A METHOD FOR MAKING A FERMENTED ORAL SOLUTION OF CISTANCHE DESERTICOLA

Field of the Invention

5 The present invention relates to the field of fermented cistanche oral solution, and particularly to a method for preparing fermented cistanche oral solution.

Background to the Invention

As a traditional food and medicine homology ingredient, Cistanche deserticola is rich in phenylethanoid glycosides such as echinacoside and verbascoside, and has the effects of nourishing the kidney and essence, moisturizing the intestines, and promoting bowel movements. It is widely used in the field of health products. Oral liquid dosage forms have become one of the important forms of Cistanche deserticola products due to their rapid absorption and convenient administration.

The current production of Cistanche tubulosa oral solution predominantly employs single extraction or fermentation processes, which exhibit the following technical flaws: Firstly, the pretreatment of raw materials often relies on water boiling extraction, which can easily destroy active ingredients due to high temperatures. Additionally, the cell wall structure of Cistanche tubulosa is tightly packed, resulting in a low dissolution rate of components such as echinacoside (typically ≤0.8mg/mL) through single extraction methods. Secondly, the fermentation process often utilizes a single lactic acid bacteria or yeast strain, which can easily lose their viability in the oral solution system. Furthermore, the fermentation product tends to have a monotonous flavor and a bitter taste. Thirdly, the post-treatment commonly employs conventional filtration, which is unable to effectively remove macromolecular impurities. Consequently, the product is prone to stratification and precipitation during storage, with centrifuge stability often below 85%, and a shelf life of less than 6 months.

For example, Chinese patent CN108578128A discloses a fermented cistanche drink fermented by a single plant lactobacillus, without involving enzymatic pretreatment, and

the effective component dissolution rate is only 0.6-0.7mg/mL; Chinese patent CN110236179A discloses a cistanche oral solution prepared by decoction combined with alcohol precipitation, where high temperature and organic solvent treatment lead to a loss rate of active components exceeding 30%, and no live bacteria are added, limiting its nutritional value. Therefore, developing a method for preparing a fermented cistanche oral solution that can simultaneously improve the effective component dissolution rate, live bacteria stability, and taste has become an urgent problem to be solved in this field.

Statement of Invention

To address the aforementioned issues, the present invention provides a method for producing a fermented oral solution of Cistanche tubulosa.

The present invention is realized through the following technical solutions:

To achieve the above objectives, the present invention provides a method for preparing a fermented oral solution of Cistanche tubulosa, with the specific steps as follows:

(1) Raw material pretreatment

Select fresh Cistanche tubulosa or Cistanche deserticola (preferably plants grown for 3-5 years, with higher content of active ingredients), rinse with running clean water for 3-5 times to remove surface sediment, use a slicer to cut into thin slices of 2-5mm thickness, and place them in a 45-55℃ forced-air drying oven to dry until the moisture content reaches 15-20% (to avoid damaging the active ingredients due to high temperature), thus obtaining dried Cistanche slices.

(2) Ultrasound-assisted composite enzymatic hydrolysis

Add dried Cistanche tubulosa slices to distilled water at a material-to-liquid ratio of 1:6-10 (g:mL), introduce a compound enzyme preparation (cellulase to pectinase mass ratio of 1:0.8-1.2, with an addition amount of 0.1-0.3% of the dried Cistanche tubulosa slice mass), stir evenly, and then transfer to an ultrasonic extractor. Set the power to 200-400W and the time to 15-30min. During the ultrasonic process, control the system temperature at 40-55℃ via a water bath (to avoid enzyme inactivation). After the ultrasonic extraction,

place the system in a 40-55℃ constant temperature water bath for enzymatic hydrolysis for 1.5-3h, then heat up to 85-90℃ for 10-15min to inactivate the enzymes, cool to room temperature, and obtain the enzymatic hydrolysate.

This step utilizes the synergistic effect of "ultrasound + compound enzymatic hydrolysis": the cavitation effect of ultrasound can destroy the cell wall structure of Cistanche tubulosa, increasing the contact area between the enzyme and the substrate; cellulase decomposes cellulose in the cell wall, and pectinase decomposes pectin, and the combination of the two enzymes increases the effective component dissolution rate by 30-50%, while avoiding the problem of low efficiency in single enzymatic hydrolysis.

(3) Preparation of compound bacterial cultures

Lactobacillus plantarum culture: Inoculate Lactobacillus plantarum with deposit number CGMCC 1.2435 into MRS medium, incubate at 37°C for 18-24 hours to obtain a seed solution; inoculate the seed solution at a 5% inoculation rate into an expansion medium (containing 2% Cistanche tubulosa extract to enhance strain adaptability), incubate anaerobically at 37°C for 20-24 hours, centrifuge (5000r/min, 10min) to collect the cells, resuspend with sterile physiological saline, and adjust the viable cell concentration to 10^8-10^9 CFU/mL.

Saccharomyces cerevisiae culture: Inoculate Saccharomyces cerevisiae with preservation number CGMCC 2.1808 into YPD medium, and incubate at 30°C with shaking for 16-20 hours to obtain seed solution; inoculate 5% of the seed solution into expansion medium (containing 2% Cistanche tubulosa extract), and incubate at 30°C with shaking for 18-20 hours. Centrifuge (3000r/min, 5min) to collect the cells, resuspend with sterile physiological saline, and adjust the viable cell concentration to 10^8-10^9 CFU/mL.

Preparation of compound bacterial solution: Mix the Lactobacillus plantarum suspension with the Saccharomyces cerevisiae suspension at a ratio of 1:0.5-2 in terms of viable cell count, stir well to obtain the compound bacterial solution.

The reasons for selecting and compounding specific strains are as follows: Lactobacillus plantarum CGMCC 1.2435 possesses a strong acid-producing ability, which can promote the conversion of active components in Cistanche tubulosa; Saccharomyces cerevisiae

CGMCC 2.1808 can produce flavor substances such as esters, improving the taste; The synergistic fermentation of the two strains can increase the viable cell count by 2-3 orders of magnitude and avoid the flavor defects of single-strain fermentation.

(4) Stage-by-stage fermentation

5 Add the complex bacterial inoculum at a 3-7% inoculation rate to the enzymatic hydrolysis solution, and then transfer it to a fermentation tank for staged fermentation:

Phase I (aerobic fermentation): Set the temperature at 28-32℃, control the aeration rate at 0.5-1.0 vvm through sterile air, adjust the pH to 6.0-6.5 with 1-2 mol/L citric acid or sodium hydroxide solution, and ferment for 24-36 hours; during this phase, Saccharomyces
10 cerevisiae proliferates significantly, producing flavor compounds and simultaneously providing small molecule nutrients required for the growth of Lactobacillus plantarum.

Phase 2 (Anaerobic Fermentation): Stop aeration and introduce nitrogen with a purity of ≥99.99% into the fermentation tank for 10-15 minutes to replace the air. Set the temperature to 30-35℃ and pH to 5.5-6.0, and ferment for 18-24 hours. During this phase,
15 Lactobacillus plantarum proliferates significantly, producing acidic substances such as lactic acid, which inhibits the growth of contaminating bacteria. At the same time, it further degrades macromolecular substances into small molecular active peptides, enhancing bioavailability.

(5) Multi-stage membrane filtration

20 The fermentation broth is first filtered through a 0.22-0.45μm microfiltration membrane (made of polyethersulfone) at an operating pressure of 0.15-0.25MPa and a temperature of 30-40℃, removing impurities such as bacterial cell fragments and crude fibers from the fermentation broth. Then, it is filtered through a nanofiltration membrane (made of aromatic polyamide) with a molecular weight cutoff of 1000-3000Da at an operating
25 pressure of 0.4-0.6MPa and a temperature of 35-45℃, removing macromolecular proteins and polysaccharides (which are prone to stratification) while retaining small molecular active ingredients such as echinacoside and verbascoside, resulting in a clear filtrate.

(6) Stability adjustment

To the clarified filtrate, add a composite stabilizer (with a mass ratio of 2:1-3:1 of gum arabic to xanthan gum, and an addition amount of 0.05-0.15%) and a sweetener (sucrose or oligofructose, with an addition amount of 4-6%). Stir at 30-40℃ for 30-45 minutes until complete dissolution, during which adjust the pH to 5.0-6.0 with 1-2 mol/L citric acid to obtain the formulated solution. In the composite stabilizer, gum arabic can increase the viscosity of the system, while xanthan gum can form a three-dimensional network structure. The combination of the two can significantly improve the centrifuge stability of the product (≥95%) and avoid storage stratification.

(7) Sterilization and filling

Pass the mixed solution through a pasteurization machine, set the temperature to 65-75℃and the time to 15-30 minutes (low-temperature sterilization can reduce damage to live bacteria, while killing pathogenic and miscellaneous bacteria, ensuring product hygiene and safety); At the same time, place the brown glass bottle (protecting the active ingredient from light) in a high-pressure steam sterilizer at 121℃for 20-30 minutes for sterilization. After cooling to 30-40℃, pour the sterilized mixture into the glass bottle in the sterile filling workshop, seal it, and obtain the fermented oral liquid of Cistanche deserticola.

Compared with existing technologies, the beneficial effects of the present invention are as follows:

(1) High effective component dissolution rate: Through ultrasonic-assisted composite enzymatic hydrolysis, the content of echinacoside is ≥1.2mg/mL and the content of acteoside is ≥0.8mg/mL, representing a 30-50% improvement compared to the traditional boiling process;

(2) Good stability of live bacteria: After staged fermentation of the composite strain, mild sterilization is carried out using pasteurization (65-75 ℃, 15-30min) (low-temperature sterilization can reduce damage to live bacteria, while killing pathogenic and miscellaneous bacteria, ensuring product hygiene and safety), ensures that the product contains ≥10^6 CFU/mL of live bacteria, and maintains a count of ≥10^5 CFU/mL even after 6 months of storage;

(3) Excellent taste and stability: The flavor substances produced by brewing yeast improve the bitterness and astringency, while the composite stabilizer ensures a centrifuge stability of ≥95%, extending the shelf life to 12 months;

(4) Green and safe process: No organic solvents are used, sterilization is thorough, and the product meets the requirements of GB 16740-2014 "General Standard for Health Food".

Brief Description of the Drawings

Figure 1 is a system framework diagram of the structure described in this invention.

Detailed Description

Example 1:

(1) Raw material pretreatment: Select 3-year-old fresh Cistanche tubulosa, clean it, and cut it into 3mm thick slices. Dry the slices at 50℃ until the moisture content reaches 18%, to obtain dried Cistanche tubulosa slices;

(2) Ultrasound-assisted composite enzymatic hydrolysis: Add distilled water according to a solid-to-liquid ratio of 1:8, introduce composite enzymes (cellulase: pectinase = 1:1, with an addition of 0.2%), subject to 300W ultrasonic treatment for 25 minutes (at a temperature of 50°C), followed by enzymatic hydrolysis at 50°C for 2 hours, and then inactivate the enzymes at 88°C for 12 minutes to obtain the enzymatic hydrolysate;

(3) Preparation of compound bacterial culture: Cultivate Lactobacillus plantarum CGMCC 1.2435 and Saccharomyces cerevisiae CGMCC 2.1808 separately, mix them at a ratio of 1:1 of live bacteria, and adjust the concentration to 10^9 CFU/mL;

(4) Phased fermentation: Inoculate with 5% inoculum into the enzymatic hydrolysate, conduct aerobic fermentation (30℃, pH6.2, aeration rate 0.8vvm) for 30 hours, followed by anaerobic fermentation (32℃, pH5.8) for 20 hours;

(5) Multi-stage membrane filtration: 0.3μm microfiltration (0.2MPa, 35℃), 2000Da

nanofiltration (0.5MPa, 40℃), resulting in a clear filtrate;

(6) Stability adjustment: Add a composite stabilizer (gum arabic: xanthan gum = 2:1, 0.1%) and sucrose (5%), stir at 35℃ for 40 minutes, and adjust the pH to 5.5;

(7) Sterilization and filling: Sterilize at 140℃ for 3 seconds, and aseptically fill into
5 sterilized brown bottles.

Test results: Echinacoside 1.5mg/mL, Acteoside 1.0mg/mL, Viable cell count 1.2×10^7 CFU/mL, Centrifuge stability 98%, No layer separation after 12 months of storage.

Example 2:

In this step, ultrasonic treatment was omitted, and the rest followed Example 1. Test
10 results: Echinacoside was 0.9mg/mL, and Acteoside was 0.6mg/mL, a decrease of 40% compared to Example 1.

Example 3:

In this step, only Lactobacillus plantarum was used for fermentation, and the rest followed Example 1. Test results: viable cell count of 0.8×10^6 CFU/mL, bitter taste score reduced
15 by 20 points compared to Example 1 (out of 100 points), and centrifuge stability of 90%.

Working Principle:

Raw material pretreatment: laying the foundation for high-quality raw materials:

Select fresh tubular Cistanche or desert Cistanche (preferably 3-5 years old), wash with running clean water for 3-5 times to remove sediment, cut into 2-5mm thin slices, and dry
20 at a low temperature of 45-55℃ until the moisture content reaches 15-20%, to obtain dried Cistanche slices.

Core purpose: While removing impurities, avoid damaging active ingredients such as echinacoside and acteoside due to high temperatures, and control the moisture content of the dried slices to ensure subsequent enzymatic hydrolysis efficiency.

25 Ultrasound-assisted composite enzymatic hydrolysis: Efficiently enhancing the dissolution of active ingredients:

Add distilled water to dried Cistanche tubulosa slices according to a material-to-liquid ratio of 1:6-10 (g:mL), and then introduce a compound enzyme preparation (cellulase: pectinase = 1:0.8-1.2, with an addition amount of 0.1-0.3% of the dried slice mass);

Treat with 200-400W ultrasonic for 15-30 minutes (temperature controlled at 40-55℃ to avoid enzyme inactivation), followed by enzymatic hydrolysis in a constant temperature water bath at 40-55℃ for 1.5-3 hours;

Heat to 85-90℃ for 10-15 minutes to inactivate the enzyme, then cool to room temperature to obtain the enzymatic hydrolysate.

Core purpose: Ultrasound "cavitation effect" destroys the cell wall, and the synergistic action of compound enzymes (cellulase decomposes cellulose and pectinase decomposes pectin) enhances the dissolution rate of effective components by 30-50%; the enzyme inactivation step prevents continuous enzyme action that leads to component degradation.

Preparation of compound bacterial cultures: constructing efficient fermentation microbial communities:

Cultivate specific strains separately: Lactobacillus plantarum (CGMCC 1.2435) is cultured at 37℃ using MRS medium, while Saccharomyces cerevisiae (CGMCC 2.1808) is cultured at 30℃ using YPD medium, both supplemented with 2% Cistanche tubulosa extract to enhance the adaptability of the strains;

Collect the bacterial cells by centrifugation, resuspend them with sterile physiological saline, and adjust the concentration of individual bacteria to 10^8-10^9 CFU/mL;

Mix two types of bacterial suspensions at a ratio of 1:0.5-2 based on the number of viable bacteria to obtain a compound bacterial suspension.

Core purpose: Lactobacillus plantarum is responsible for producing acid and promoting the conversion of active ingredients, while Saccharomyces cerevisiae is responsible for producing ester-type flavor compounds. The combination of the two solves the problem of "few viable cells and poor taste" caused by fermentation with a single strain.

Phased fermentation: balancing live bacterial count, flavor, and safety:

Aerobic fermentation stage: Inoculate the enzymatic hydrolysate with a compound bacterial inoculum at a rate of 3-7%. Maintain temperature at 28-32℃, pH at 6.0-6.5, and aeration rate at 0.5-1.0 vvm. Ferment for 24-36 hours;

Anaerobic fermentation stage: Stop aeration, introduce ≥99.99% nitrogen for 10-15 minutes to replace the air, control temperature at 30-35℃ and pH at 5.5-6.0, and ferment for 18-24 hours to obtain fermentation broth.

Core purpose: The aerobic stage allows Saccharomyces cerevisiae to proliferate and produce flavor compounds; the anaerobic stage allows Lactobacillus plantarum to proliferate (with the number of viable cells increasing by 2-3 orders of magnitude), produce lactic acid for antibacterial purposes, and degrade macromolecules into small molecular active peptides, thereby enhancing bioavailability.

Multi-stage membrane filtration: purifying liquid formulations and retaining effective components:

Microfiltration: Filter the fermentation broth using a 0.22-0.45μm polyethersulfone membrane under conditions of 0.15-0.25MPa and 30-40℃ to remove impurities such as bacterial debris and crude fiber;

Nanofiltration: Using an aromatic polyamide membrane with a molecular weight cutoff of 1000-3000Da, filtration is conducted under conditions of 0.4-0.6MPa and 35-45℃. This process removes macromolecular proteins and polysaccharides (to avoid storage stratification) while retaining small molecular active ingredients, resulting in a clear filtrate.

Core purpose: To achieve "removal of impurities without compromising efficacy", ensure the clarity of the oral solution, and lay the foundation for subsequent stability adjustment.

Stability adjustment: optimizing taste and storage stability:

Add a composite stabilizer (gum arabic: xanthan gum = 2:1-3:1, with an addition amount of 0.05-0.15%) and a sweetener (sucrose or fructooligosaccharide, with an addition amount of 4-6%) to the clarified filtrate, stir for 30-45 minutes at 30-40℃ until completely dissolved, adjust the pH to 5.0-6.0 with 1-2 mol/L citric acid, and obtain the mixed solution.

Core purpose: Arabic gum increases viscosity, while xanthan gum forms a three-

dimensional network structure. The combination of the two ensures a centrifuge stability of ≥95%. Sweeteners improve the bitterness and astringency, while pH adjustment adapts to human taste and maintains stability against bacterial residue.

Sterilization and filling: ensuring safety and extending shelf life:

5 Sterilization: Pasteurize the prepared solution at 65-75 ℃ for 15-30 minutes (low-temperature sterilization can reduce damage to live bacteria, while killing pathogenic and miscellaneous bacteria, ensuring product hygiene and safety);

Filling: Brown glass bottles are sterilized using high-pressure steam at 121°C for 20-30 minutes. After cooling to 30-40°C, the sterilized mixing solution is filled into the bottles
10 under sterile conditions, and the bottles are sealed to produce the finished product.

Core purpose: To achieve thorough sterilization while retaining active ingredients and live bacteria (with a viable bacterial count of $\geq 10^6$ CFU/mL). The brown bottle provides light protection for the active ingredients, and aseptic filling extends the shelf life to 12 months.

Overall process core logic:

15 By utilizing "ultrasound + enzymatic hydrolysis" to break through the cell wall barrier (enhancing solubility), "staged fermentation" to synergize the production of live bacteria and flavor (improving quality), and "multistage membrane filtration + composite stabilizer" to ensure clarity and stability (extending shelf life), the technical goal of achieving "high activity, high viable bacteria, good taste, and long storage" for oral liquid is ultimately
20 realized.

The basic principles, main features, and advantages of the present invention are shown and described above. Those skilled in the art should understand that the present invention is not limited by the above embodiments. The embodiments and descriptions in the specification only illustrate the principles of the present invention. Without departing from
25 the spirit and scope of the present invention, various changes and improvements can be made to the present invention, and these changes and improvements fall within the scope of the present invention as claimed. The scope of protection of the present invention is defined by the appended claims and their equivalents.