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# (54) METHOD FOR EXTRACTING SQUALENE FROM GRAPE SKIN DREGS.

(57) Disclosed is a method for extracting squalene from grape skin dregs. The method comprises the following steps: (1) pre-processing grape skin dregs: putting the grape skin dregs in an oven of 70 degrees for drying, then putting the dried grape skin dregs into a crusher for treatment for 5 min, and crushing seeds, seed coats and some fruit stalks in the skin dregs into powder for subsequent organic solvent leaching treatment; and (2) extracting squalene from the grape skin dregs powder obtained in step (1) by taking a non-polar organic solvent such as chloroform, petroleum ether or n-hexane as the extraction solvent and using an ultrasonic-assisted organic solvent extraction method as the extraction process. According to the present invention, the grape skin dregs are used as a raw material, so that the problems of scarce squalene sources and reuse of wine brewing by-product waste are solved; moreover, the process flow is simple, the extraction efficiency is high, and the acquisition cost of the squalene is greatly reduced.

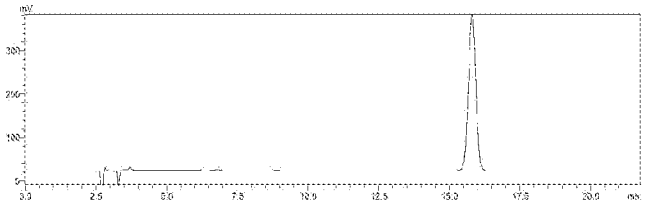

Fig. 1

# METHOD FOR EXTRACTING SQUALENE FROM GRAPE SKIN DREGS

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## TECHNICAL FIELD

The present invention relates to the technical field of squalene preparation methods, in particular to a method for extracting squalene from grape skin dregs.

## BACKGROUND

Squalene, the biosynthetic precursor of steroid and terpenoid, is a non-toxic marine bioactive substance with the efficacy of preventing and treating diseases.

The squalene is widely distributed and can be found in animals and plants, among which deep-sea sharks contain the most squalene in their bodies, which is related to their living environment. As precious protected animals, the deep-sea sharks have a very low growth rate and reproduction rate. However, the production of squalene in large quantities will lead to the ecological imbalance of deep-sea sharks, so it is an inevitable trend to extract the squalene from other renewable resources.

In recent years, there have been reports of squalene extraction with the plants, such as, Chinese patent (patent application No.200910069670.8), entitled “METHOD FOR EXTRACTING HIGH-PURITY SQUALENE FROM OLIVE OIL”, Chinese patent (Patent Application No.201010190275.8), entitled “METHOD FOR EXTRACTING SQUALENE FROM DEODORIZED DISTILLATE OF VEGETABLE OIL”, and Chinese patent (patent application No.200410022116.1), entitled “SIRAITIA GROSVENORII SQUALENE AND PREPARATION METHOD THEREOF”. However, it is found that the accumulation of squalene-containing plant resources is small, and the cost of raw materials is high, which makes it difficult to extract the squalene from the raw plant materials for development and utilization.

Squalene is used more and more widely, and the market demand is increasing, the limited squalene resources can no longer meet people's needs, so it is of great significance to explore new squalene resources.

## BRIEF SUMMARY OF THE INVENTION

The objective of the present invention is to provide a method for extracting squalene from by-products in the grape brewing industry, which uses grape skin dregs as raw material and uses an ultrasonic-assisted extraction method to extract the squalene to effectively solve the problems of scarce squalene sources and the reuse of by-product waste in wine brewing.

In order to achieve the above objective, the technical solution used by the present invention is as follows:

A method for extracting squalene from grape skin dregs comprises the following steps:

1. pre-processing the grape skin dregs: putting fresh grape skin dregs with fresh wine drained into an oven at 70°C for drying same overnight to a constant weight; putting the dried grape skin dregs in a high-speed crusher for crushing same into powder; packing the powder in a sealed bag, and storing the powder in a dryer for use;

2. extracting squalene in the skin dregs from the powder obtained in step 1 by taking petroleum ether, chloroform or n-hexane as an extraction solvent and using an ultrasonic-assisted extraction method;

3. centrifuging a suspension obtained in step 2 at 10,000 r/min for 10 min to obtain a clear squalene extract; and removing the organic solvent by means of rotary evaporating the leaching liquid in a rotary evaporator to obtain crude squalene; and

4. detecting the crude squalene obtained in step 3 by utilizing high-performance liquid

chromatography, the peak retention time on a chromatogram of the crude squalene is completely consistent with that of a squalene standard sample, and the content is 1.985 mg/g-3.561 mg/g.

As a further improvement of the present invention, the extraction method in step 2 is: weighing 100 g of processed grape skin dregs; respectively adding the chloroform, the petroleum ether or the n-hexane according to a ratio of material to liquid of 1:1, 1:3, 1:5, 1:7 and 1:9; and placing in a constant-temperature water bath oscillator of 200 r/min at 37°C for extracting same for 3-20 h, wherein ultrasonic power is 85 W and ultrasonic time is 10 min, 20 min, 30 min and 45 min.

As a further improvement of the present invention, a detection method in step 4 is, a liquid chromatography column is LiChrospher® 100 RP-18 endcapped (250 mm×4.6 mm, 5 μm); a mobile phase is acetonitrile-acetone (60:40, vol/vol); a detection wavelength is 208 nm; a flow rate is 1.0 ml/min; a column temperature is 30°C; and a sample size is 10 μL.

The beneficial effects of the present invention are:

1. the wastes in the field of wine brewing are used as the raw material, which solves the problems of high cost and scarce sources of the raw material of squalene extraction. The depletion of marine fishery resources limits the process of extracting squalene from sharks in the future, while, the accumulation of squalene-containing plant resources is small, which makes it difficult to develop and utilize the plant resources;

2. a large number of by-products such as the skin dregs are produced in a wine production process, and the process flow of the present invention is simple, the extraction efficiency is high, and the acquisition cost of the squalene is greatly reduced;

3. the grape skin dregs are used as the extraction raw material, the problem of the reusing by-product waste of the wine brewing is solved.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

In order to explain the embodiments of the present invention or the technical solutions in the prior art more clearly, the accompanying drawings required in the embodiments or the description of the prior art will be briefly introduced below. Obviously, the accompanying drawings in the following description are only some embodiments of the present invention, and a person of ordinary skill in the art can still derive other drawings according to these accompanying drawings without creative efforts.

Fig. 1 is a high-performance liquid chromatogram of a squalene standard sample of the present invention; and

Fig. 2 is a high-performance liquid chromatogram of a squalene in the extract of the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

To make the objectives, technical solutions and advantages of the present invention clearer, the present invention will be further described in detail with reference to the accompanying drawings and specific embodiments. It should be understood that the specific embodiments described herein are only used to explain the present invention, and are not used to limit the present invention.

Embodiment 1

100 g of grape skin dregs powder is taken and put into a triangular flask, and 100 mL chromatographic grade n-hexane is added. The triangular flask is placed on an ultrasonic crusher with power of 85 W and the ultrasonic time is 10 min, and put in a

constant-temperature water bath oscillator of 200 r/min at 37°C for extraction for 3 h. Then, a suspension is centrifuged at 10,000 r/min for 10 min to obtain a supernatant. The supernatant is placed on a rotary evaporator to remove all solvents to obtain crude squalene. With reference to Fig. 1 and Fig. 2, the extraction amount of the squalene is 1.985 mg/g dry weight of the grape skin dregs by high-performance liquid chromatography.

Embodiment 2

100 g of grape skin dregs powder is taken and put into a triangular flask, and 300 mL chloroform is added. The triangular flask is placed on an ultrasonic crusher with power of 85 W and the ultrasonic time is 20 min, and put in a constant-temperature water bath oscillator of 200 r/min at 37°C for extraction for 5 h. Then, a suspension is centrifuged at 10,000 r/min for 10 min to obtain a supernatant. The supernatant is placed on a rotary evaporator to remove all solvents to obtain crude squalene. The extraction amount of the squalene is 2.45 mg/g dry weight of the grape skin dregs by high-performance liquid chromatography.

Embodiment 3

100 g of grape skin dregs powder is taken and put into a triangular flask, and 500 mL chloroform is added. The triangular flask is placed on an ultrasonic crusher with power of 85 W and the ultrasonic time 45 min, and put in a constant-temperature water bath oscillator of 200 r/min at 37°C for extraction for 20 h. Then, a suspension is centrifuged at 10,000 r/min for 10 min to obtain a supernatant. The supernatant is placed on a rotary evaporator to remove all solvents to obtain crude squalene. The extraction amount of the squalene is 3.35 mg/g dry weight of the grape skin dregs by high-performance liquid chromatography.

It should be understood that although the present description is described in terms of

the implementation, not every implementation includes only one separate technical solution, and such a description mode of the description is merely for the sake of clarity. A person skilled in the art should take the description as a whole, and the technical solutions in all the embodiments may be appropriately combined to form other implementations that can be understood by a person skilled in the art.

## Claims

1. A method for extracting squalene from grape skin dregs, characterized by comprising the following steps:

(1) pre-processing grape skin dregs: drying the grape skin dregs in an oven of 70°C; putting the dried grape skin dregs into a high-speed crusher for crushing same into powder; packing the powder in a sealed bag; and storing the powder in a dryer for use;

(2) extracting squalene from the grape skin dregs powder obtained in step (1) by taking a non-polar organic solvent such as chloroform, petroleum ether or n-hexane as the extraction solvent and using an ultrasonic-assisted organic solvent extraction method as the extraction process;

(3) centrifuging a leaching liquid obtained in step (2) at 10,000 r/min for 10 min to obtain a clear squalene leaching liquid; and vacuum concentrating by using a rotary evaporator and thoroughly removing the solvent to obtain crude squalene from the grape skin dregs; and

(4) detecting the crude squalene obtained in step (3) by utilizing a high-performance liquid chromatography, and the peak retention time on a chromatogram of the crude squalene is completely consistent with that of a squalene standard sample, and the content is 1.985 mg/g-3.561 mg/g.

2. The method for extracting squalene from grape skin dregs according to claim 1, characterized in that, the extraction method in step (2) comprises: weighing 100 g of processed grape skin dregs; respectively adding non-polar organic solvents such as the chloroform, petroleum ether or the n-hexane according to a ratio of material to liquid of 1:1, 1:3, 1:5, 1:7 and 1:9; and placing in a constant-temperature water bath oscillator of 200 r/min at 37°C for extracting same for 3-20 h, wherein ultrasonic

power is 85 W and ultrasonic time is 10 min, 20 min, 30 min and 45 min.

3. The method for extracting squalene from grape skin dregs according to claim 1, characterized in that, a detection method in the step (4) is, a liquid chromatography column is LiChrospher® 100 RP-18 endcapped (250 mm×4.6 mm, 5 μm); a mobile phase is acetonitrile-acetone (60:40, vol/vol); a detection wavelength is 208 nm; a flow rate is 1.0 ml/min; a column temperature is 30°C; and a sample size is 10 μL.

# Revendications

1. Procédé d'extraction de squalène à partir de marc de peau de raisin, caractérisé en ce qu'il est : les étapes sont les suivantes :

(1). Prétraitement du marc de peau de raisin : le marc de peau de raisin est séché dans un four à 70°C, chargé dans un broyeur à grande vitesse et réduit en poudre, emballé dans des sacs scellés et conservé dans un dessiccateur pour être utilisé ;

(2). Extraction de squalène à partir de poudre de marc de peau de raisin obtenu dans l'étape (1) par extraction par solvant organique assistée par ultrasons en utilisant un solvant organique non polaire tel que le chloroforme, l'éther de pétrole ou le n-hexane comme solvant d'extraction ;

(3). L'extrait obtenu à l'étape (2) a été centrifugé à 10000r/min pendant 10min pour obtenir un extrait de squalène clarifié ; concentré sous vide à l'aide d'un évaporateur rotatif pour éliminer le solvant et obtenir le squalène brut de marc peau de raisin ;

(4). Le squalène brut obtenu dans l'étape (3) a été analysé par chromatographie liquide à haute performance et le temps de rétention des pics sur le spectre était en parfait accord avec celui de l'échantillon de squalène, la teneur était dans la gamme de 1,985 mg/g-3,561 mg/g.

2. Procédé d'extraction de squalène à partir de marc de peau de raisin selon la Revendication 1, caractérisé en ce qu'il est : le procédé d'extraction dans l'étape (2) consiste à : peser 100g de marc de peau de raisin traité, ajouter des solvants organiques non polaires tels que le chloroforme, l'éther de pétrole ou le n-hexane à un rapport matière/liquide de 1:1, 1:3, 1:5, 1:7, 1:9, respectivement, une puissance

ultrasonique de 85W, un temps ultrasonique de 10, 20, 30, 45 min, et placé dans un oscillateur thermostatique à bain-marie à 200 r/min à 37°C pendant 3-20 h pour la macération.

3. Procédé d'extraction de squalène à partir de marc de peau de raisin selon la Revendication 1, caractérisé en ce qu'il est : le procédé de détection dans ladite étape (4) est : colonne de chromatographie liquide : LiChrospher® 100 RP-18 endcapped (250 mm×4,6 mm, 5 μm) ; phase mobile : acétonitrile-acétone (60:40, vol/vol) ; longueur d'onde de détection : 208 nm ; débit : 1,0 mL/min ; température de la colonne : 30°C ; volume d'injection : 10 μL.

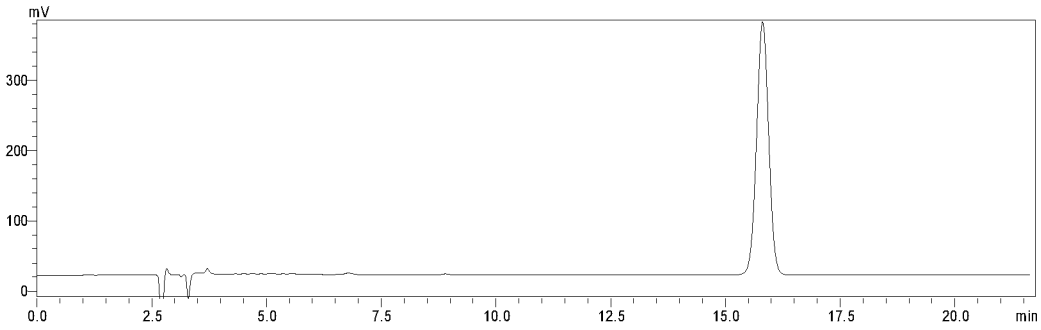


Fig. 1

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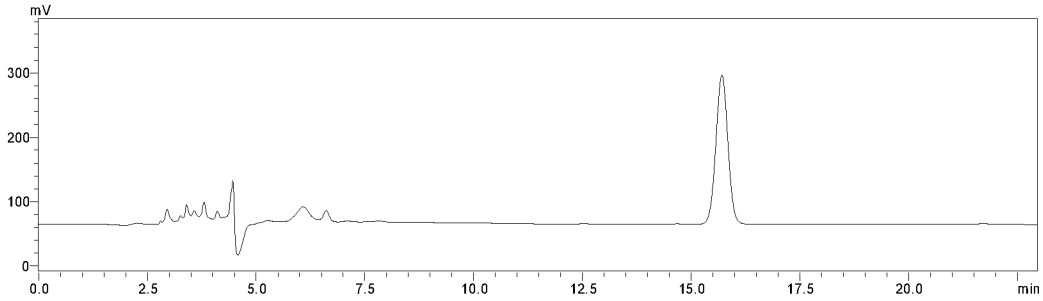


Fig. 2