtained in step a) with a solvent and removing the solvent after the extraction to obtain a product comprising genipin; and
- c) Reacting the product comprising genipin obtained in step b) with an amino acid and/or a salt thereof under an atmosphere of inert gas, and
- 20 d) Introducing oxygen after genipin is consumed to produce the gardenia blue pigment; and
- e) Optionally, purifying the gardenia blue pigment produced in step d).

In the process, the geniposide used as raw material may be from various sources. It may be obtained by extracting the fruit *Gardenia Jasminoides Ellis* by any known process, for example, that as disclosed in Chinese patent publication CN102732050A. In addition, geniposide powders,

25 which contain about 20wt% to about 70wt% of geniposide and are commercially available, and the waste stream from the gardenia yellow production, which contains about 40wt% of geniposide and is also commercially available, may be used into the process directly or after simple refining. (see CN103509368A, CN103525883A etc.)

The glycosidase is an enzyme under EC 3.2.1 according to the Recommendations of the

30 Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes by the Reactions they Catalyse. Preferably, the glycosidase is cellulase (EC 3.2.1.4) and β -glucosidase (EC 3.2.1.21). The examples of the glycosidase include but are not limited to cellobiase commercially available from Sunson Biotechnology Co. Ltd. (Guangzhou, China), Cellulase 4000 commercially available from DSM

35 (China) Ltd. (Shanghai, China), and Rapidase[○],^R commercially available from DSM (China) Ltd. (Shanghai, China). The glycosidase may be added into the reaction of the step a) in an amount in the range of from 0.01 g to 1.0 g, preferably in an amount in the range of from 0.1 g to 0.9 g, more preferably in an amount in the range of from 0.3 g to 0.8 g, per 1 g of geniposide.

The treatment of the step a) may be carried out at a pH in the range of from 3.0 to 6.5, preferably at a pH in the range of from 3.6 to 6.0, and more preferably at a pH in the range of from 4.0 to 4.6. Preferably, the treatment of the step a) is carried out in a buffer solution which can provide the above pH ranges. Such buffer solution is known in the art, and the examples include but are not limited to an aqueous HCOOH/NaOAc solution or an aqueous citric acid/Na₂HPO₄ solution. The buffer solution may be added in an amount in the range of from 8 mL to 80 mL, preferably in an amount in the range of from 10 mL to 70 mL, more preferably in an amount in the range of from 15 mL to 50 mL, per 1 g of geniposide.

The treatment of the step a) may be carried out at a temperature in the range of from about 20°C to about 60°C, preferably at a temperature of about 50°C.

The reaction of the step a) may last about 2 hours to about 30 hours, preferably 4 hours to 10 hours, and more preferably less than 8 hours. The obtained hydrolysate contains genipin as main component and other components. The hydrolysate can be used for the extraction in step b) directly.

In the prior process, the hydrolysate obtained from the step a) is normally reacted with an amino acid directly to produce gardenia blue pigment. However, the gardenia blue pigment obtained from the prior process is dark and not good for some industrial applications such as beverages. The inventors of the present invention surprisingly discovered that an additional extraction of the step b) results in sky blue color, a bright blue pigment which is more applicable for industrial applications like beverages.

Any person skilled in the art could understand that, the solvent used for extraction in the step b) is important and may be any one suitable for the purpose of the invention, and examples include but are not limited to diethyl ether, ethyl acetate, butanol, a mixture of butanol with petroleum and/or hexane, or mixtures thereof. In the embodiment that the solvent is a mixture of butanol with petroleum and/or hexane, the volume ratio of butanol with petroleum and/or hexane is in the range of from 1:5 to 5:1, preferably in the range of from 1:3 to 3:1, and more preferably in the range of from 1:2 to 2:1. Preferably, the solvent used in the step b) is ethyl acetate.

The amount of the solvent used in the step b) may be from 0.5 mL to 5 mL, preferably from 1.0 mL to 3 mL, per 1 mL of the hydrolysate. The extraction of the step b) may be carried out at a temperature in the range of from 10°C to 60°C, preferably at room temperature. According to the present invention, the extraction of the step b) may be repeated two to four times.

The product comprising genipin is obtained after the organic phases are collected and the solvent is removed in the step b). The solvent may also be recycled. The extraction procedures and the procedures for collecting solvents and removing/recycling solvents during the extraction are known to the person skilled in the art. Therefore, they are not discussed in more detail here.

As known in the art, genipin reacts with an amino acid or a salt thereof in one step to provide gardenia blue pigment. Different from the known process, the present invention provides an

improved process with a two-steps color reaction to produce the gardenia blue pigment. As steps c) and d) of the process of the present invention, the product comprising genipin obtained in the step b) is reacted with an amino acid or a salt thereof under an atmosphere of inert gas at first, and then oxygen is introduced after the genipin is consumed to produce the gardenia blue pigment. By the two-steps color reaction, the inventors of the present invention surprisingly discovered that the process of the present invention provides more consistent and stable gardenia blue pigment with higher color value, and the color reaction time is reduced significantly from more than 30 hours to less than 10 hours.

In the step c) of the present invention, the amino acid may be selected from the group consisting of glutamate, phenylalanine, histidine, leucine, isoleucine, arginine and mixture thereof. The salt may be any alkali metal salt such as sodium salt. Preferably the salt is sodium glutamate. The inventors of the present invention discovered that the amino acid and the salt used in the step c) are also important because they can provide sky blue color as disclosed in the present invention. Preferably, the amino acid used in the step c) is sodium glutamate.

In the step c) of the present invention, the amino acid may be added in an amount of 0.5-10 moles, preferably 0.8-6 moles, and more preferably 1-3 moles, per 1 mole of genipin in the reaction. Preferably, the amino acid is added in an aqueous solution.

In one embodiment, the product comprising genipin obtained from the step b) is dissolved in a water-soluble solvent to react with the amino acid in the step c). The water-soluble solvent may be any one known in the art that can dissolve the product comprising genipin and examples include but are not limited to C₁₋₁₀ alkanols such as methanol and ethanol, and C₃₋₁₀ ketones such as acetone. Preferably, the water-soluble solvent is methanol, ethanol or acetone or any mixture thereof. In one preferable embodiment of the process, the product comprising genipin obtained from the step b) is reacted with an aqueous solution of the amino acid in the absence of any organic solvent in the step c). No organic solvent process would be preferred in the industry because of safety advantage and others like easy-to-workup, reduced organic solvent residue and low cost.

The inert gas used in step c) may be any gas that cannot react with any material in the reaction liquid. The example of the inert gas includes but is not limited to nitrogen gas, argon gas and helium gas.

The reaction of the step c) may be carried out at about 30°C to about 100°C, preferably about 60°C to 80°C, such as 60°C, 65°C, 70°C and 75°C. The progress of the reaction can be monitored by any known method, such as HPLC and TLC.

Preferably, the step c) is carried out at a pH value in the range of from 7.0 to 11. In some embodiments, a base selected from but not limited to NaOH, KOH, NaCO₃ and NaHCO₃ is added to adjust the reaction mixture of the step c) to an appropriate pH value.

After the genipin in the reaction liquid is consumed, preferably after all the genipin is consumed, oxygen is introduced into the reaction liquid to produce the gardenia blue pigment as step d) of the present invention. The oxygen may be introduced as pure oxygen, air and/or diluted oxygen or air. Preferably, the oxygen is introduced as air.

5 The reaction of the step d) of the present invention may continue till the desired gardenia blue is obtained. Preferably, the reaction lasts from 4 hours to 20 hours, more preferable from 6 hours to 15 hours, under the same range of temperature as the step c). The reaction may be terminated or quenched by any know method in the art, for example, by introducing vacuum or inert gas when the desired gardenia blue is obtained.

10 After the reaction is complete, the gardenia blue pigment can be obtained as a solid by removing the organic solvent and water in the reaction mixture. Accordingly, the process of the present invention optionally further comprises the step of removing the solvent and water to provide a solid of the gardenia blue pigment by, for example, lyophilization or spray drying.

15 Optionally, the obtained gardenia blue pigment can be purified further by any procedures known in the art such as ultrafiltration to obtain an even purer gardenia blue pigment. The process of the present invention produces the gardenia blue pigment which is sky blue, brighter than the blue color such as ultramarine blue produced by the known processes and thus more popular for some industrial applications such as beverages.

20 In addition, the obtained gardenia blue pigment of the present invention has a color value of >100, that means low dosage is needed in applications and more stable in beverage. Further, by an additional extraction step, the obtained gardenia blue pigments can be easily separated and purified from the reaction mixture without complicated operations. Furthermore, by the two-steps color reaction, the process of the present invention becomes easy-to-workup and has reduced reaction time, and the obtained gardenia blue pigment is more consistent.

25 In the present invention, color (lightness, chroma, and hue) of the gardenia blue pigment is determined with a HunterLab Ultra Scan Pro spectrophotometer (Hunter Associates Laboratory, Reston, VA, USA) and expressed on basis of the CIELAB color scale. The mode used is RSIN which stands for Reflectance - Specular Included. The small area view (SAV) with a diameter of 4.826 mm (0.190 inch) is chosen. Color measurements are carried out after CIE guidelines
30 (Commission International d'Eclairage). Values can be expressed as planar coordinates as L*, a*, b* with L* being the measuring values for lightness, with a* being the value on the red-green axes and b* being the value on the yellow-blue axes.

The Chroma (C*) sometimes called saturation describes the vividness or dullness of a color which can be calculated as followed:

35 $C^* = \sqrt{a^{*2} + b^{*2}}$

The angle called hue (h) describes how we perceive an object's color and can be calculated as followed:

$$h = \tan(b/a)^{(-1)}$$

Instruments settings:

- 5 • Color scale is the CIE L*a*b*/L*C*h
- Light source definition: D65 daylight equivalent
- Geometry: Diffuse/8°
- Wavelength: scan 350-1050 nm
- Sample measurement area diameter: 4.826 mm
- 10 • Calibration mode: Reflection/ Specular-included

In the present invention, the maximum absorption wavelength ($\lambda_{\max}$) and the color value are measured according to the national standard GB 28311-2012 of China.

The present invention is illustrated further by the following Examples. These Examples are not intended to limit the invention in any way.

15 Examples

Example 1

- 420 g liquid geniposide (40wt%) purchased from He'nan ZhongDa Hengyuan Biotechnology Co., Ltd. (He'nan, China) was added into 2.9 L of an aqueous citric acid/ Na_2HPO_4 buffer solution (pH 4.0), the resulting mixture was heated to 50°C. 50.4 g of cellobiase purchased from Sunson
- 20 Biotechnology Co. Ltd. (NingXia, China) was further added to the preheated solution for reaction for 10 hours under 50°C. After filtration to remove insolubles, the reaction mixture was extracted with 2.9 L ethyl acetate twice. The organic phase was concentrated under vacuum to remove part of ethyl acetate. About 560 g of ethyl acetate solution was left for further precipitation at about 50°C, 200 mbar to offer 58.9 g crude genipin powder with 98.0% HPLC purity.
- 25 In a reaction flask with 4.5 g of the above genipin powder, 67 ml of ethanol was used to dissolve the powder. 4.42 g sodium glutamate was dissolved in 67 ml of deionized water and charged to the flask with genipin solution. The resulting mixture was stirred under nitrogen at 75°C for about 4.5 hours when genipin totally disappeared by NMR monitoring. Then nitrogen in the system was blown away by air and the resulting mixture was stirred in the presence of air for another 6 hours
- 30 at 75°C. After removing ethanol under vacuum, freeze drying was performed to offer about 7.8 g of gardenia blue pigment as solid powder.

The obtained gardenia blue was dissolved in deionized water to provide a test solution for color test under the maximum absorbance of 0.337. The test results of $\lambda_{\max}$, color value and L^* , a^* , b^* and h values are summarized in table 1.

Example 2

- 5 3 g of the genipin produced in Example 1 and 2.95 g of sodium glutamate were added to 90 ml of deionized water. The resulting mixture was stirred under nitrogen at 75°C for about 4.5 hours when genipin totally disappeared by NMR monitoring. Then nitrogen in the system was blown away by air and the resulting mixture was stirred in the presence of air for another 8 hours at 75°C. The resulting solution was freeze dried to offer about 5.2 g of gardenia blue as solid powder.
- 10 The obtained gardenia blue was dissolved in deionized water to provide a test solution for color test under the maximum absorbance of 0.337. The test results of $\lambda_{\max}$, color value and L^* , a^* , b^* and h values are summarized in table 1.

Table 1

Example No.	Color value (E)	$\lambda_{\max}$	L^*	a^*	b^*	c^*	h
1	135.79	598	84.88	-9.22	-10.85	14.24	229.66
2	100.45	598	85.48	-9.96	-10.36	14.37	226.14

15 Example 3

- 81 kg of liquid geniposide (40wt% geniposide) purchased from Qianjiang Green Sea Treasury Biotechnology Co., Ltd. (Hubei, China) was added into 565 L of an aqueous citric acid/ Na_2HPO_4 buffer solution (pH 4.0), the resulting mixture was heated to 50°C. 24.6 kg of cellobiase purchased from Sunson Biotechnology Co. Ltd. (NingXia, China) was further added to the preheated solution
- 20 for reaction for 10 hours under 50°C. After centrifugation to remove insolubles, the reaction mixture was extracted twice with 650 L of ethyl acetate. The combined organic phase was concentrated under vacuum to remove part of ethyl acetate to left 150 L of ethyl acetate solution. 730 ml of the ethyl acetate solution was further precipitated at about 50°C, 200 mbar to offer 38.3 g crude genipin powder with 91.15% HPLC purity.
- 25 20 g of the above genipin powder and 18.02 g of sodium glutamate were added to 600 ml of deionized water. The resulting mixture was stirred under nitrogen at 75°C for about 4.5 hours when genipin totally disappeared by NMR monitoring. Then nitrogen in the system was blown away by air and the resulting mixture was stirred in the presence of air for another 8 hours at 75°C. The resulting solution was freeze dried to offer about 35.5 g of gardenia blue as solid powder with
- 30 $\lambda_{\max}$: 598 nm, and color value: 110.87.

Example 4 - comparative

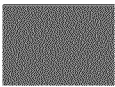
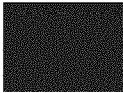
8 g of geniposide powder (60wt% geniposide) purchased from YuanYang Bio-engineering Co., Ltd.(Xi'an, China) was added into 64 ml of an aqueous citric acid/ Na_2HPO_4 buffer solution (pH 4.6). 240 mg of Cellulase 4000 purchased from DSM (China) Ltd. (Shanghai, China) was further added for reaction for 24 hours at 50°C. Then 2.0 g of sodium glutamate was added into the reaction mixture for 96 hours at 50°C.

The reaction mixture was filtered, purified by ultrafiltration, and then lyophilized to obtain 2.8 g of gardenia blue pigment as solid powder, with a maximum absorption wavelength of 591 nm and a color value of 11.25.

Example 5

- 10 The obtained gardenia blues obtained from above examples 1-3 and the comparative examples 4 were compared. According to the Industrial International Standard Color Chart ("IISCC"), the gardenia blue pigments obtained from examples 1-3 are called "sky blue" while the gardenia blue pigment obtained from the comparative example 4 is called "ultramarine blue". Table 2 indicates the results. Obviously, the "sky blue" is brighter than the "ultramarine blue".

15 **Table 2**

No.	called in IISCC
Examples 1-3	 Sky blue
Example 4	 Ultramarine blue

Claims

1. A process for the preparation of gardenia blue pigment, comprising the following steps:
 - a) Treating geniposide with a glycosidase to obtain a hydrolysate; and
 - b) Extracting the hydrolysate obtained in step a) with a solvent and removing the solvent after the extraction to obtain a product comprising genipin; and
 - c) Reacting the product comprising genipin obtained in step b) with an amino acid and/or a salt thereof under an atmosphere of inert gas, and
 - d) Introducing oxygen after genipin is consumed to produce the gardenia blue pigment; and
 - e) Optionally, purifying the gardenia blue pigment produced in step d).
2. The process of claim 1, wherein the glycosidase is cellulase (EC 3.2.1.4) or β -glucosidase (EC 3.2.1.21).
3. The process according to any one or more of the preceding claims, wherein the treatment of step a) is carried out at a pH in the range of from 3.0 to 6.5, preferably at a pH in the range of from 3.6 to 6.0, and more preferably at a pH in the range of from 4.0 to 4.6.
4. The process according to any one or more of the preceding claims, wherein the solvent used in the step b) is diethyl ether, ethyl acetate, butanol, a mixture of butanol with petroleum and/or hexane, or mixtures thereof.
5. The process according to any one or more of the preceding claims, wherein the solvent is used in an amount in the range of from 0.5 mL to 5 mL, preferably from 1.0 mL to 3 mL, per 1 mL of the hydrolysate.
6. The process according to any one or more of the preceding claims, wherein the amino acid used in step c) is selected from the group consisting of glutamate, phenylalanine, histidine, leucine, isoleucine, arginine and mixture thereof.
7. The process according to any one or more of the preceding claims, wherein the salt is alkali metal salt such as sodium salt, preferably the salt is sodium glutamate.
8. The process according to any one or more of the preceding claims, wherein in the step c) the product comprising genipin obtained from step b) is reacted with the amino acid in a water-soluble solvent or in the absence of any organic solvent.
9. The process according to any one or more of the preceding claims, wherein the inert gas is nitrogen gas, argon gas or helium gas.
10. The process according to any one or more of the preceding claims, wherein the reaction of the step c) is carried out at about 30°C to about 100°C, preferably about 60°C to 80°C, such as 60°C, 65°C, 70°C and 75°C.

11. The process according to any one or more of the preceding claims, wherein the oxygen in the step d) is introduced as pure oxygen, air and/or diluted oxygen or air.

12. The gardenia blue pigments obtainable from the process according to any one or more of the preceding claims.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/070421

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12P17/16 C09B61/00 C09B67/54 A23L5/43
ADD.

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)

C12P C09B A23L

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)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/041500 A1 (DSM IP ASSETS BV [NL]) 24 March 2016 (2016-03-24)	12
Y	page 3, lines 14-19; claims; examples -----	1-11
X	WO 2013/070682 A1 (WILD FLAVORS INC [US]; WU SHAOWEN [US]; HORN GREGORY [US]) 16 May 2013 (2013-05-16)	12
Y	paragraph [0030]; claims; examples -----	1-11
X	CN 105 624 198 A (HENAN ZHONGDA HENGYUAN BIOTECHNOLGY CO LTD) 1 June 2016 (2016-06-01)	12
Y	the whole document -----	1-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search

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Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 2016041500	A1	24-03-2016	CN	106715703 A		24-05-2017
			EP	3221462 A1		27-09-2017
			JP	2017527282 A		21-09-2017
			KR	20170059993 A		31-05-2017
			US	2017260394 A1		14-09-2017
			WO	2016041500 A1		24-03-2016

WO 2013070682	A1	16-05-2013	AU	2012335907 A1		29-05-2014
			CA	2863810 A1		16-05-2013
			CL	2014001195 A1		27-02-2015
			CN	104168775 A		26-11-2014
			EA	201490824 A1		30-10-2014
			EP	2775861 A1		17-09-2014
			EP	3238550 A1		01-11-2017
			JP	6152384 B2		21-06-2017
			JP	2014532441 A		08-12-2014
			JP	2017169584 A		28-09-2017
			NZ	624662 A		29-05-2015
			US	RE46314 E		21-02-2017
			US	2013115252 A1		09-05-2013
			WO	2013070682 A1		16-05-2013

CN 105624198	A	01-06-2016	NONE			
