MINISTRY OF EDUCATION AND TRAINING
NHA TRANG UNIVERSITY

NGUYỄN THỊ THĂNG LONG

RESEARCH RECEIVING AND MAKING FROM INULIN POWDER FROM DANGSHEN TUBERS (*CODONOPSIS JAVANICA*) GROW NATURALLY IN LAC DUONG DISTRICT - LAM DONG PROVINCE

SUMMARY Ph.D. THESIS

KHANH HOA - 2021

The thesis is completed at the Nha Trang University

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The thesis is defended at the Nha Trang University Council, meeting at the Nha Trang University at the hour 8 Date 28 Month 11 Year 2021

The thesis can be found at: National Library and the Nha Trang University Library

SUMMARY OF THE DOCTORAL THESIS'S NEW CONTRIBUTIONS

Thesis topic: "Research on extracting and making inulin powder from Dangshen 's roots (*Codonopsis javanica*) naturally growing in Lac Duong district, Lam Dong province".

Mayor: Post Harvest Technology **Code:** 9540104

Ph.D. Candidate: Nguyễn Thị Thăng Long **Year**: 2015-2019

The scientific advisors: Assoc. Prof. Dr. Vũ Ngọc Bội

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Content:

The thesis has obtained some new results added to the field of research, spray drying to create inulin powder from Dangshen (Codonopsis javanica) and making synbiotic preparations from inulin for application in functional foods:

1)The thesis has researched and determined for the first time the morphological and structural features of the isotropic root tissue (Codonopsis javanica) that grows naturally in Lac Duong - Lam Dong as the basis for age discrimination and timing. Harvesting Dangshen roots. On the other hand, the thesis also determined that 3-year-old Dangshen root has ingredients that meet Vietnamese pharmacopeia standards in 2017: Total sugar content: (10.08 ± 0.88) ^oBx, Total extract content:(58.2 ± 1.57) g/100g; Total mineral content: (5.50 ± 0.17) %, containing inorganic impurities and heavy metals.

2)The thesis has first time researched and optimized the inulin extraction process from Codonopsis javanica that naturally grows in Lac Duong Lam Dong: the extraction solvent is distilled water of two times, extraction temperature 71^oC, extraction time, 36 minutes, and the ratio of solvents / raw materials is 47ml/g with 23.93 % inulin extraction efficiency, 26.96 % fructan extraction efficiency, and 61.35 % of total dissolved extraction efficiency. The thesis also identifies the right factors for inulin precipitation: the appropriate temperature to concentrate the extraction before precipitating to 16 ^oBx is under 55^oC, the suitable precipitation agent is ethanol, the concentration of ethanol is fit. The content for inulin precipitation is 80%, and ethanol concentration for fructan precipitation is 90%, temperature for inulin precipitation is (6±1)°C with average precipitation efficiency of 95.53%.

3) The thesis studies for the first time on purification and determination of the molecular structure characteristics of inulin obtained. The study results showed that it is possible to purify inulin from crude extracts by recrystallization six times, and the purified inulin collection efficiency is (75.85±0.84) %. The purified inulin obtained from Codonopsis javanica naturally grows in Lac Duong-Lam Dong includes two molecules of Fructose polysaccharide: a molecule with a mass of 3.193 Da, about 19-23 monomers, depict at 96.448 %, and one the molecule has a molecular weight of 1.112.892 Da, accounting for 3.552%.

4) The dissertation identifies for the first time some parameters suitable for the spray inulin powdered in Codonopsis javanica. The drying aid is maltodextrin, with the appropriate additional

maltodextrin ratio of 10 %. The inlet temperature is 185°C, and the drying chamber air temperature is 85°C; The compressed air pressure is 3 atm, the input pump speed is 10ml/p, which corresponds to the injector speed of 16000 rpm. The powder obtained had an inulin content of (445.90 ± 2.79) mg/g; Fructan content reached (469.40 ±1.61) mg/g, pH (5.18 ± 0.01), mineral content (4.82 ± 0.07) %. Solubility: 1/ 9.5 ± 0.5 / 15 (g / ml/ min; Humidity (6.06 ± 0.27) % and free of inorganic impurities and heavy metals. particle size (882.2±101.4) ηm, particle density 100%, dispersion 0.497 PDI, melting temperature over 600°C. The powder meets food safety standards according to current regulations of the Ministry of Health for functional food.

5) The thesis has done for the first time by using inulin powder from Dangshen to make synbiotic products to apply in food and obtained some results Dangshen spray- powder containing Inulin has strong prebiotic properties with a dose of 4% (w/w- equivalent to 1.8 -2% based on inulin content) can stimulate cell proliferation (CFU) of 8 strains (two of L. acidophilus, L. plantarum, L. rhamnosus, B. longum, B. lactic, two of Enterococcus faecalis) from 1.4 ÷ 11.5 times, in which, L. acidophillus (M7) had the highest fertility rate of 2.3 x10^{11}CFU/ g and Enterococcus faecalis (M4) had the lowest fertility rate of 7x10 ^{6} CFU/ g.

6) The thesis first has identified the synbiotic mixing formula for the first time: Inulin powder 0.514 g/g mixed with L. acidophillus biomass 0.33 g/g (density 5x10^{10}CFU/ g); 0.128 g/ g L. plantarum biomass (density 2x10^{11}CFU/ g); 0.033 g/g B. longum (density 8 x10^{10}CFU / g) and 0.1 g/g of B. lactics biomass (density 10^{11}CFU/ g).

7) The thesis tests synbiotic products for the first time in mice. Results, synbiotic's doses of 1.2 g/ kg and 2.4 g/ kg using for 14 or 28 days were not acute toxicity, semi-chronic toxicity to the liver's function, and to the kidney in test rats. Synbiotic has immunostimulating properties as well as the ability to treat diarrhea in mice.

DIRECTORS **Ph.D. Student**

Assoc. Prof. Dr. Vũ Ngọc Bội **Assoc. Prof. Dr. Đào Xuân Vinh** **Nguyễn Thị Thăng Long**

THE SUMMMARY OF THE THESIS

INTRODUCTION

1. The necessary of the thesis

The herbs extract substances with outstanding features of being non-toxic, having no side effects, and multi-effects should be used as functional foods and gradually as a substitute for medicines.

On October 30 in 2013, the Prime Minister Issued Decision 1976/QD-TTG on "Overall development of medicinal herbs to 2020 and orientations to 2030", which focused on 28 indigenous medicinal herbs, including Dangshen (*Codonopsis javanica*). According to this trend, researchers are interested in the study of extracting and acquiring pure substances from plants in general and naturally growing railway to improve the effectiveness of herbal application in all fields.

Special natural conditions made 1664 Lam Dong province has 237 species of plant families, representing nearly 50% of medicinal plants in the country. Besides, Lam Dong province is being also planned as herbal of the primary region.

For the above reasons, the project of Ministry level has been implemented by me of the research about "Studying the chemical composition of the Đanshen (*Codonopsis javanica*) in Lam Dong and the application on functional foods". Code B2018-DLA-01. Basing on funding from the Ministry-level project, I made the thesis on "Research on inulin collection and production from *Codonopsis javanica* naturally growing in Lac Duong - Lam Đong province".

2. The goal of the thesis

Determine the time of harvesting the natural growing Dangshen roots.

Research on inulin extraction, purification, and characterization of inulin molecular structure in Dangshen tubers naturally growing in Lac Duong district, Lam Dong province.

Research on spray drying to create Dangshen powder and experiment to create a synbiotic powder from spray-drying powder, oriented application in food.

3. Object and scope of research

Object: The raw materials used in the harvesting time study were fresh, mixed at random collection.

The raw materials used to extract Inulin are Dangshen 's residue after extraction of other ingredients by organic solvents (n-hexane, ethanol).

4. Research scope

1) Research to determine the harvesting time of wild Dangshen in Lac Duong district, Lam Dong province.

2) Optimizing research on inulin extraction from naturally grown Dangshen

3) Purification study and characterization of the inulin molecular structure obtained.

4) Research on spray drying to create inulin powder.

5) Testing using spray-dried Dangshen powder to create a synbiotic powder, oriented application in food.

5. Research Methodology

The thesis uses the standard research methods of the World and Vietnam in the study of Inulin intake from Codonopsis javanica, applying mathematics to optimize to ensure the experimental results are high reliability.

6. The structure of the thesis

The thesis includes 141 pages, of which 31 pages of overview, 21 pages of research methods, 87 pages of research results, 2 pages of conclusions, 51tables of data, 64 pictures, 181 references (30 pages in Vietnamese, 151 documents in English) and appendix 25 pages.

CHAPTER 1. LITERATURE REVIEW

1.1. Introduction about the Dangshen and inulin

The Dangshen belongs to the genus Codonopsis. Out of 47 species announced, only two appear in Vietnam. The Dangshen is narrow distribution in some areas with altitudes above 800-2000. This specie is on the list of Vietnamese Red Book. There is only one species of *C. javanica* and is the type species in Lam Dong province.

Inulin is a straight-chain fructan-type polysaccharide, composed of 95% of the fructose and a stored carbohydrate. The link in the circuit is a β-D bond (2,1). When in the long-chain form, there is a DPn (Degree polymer) of $10 \leq n \leq 70$) units, the average of DPav 25 is called Inulin, when in the short-chain type, it is called FOS (fructooligosaccharides) ($3 \leq n \leq 10$). The DP of the Inulin of plant origin is relatively low (max <200).

Due to the change in DP in the chain length, so the molecular weight of inulin varies from 3500 to 5500Da. The molecular weight of Inulin (Fructan type) is from $5.0x10^2$ to $1.3x10^4$ Da, and Levan (Fructan type) branched from $1x10^4$-$1x10^8$ Da. Inulin is hydrolyzed in Endo and Exo by enzyme inulinase.

Because of the β-linked at the C2 position of the fructoses and there is one glucose at the end of the chain-linked by α (1-2), the Inulin/FOS is no-digested by the α-glucosidase enzymes, maltase-isomaltase, sucrase in the human and animal gastrointestinal tract. Therefore, it has been classified into a kind of non-digestible soluble fiber (NDO - non-digestible oligosaccharide).

1.2. Detailing technology, manufacturing, and cleaning inulin

There are many methods of extracting herbal inulin that has been studied but basically on the principle of liquid-liquid and solid-liquid extraction. The extraction process also affects the DP of Inulin. Inulin's DP is one of the most important parametersthat determine other physicochemical and biological properties, so technological factors in the extraction process need to be determinate.

The inulin separation process basically involves three steps: Extraction of water-soluble ingredients including inulin; 2. Eliminate impurities and low DP inulins; 3. Spray (dry) dry. Inulin purification goes through two stages. Stage 1: extraction and purification through calcification of carbonates; Stage 2: precipitation and filtration combined with demineralization through ion resin exchange and decolorization with activated carbon.

1.3. Spraying for making inulin/FOS powder

When spray-drying, the properties of the drying aid are a nesassary factor. There are many different carriers with different advantages and disadvantages, so it is necessary to investigate and choose the appropriate substance for the purpose and the material. The spray-drying parameters influence the various

chemical and physical properties of the powder. The properties of the spray-dried powder depend on the physical and chemical properties of the raw material, design parameters, and dryer performance to feed flow rate, injection rate, and the inlet air temperature. The drying efficiency could be increased by the right selection of spray drying parameters that are both inlet temperature and drying air temperature, process temperature, carrier type.

Inulin is multi-functional, capable of applications in medicine, food. It has immunomodulatory, immunomodulating, antipruritic agent properties; Inulin is multi-functional, capable of applications in medicine, food. It has been shown to have immunomodulatory, immunomodulating, antipruritic agent properties; Increasing intestinal probiotic bacteria population should act as a prebiotic; Fights intestinal infections; Irritable bowel disease; fight against colon cancer and ulcerative colitis IBD (inflammatory bowel diseases - ulcerative colitis and Crohn's disease), increasing the secretion of anti-inflammatory cytokines. Prevent hypertension (antihypertensive); reduce triglycerides in the blood, prevent cardiovascular disease; anti-obesity diabetes management; As a vaccine adjuvant, increases calcium and magnesium absorption but does not affect the balance of other minerals; Bone mineralization in healthy young people fights osteoporosis but does not cause mutations, cancer or teratogenicity. Inulin has been used in the production of functional foods, as synthetic materials in nutritional foods and drugs. Inulin is a renewable raw material for the production of bioethanol, fructose syrup, unicellular protein, and single-cell oil, obtaining FOS and other useful products.

From research on Inulin in the country and around the world, it shows that inulin has many properties suitable for use in medicine, food such as naturally sourced from non-toxic Dangshen and many effects including prebiotic properties. Currently, inulin application studies in Dangshen have not been fully studied, although there are some studies proving that Inulin has multiple effects.

CHAPTER 2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Materials

Codonopsis javanica (Blume) Hook.f.) raw materials grow wild in 3 years of Da Sa; Da Nhim; Da Chais, Lac Duong district, Lam Dong province.

2.1.2. Carrier

Carriers (drying aids, additives): Maltodextrin $C_{6n}H_{(10n+2)}O_{(5n+1)}$. Dextrin $(C_6H_{10}O_5)_n$ (INS 1400); Gum Arabic (INS 414) provided by Nam Giang Company Limited.

2.1.3. Microorganisms

The project using 6 strains of lactic acid bacteria of different origins are included in the list of probiotic bacteria species according to TCVN 9633: 2013. *E. faecalis* (M1) and *L. acidophillus* (M2) by Vietnam-Australia Biological Technology Co., Ltd, TP. Provided by Nha Trang, Khanh Hoa province;

L. rhamnosus (M3), *E. faecalis* (M4) and *L. acidophillus* (M5), *L. plantarum* (M6) from Legend BIO Company Limited, Hanoi;

Strain *L. acidophillus* (M7) from the Vietnam Seed Museum of VSV (VTTC); Race *B. longum* (YK1), *B. lactic* (YK2) from Microbiology Laboratory, Department of Biology, Dalat University.

2.2. Research methods

2.2.1. General research scheme

During the research process, the experiments were arranged according to the general research scheme (Fig.2.3).

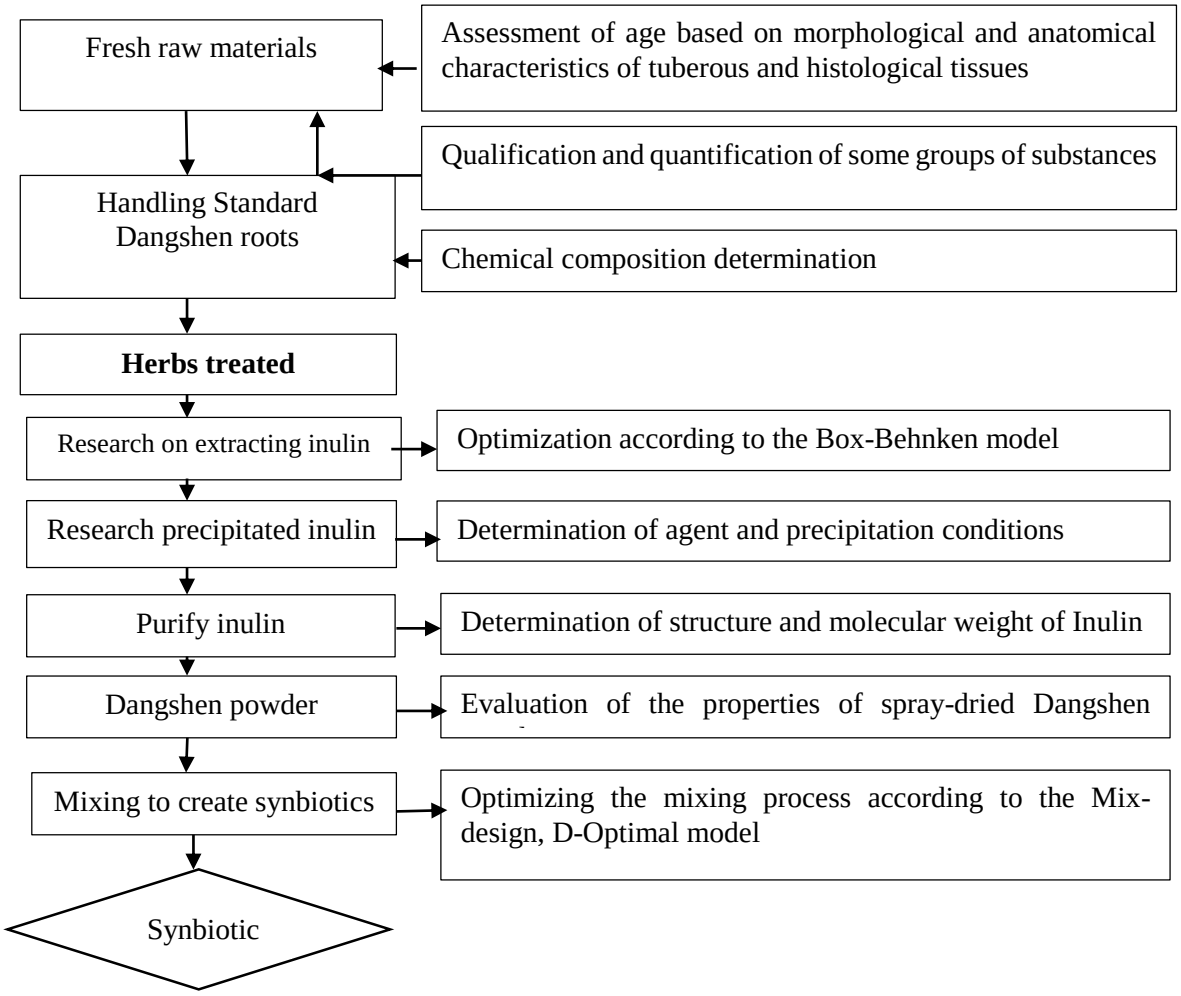


Figure 2.3. General research scheme

The thesis conducts research to identify each stage for the research process. Specifically, the classification of fresh ingredients is based on its morphological and microscopic characteristics to determine the harvesting age and the type of raw materials that meet the medicinal material standards. From the herbal residue extracted with organic solvents, the precipitation agents will be selected, and the inulin of extraction process will be optimized. On that basis, purified research to determine the inulin molecular structure of the Lam Dong Dangshen. After that, researching on creating powdered spray drying from inulin extracts and checking the quality of the powder and prebiotic activity of the powder. Finally, the synbiotic preparation was tested by optimizing the mixing ratio of the obtained prebiotic biomass and the previously generated probiotic.

2.2.2. Methods of analysis of Dangshen root morphology, histology, and chemistry

+ Sampling and sample processing: according to WHO (1992) and Vietnam Pharmacopeia (2017).

Morphological analysis is based on the description of Vietnam Pharmacopoeia, V (2017) and according to Zhang's study (2017).

+ Determining the age of the year through the annual growth cycle according to Schweingruber (2005).

+ Measurement of xylem, phloem (µm) diameter is measured with a ruler in a specialized software of the Olympus SZX 7 stereo microscope, Japan.

+ Qualitative composition of substances in n-hexane extract, in alcohol extract according to Sawant and Cs (2013). Tannin qualitative according to WHO (1992).

+ Essential Oil Qualitative; Anthranoid (Borntraeger reaction) according to Nguyen Thi Kim Phung (2007) Qualitative Inulin; Fructan by WHO Powdered Method (1992).

+ Qualitative inulin by TLC thin layer chromatography according to Yevtifieieva and Cs (2016)

+ Determination of moisture; Determination of dry matter; Content of total dissolved extracts; Total mineral content and acid-insoluble mineral. All identified by WHO (1992)

+ The total sugar content (% Bx) is determined according to TCVN 7771: 2007 (ISO 2173: 2003).

+ The heavy metal elements Hg, As, Cd and Pb are determined by atomic absorption spectroscopy according to TCVN 7602: 2007 / (AOAC 972.25).

+ Determining the standard raw materials according to Vietnam Pharmacopoeia, V (2017).

+ Quantification of polyphenols according to Singleton et all (1999). Gallic acid as a standard. Perform photometric measurements at 755 nm.

+ Quantification of Flavonoids according to Woisky et all. Quercetin as a standard. Perform photometric measurements at 430 nm.

+ Determination of FRAP reduction activity according to Cuong et all (2015). $FeSO_4$ as a standard. Perform photometric measurements at 699 nm.

+ Determination of ABTS reduction activity according to Roberta et all (1999).

+ Quantification of total TAC elimination capacity according to Prieto et all (1999). Vitamin C as a standard. Perform photometric measurements at 694 nm.

+ Quantification of Fructan / Inulin by UV-VIS color comparison spectroscopy according to Pencheva and Cs (2012). Resorcinol - Thiure Color Complex. Fructose is used as a standard. Perform photometric measurements at 483 nm.

+ Total saccharide content (ScTP) was determined by the phenol acid method according to Chen et all (2011). Glucose is used as a standard. Perform photometric measurements at 490 nm.

+ Inulin purification by recrystallization method. Purity was checked by Thin plate chromatography TLC according to Yevtifieieva et al (2016).

+ Determination of molecular weight of Inulin by high-performance molecular sieve chromatography (Gel chromatography).

+ Determination of inulin structure by IR infrared spectroscopy, MNR spectrum, two-dimensional nuclear magnetic resonance spectrum HSQC, HMQC.

+ Evaluation of the physical properties of spray drying powder is determined by the method of dynamic light scattering (DLS) according to ISO 22412 (2008) (Technical Committee ISO / TC 24, 2017).

+ The molten heat was measured by the automatic thermometers of the HS-60. (The USA).

+ The pH value is measured with a pH measuring device. Japan.

+ Solubility is done according to the method of Pharmacopoeia VN (2017) or QCVN-3/7/2919.The quantitative method of microorganisms.

+ The composition and characteristics of spray-dried inulin powder of dried ginseng root are applied according to Decision 46/2007/ of the Ministry of Health on Functional foods.

+ Determination of microorganism content by the method of counting colonies MPN (Most Probable Number) according to Decision No. 929 / QD-ATTP. November 8, 2017.

+ Determination of the total number of aerobic bacteria (CFU/g) according to TCVN 4884-1: 2005.

+ Determination of the total number of E. coli bacteria (CFU / g) according to TCVN 7924-2: 2008

+ Identify Cl. perfringens (CFU / g) according to TCVN 4991: 2005.

+ Determine Salmonella spp (yes or KPH / 25g) according to TCVN 4829: 2005.

+ Total number of spores of yeasts and molds (CFU / g) according to TCVN 8275-1 & 2: 2010.

2.3. Method of collecting and data processing

According to the statistical method. Each experiment was three times, each time three of the samples, and the results were the average of the experiments. Calculate and plot the graph, process the resulting according to the statistical comparison test of mean values between groups, analyze variance ANOVA by using Minitab 18.0 software. Data analysis and optimized design using Design Expert 12.0 software (USA).

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Determination of raw materials planning time in lac duong district - Lam dong province

3.1.1. Effect of age on morphology and tissue structure of Dangshen 's roots

3.1.1.1. Effect of age on morphology and tissue structure of Dangshen 's roots

The research results show that it is possible to rely on the morphological and histological characteristics of oogonium to determine the age of the raw materials. For example:

- One-year-old stage: Dangshen tubers have no scars on the short root neck. The color of the root is ivory white, the skin is thin. They look succulent, with no branched. It also has a mildly sweet taste. When analyzing the tissue, the xylem part of the root is round, fan-shaped wood rays are not clear.

- Two-year-old stage: Dangshen root has two scar rings on the root neck, the root neck is short, ivory white, the skin is a bit thick, it doesn't look succulent, the roots are branched with tuo branches and have a light sweet taste. When dissecting the tissue, the xylem part of the tuber is round, the wood rays are fan-shaped, and there are 1 to 2 wood rays that spread to the parenchyma of the shell.

- Three-year-old stage: Dangshen root has three scar rings on the root neck, long root neck, ivory white, thick skin, firm-hand, three-branched roots. The shape of the xylem begins to be hexagonal, the wood rays are fan-shaped, there are two to three wood rays that spread to the parenchyma of the shell.

- Four-year-old stage: Dangshen root has four scar rings on the root neck. The root neck is the long, ivory white, thick, rough shell, and firmly in hand. The roots are divided into 4 branches. The shape of the xylem is distorted hexagonally, the wood rays are fan-shaped, there are 3 - 4 wood rays that spread to the outer part of the shell parenchyma.

3.1.1.2. The influence of age on the diameter size of the root Dangshen

Table 3.1. The influence of age on the diameter size of the Dangshen root

No	Years of age (years)	Number of samples (tubers)	Average tuber diameter (cm)
1	1	523	$0,64^{d} \pm 0,29$
2	2	548	$1,09^{c} \pm 0,19$
3	3	609	$1,66^{b} \pm 0,23$
4	4	162	$2,24^{a} \pm 0,30$

Note: Different letters in the same column are different (p <0.05).

The results of evaluating the diameter size of 1842 Dangshen tubers of the four different ages (Table 3.2) show that the diameter of the tuber and the age of the Dangshen are related and can rely on the size of the bulb to confirm their age.

3.1.1.3. Effect of age on the ratio between xylem and phloem diameter

Table 3.2. The results of tissue anatomy analysis of the tuberous Dangshen under a microscope

No	Years of age (years)	Number of slices (slices)	Xylem diameter (mm)	Phloem diameter (mm)	Diameter ratio of xylem / phloem (%)
1	1	251	$2,7^{d} \pm 0,5$	$4,65^{d} \pm 0,74$	$58,90^{a}$
2	2	220	$3,6^{c} \pm 0,7$	$8,09^{c} \pm 2,14$	$47,23^{b}$
3	3	198	$5,0^{b} \pm 1,1$	$15,64^{b} \pm 2,60$	$32,69^{d}$
4	4	114	$7,7^{a} \pm 2,6$	$17,39^{a} \pm 2,56$	$42,31^{c}$

Note: Different letters in the same column are different (p <0.05).

From the analytical results (Table 3.2), it shows that the roots of the amygdala have growth rings and can be based on the anatomical structure of the isotonic root tissue to distinguish the age of Dangshen . The results of qualitative analysis of some groups of organic matter in 3-year-old Dangshen extract (Table 3.3) shows: In the tuberous Dangshen (DS) contains flavonoids, organic acids, amino acids, polysaccharides, alkaloids, essential oils, phytosterols, fats, coumarin, anthranoid, reducing sugars and no carotenoids, saponins, and tannins.

The qualitative Inulin reaction in the roots of the rhizosphere showed the orange-yellow reaction, and the inulin qualitative in the research samples by TLC showed that the Rf inulin coefficient of the crude powder was equivalent to that of the standard Inulin (Merck).

3.1.2. Quantification of substances

3.1.2.1. Identifying groups of substances

Qualitative results of some groups of organic substances present in 3-year-old Dangshen extract (Table 3.3).

Table 3.3. The results of the qualitative analysis of some groups of organic matter in 3-year-old Dangshen extract

No	Active group	Qualitative reaction	Results	Basic conclusions
1	Fatty	Blurred residue left on the filter paper	+	Positive
2	Oils	Smells good	+	Positive
3	Phytosterol	Reaction Salkowski	+	Positive
4	Carotenoid	H_2SO_4 concentrated	-	negative
5	Polyphenol	Ferric Chloride Test	+++	Positive
6	Flavonoid	Reacts with alkali NaOH 10%	++	Positive
7	Coumarin	The reaction opens and closes the lactone ring	++	Positive
		Fluorescence under ultraviolet light	+	
8	Saponin	Foaming phenomenon	-	Negative
9	Organic acids	Na_2CO_3	+	Positive
		Change color litmus paper	+++	
10	Acid amin	eagents Ninhydrin	+++	Positive
11	Reducing sugar	Reagents Fehling	+	Positive
12	Polysaccharid	Reagents lugol	+++	Positive
13	Inulin, fructan	Reagents Resorcinol - Thiure Thin layer chromatography (TLC)	+++ +++	Positive
14	Alcaloid	Reagents Mayer	++	Positive
		Reagents Bouchardat	+++	
		Reagents Dragendorff	++	
15	Anthranoid	Reagents Borntraeger	+	Positive
16	Tanin	Reagents $FeCl_3$	-	Negative

Notes: - reaction negative, + positive reaction. ++ clear positive reaction, +++ obvious positive reaction

In Dangshen root contains groups of flavonoids, organic acids, amino acids, polysaccharides alkaloids, essential oils, phytosterols, fats, coumarins, anthranoids, reducing sugars, and no carotenoids, saponins, and tannins.

The qualitative reaction of Inulin in the roots of Dangshen root gives a yellow-orange reaction and the qualitative inulin by TLC showed that the Rf inulin of the raw powder sample was equivalent to the Rf of the standard Inulin (Merck).

3.1.2.2. Quantification of substances

Quantitative analysis of some substances according to the regulations on herbal quality management according to WHO (1992). The results are as follows:

+ **About sugar content:** The trend decreases with increasing age. The level of the first year (16.48%) decreased gradually to 127.2%, continued to decrease to 10.08 % in the third year, and the lowest in the 4th year (7.03)%.

+ **Regarding the content of soluble extracts:** Trends in decreasing fluctuation with increasing age. The level of the first year (68.63^{a}) % decreased gradually to 61.61^{b} %, continued to decrease to 54.24^{c} % in the third year, and the lowest in the 4 th year (52.72^{d})%.

+ **About total mineral content:** The trend of total mineral content is proportional to the age of the year. The level of the first year (3.33^{d}) %gradually increases to 4.09^{c} %, continues to increase to 5.5^{b}% in year 3, and is highest in the 4th year (6.36^{a})%.

+ Inulin content: Inulin content in different age periods follows the rule of non-linear sugar level 2, the concentration peaks at the 3rd year ($222,24^{a}$) mg/g DW, this figure second highest at the 4th year (127.41^{b}) mg /g, lower inulin content in year 2 (198.22^{c}) mg/g DW and lowest at one year of age (169.82^{d}) mg/g DW.

The correlation between inulin content and growth stages follows the equation:

$$\text{Inulin content (mg fructose / g DW)} = 118.1 + 58.85X - 8.44X^2 \ (R^2\ 0.96).$$

About the content of some heavy metals and inorganic impurities

The results of heavy metal analysis by AAS atomic absorption spectroscopy showed that Dangshen tubers do not contain heavy metals (Hg; Pb; As; Cd) and inorganic impurities.

About polyphenol and flavonoid content.

The correlation between the flavonoid polyphenols content and the growth time in the Danghen root ($R^2 > 0.90$) is shown by the following regression equation:

Polyphenol content (mg GAE / DW) = $0.4 + 0.59X-0.09X^2$ (R^2 0.92)

Flavonoid content (mg QE / DW) = $0.17 + 0.42X- 0.06X^2$ (R^2 0.98)

Table 3.5. The polyphenol content and antioxidant activity of isotonic extract at different ages

Active ingredient	**Analysis results**			
	1 year old bulb	**2 year old bulb**	**3 year old bulb**	**4 year old bulb**
Polyphenol content (mg acid garlic /g DW)	$09.2^{d} \pm 0.04$	$1.18^{c} \pm 0,05$	$1.39^{a} \pm 0.055$	$1.29^{b} \pm 0.03$
Flavanoid content (mg quercetin /g DW)	$0.54^{d} \pm 0.02$	$0.74^{c} \pm 0.015$	$0.87^{a} \pm 0.007$	$0.82^{b} \pm 0.016$
Free radical scavenging activity (%)	$4.62^{c} \pm 0.26$	$10.78^{b} \pm 0.93$	$15.65^{a} \pm 1.98$	$9.7^{b} \pm 0.45$
Iron reduction activity (mg $FeSO_4$ /g DW)	$10.36^{d} \pm 0.88$	$12.74^{c} \pm 0.32$	$15.39^{a} \pm 0.53$	$14.17^{b} \pm 0.34$
Total antioxidant activity (mg ascorbic acid /g DW)	$10.99^{d} \pm 0.95$	$15.76^{c} \pm 0.85$	$23.64^{a} \pm 0.57$	$18.93^{b} \pm 0.1$

Different letters in the same row represent a significant difference. ($p < 0.05$).

Research results (Table 3.5) show: Growth time has a strong effect on the accumulation of inulin, polyphenols, flavonoids, total sugars, and minerals, as well as total antioxidant, iron reduction, and free radical scavenging activity contained in extracts of Dangshen root.

Content of inulin, polyphenols, flavonoids, total sugar, total antioxidant activity, iron reduction activity, and free radical scavenging activity are highest at three years of age.

Dangshen tubers do not contain heavy metals and inorganic impurities. The thesis chooses the 3-year-old railway root as raw material for the research process.

3.1.3. Evaluation of the quality of Dangshen root growing in Lac Duong - Lam Dong

Research results (Table 3.6) show that 4-year-old Dandelion root has a total mineral content exceeding the regulations of the Vietnam Pharmacopoeia (>6%) while at the age of 1, 2, and 3 years all meet the standards. VN Pharmacopoeia Standard (2007). The Dangshen root of all ages does not contain heavy metals and inorganic impurities. However, 3-year-old tubers had the highest content of inulin and polyphenols. Therefore, the thesis decided to choose 3-year-old ginseng roots as raw materials for the research process.

Table 3.6. Content of Dangshen root grown in Lac Duong - Lam Dong

Indicators	Standards of VN Pharma	Age (years)			
		1	2	3	4
Humidity (%)	<15	< 5	< 5	< 5	< 5
Qualitative reaction	+	+	+	+	+
Total dissolved extracts (%)	> 35	$68,63^{a} \pm 1,43$	$61,61^{b} \pm 1,54$	$58,24^{c} \pm 1,57$	$52,72^{d} \pm 1,42$
Total mineral (%)	< 6	$3,33^{d} \pm 0,36$	$4,09^{c} \pm 0,26$	$5,50^{b} \pm 0,17$	$6,36^{a} \pm 0,09$
Inulin content (mg/g)	Not regulated	$169,82^{d} \pm 3,01$	$198,22^{c} \pm 3,19$	$222,24^{a} \pm 3,54$	$217,41^{b} \pm 2,94$
Polyphenol content (mg/g)	Not regulated	$0,92^{d} \pm 0,04$	$1,18^{c} \pm 0,05$	$1,39^{a} \pm 0,055$	$1,29^{b} \pm 0,03$
Minerals insoluble in acid (%)	< 2	-	-	-	-
Inorganic impurity (%)	< 1	-	-	-	-
Heavy metal content (ppm): Pb; Cd; Hg và As	$< 20/10^{6}$	Not detected	Not detected	Not detected	Not detected

Different letters in the same row represent a significant difference, p <0.05

*Note: * (Ministry of Health, 2017); (+) Positive; (-) Negative*

3.2. Research on optimization of inulin extract

3.2.1. Optimization of inulin extraction according to the Box-Behnken model

*** Define the regression model**

From 2D and 3D contour graphs (Figure 3.18) showing the effects of temperature, time, and the ratio of raw solvents on extraction efficiency of inulin, fructans, and dissolved extracts, choose the optimal range of extraction mode. The output is temperature (60-80^{o}C, time (20-50) minutes, ratio DM / NL (20-60) ml / g.

Regression equation of Inulin content: $Y_1=234.87-3.42X_1+8.98X2+27.78X_3 - 1.61X_1X_2 -6.49X_1X_3 - 7.19X_2X_3 -85.10X_1^2 -45.42X_2^2 - 30.72X_3^2$ (1)

Regression equation of Fructan content: $Y_2=267.42 - 9.56X_1 +12.25X_2+28.52X_3-10.04X_1X_2 - 9.70X_1X_3 -8.59X_2X_3 -94.86X_1^2 -58.66X_2^2 -35.79X_3^2$ (2)

Regression equation of the dissolved extract content: $Y_3=57.46 + 3.76X_1 + 2.21X_2 + 5.21X_3 + 2.56X_1X_2 + 1.75X_1X_3 - 3.50X_2X_3 - 4.37X_1^2 - 6.82X_2^2 - 7.12X_3^2$ (3)

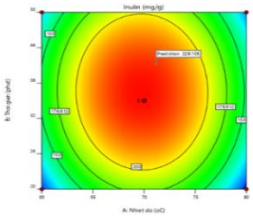
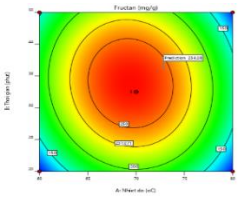
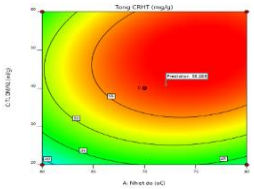
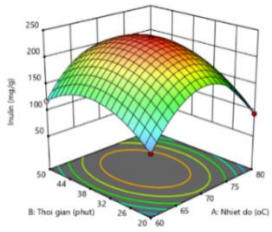
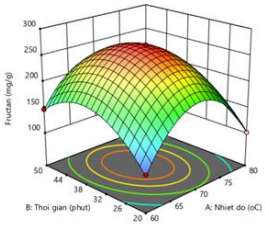
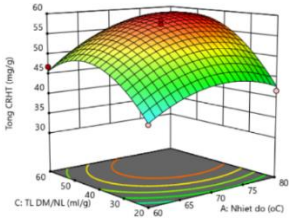
 a1. Contour lines show the effects of time and temperature on inulin extraction efficiency.	 a2. Contour lines show the effects of time and temperature on Fructan extraction efficiency.	 a3. Contour represented the influence of temperature and rate of DM / NL to efficiently extract total soluble extracts.
A. 2D model		
		
b1. Responsive surface of the inulin content	b2.Responsive surface of the fructan content	b3.Surface response of total dissolved extract content
B. 3D model		

Figure 3.18. 2D (A), 3D (B) models predict the optimal point of the target functions under the influence of input factors.

*** Determine the optimal parameters for inulin, fructan, total dissolved extracts.**

The study results showed that the optimal conditions for extracting Inulin, Fructan, and hole dissolved extracts from dried Dangshen at 71^oC temperature, extraction time 36 minutes, and solvent/material ratio was 47ml/g. Inulin extraction efficiency in railway tubers was 23.93%, Fructan 26.96%, and total dissolved extracts reached 61.35% and extracted one time.

3.2.2.Proposed process for extracting inulin from Dangshen 's roots

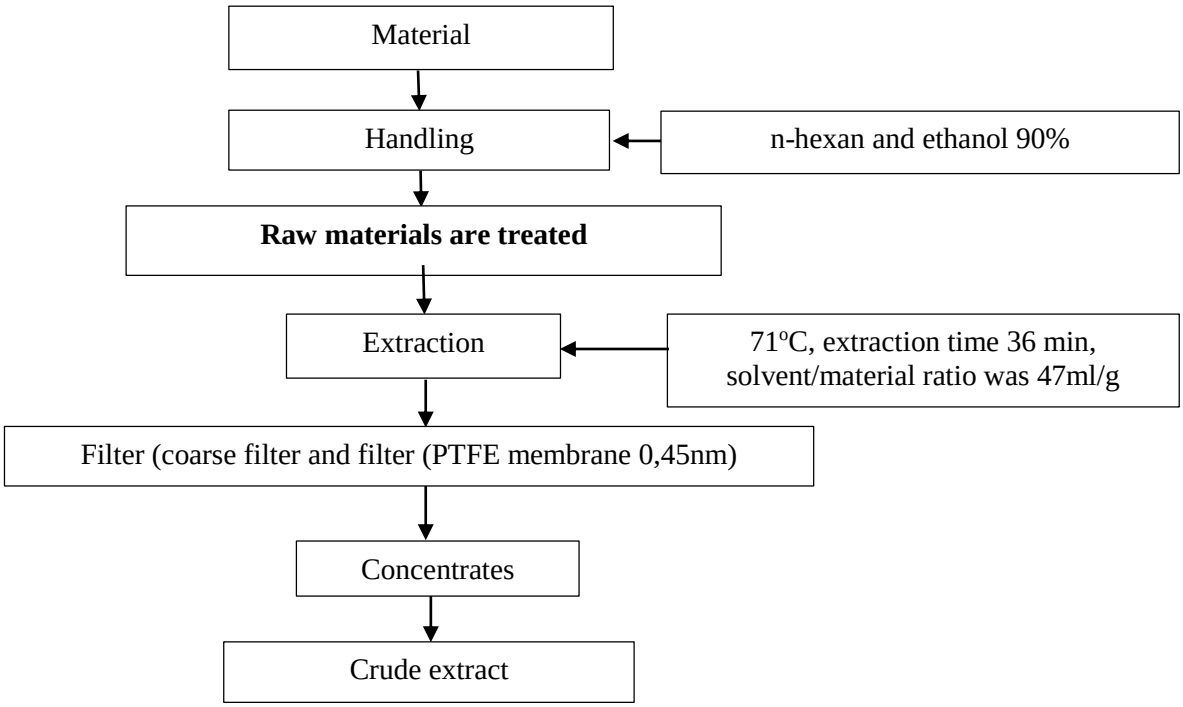


Fig 3.21. The process of extracting inulin from natural Dangshen roots

3.3 Determination of some suitable parameters for the precipitation of inulin from the extract

3.3.1. Determination of solvent and solvent concentration

The research results (Table 3.7) show that ethanol and acetone as the solvent 2 precipitates obtained inulin, fructan, and tubers saccharide in water extracts of Dangshen tubers with the highest levels. However, acetone is a toxic solvent and has a higher cost than ethanol. Therefore, the thesis decided to choose ethanol as inulin and fructan precipitation solvents in the later research process.

Table 3.7. Effects of solvent and solvent concentration on total saccharide, inulin, and fructan content obtained after precipitation

Solvent	Concentration 80%			Concentration 90%		
	Polysaccharide content totality (mg/g DW)	Inulin content (mg/g DW)	Efficiency precipitated Inulin (%)	Saccharide content totality (mg/g DW)	Fructan content (mg/g DW)	Efficiency Precipitate fructan (%)
Aceton	$244.92^{a} \pm 5.28$	$232.92^{a} \pm 5,02$	95.10	$312.43^{a} \pm 12.73$	$301.81^{a} \pm 12.30$	96.60
Ethanol	$233.07^{a} \pm 4.97$	$222.66^{a} \pm 5,09$	95.53	$294.08^{a} \pm 6.51$	$278.90^{b} \pm 2.16$	94.84
Ethyl acetate	$57.58^{b} \pm 6.20$	$46.50^{b} \pm 3.87$	80,75	$118.08^{b} \pm 5,44$	$98.65^{c} \pm 5.41$	83.54
n-butanol	$25.58^{c} \pm 3.79$	$21.54^{c} \pm 2.84$	84.19	$44.83^{c} \pm 2.84$	$36.99^{d} \pm 2.39$	82.51
n-hexan	$17.92^{c} \pm 0.58$	$12.32^{c} \pm 1.92$	72.42	$33.33^{c} \pm 2.92$	$24.25^{d} \pm 2.61$	72.75

Note: Different letters in the same column are different ($p < 0.05$).

3.3.2. Determination of ethanol concentration

Study results showed that EtOH solvent concentration 80% used to precipitate Inulin and 90% concentration used to precipitate Fructan.

3.3.3. Determination of the concentration temperature of inulin extract from Dangshen .

Research results show that the concentration should be set below 65^{o}C, specifically 55^{o}C. When the vacuum is concentrated at a temperature higher than 55^{o}C, it affects the inulin content.

3.3.4. Determine the temperature of the inulin precipitate from the extract

Table 3.12. Inulin and Fuctan precipitation temperatures

Temperatures (^{o}C)	Water solvent (Control)	Solvent concentration EtOH			
		80%		90%	
	Inulin content (mg/g DW)	Inulin content (mg/g DW)	Efficiency (%)	Fructan content (mg/g DW)	Efficiency (%)
(6±1)	22,33^{c} ±2,04	222,66^{a} ± 5,09	95,53	278,90^{a} ±2,16	94,84
- (11±1)	36,98^{b} ±2,43	224,19^{a} ± 8,71	96,19	279,67^{a} ± 4,80	95,10
- (17±1)	86,23^{a} ±1,66	224,21^{a} ± 5,38	96,20	279,73 ^{a} ±6.16	95,12

Note: Different letters in the same column are different (p <0.05).

The research results (Table 3.7and 3.12) show that factors such as solvent, solvent concentration, precipitation storage temperature, extract concentration temperature affect the obtained inulin and fructan content. Ethanol solvent at 80 and 90% concentration, storage temperature for precipitation collection (6 ± 1)°C and concentration temperature of 55^{o}C are the parameters that help to recover the highest inulin content, so it is selected for research studies next in the thesis.

3.4. Clean-up study and characteristics of factory structure of inulin

3.4.1. Inulin purification study

Table 3.13. Inulin content and purification

Precipitate times..	**Saccharide content before precipitation**	**Pure inulin content (mg/g pourder)**	**Recovery efficiency after the purification times…(%)**
4	816.67^{a}±28.27	730.61^{a} ±4.02	89.46^{c} ±0.89
5	730.61^{a} ±4.02	663.95^{b}±29.63	90.88^{b}±4.99
6	663.95^{b}±29.63	613.07^{c}±17.52	92.34^{a}±0.84

Note: Different letters in the same column are different (p <0.05).

Check the purity of the purified sample from crude powder by TLC. The results showed that the Rf of inulin in the studied sample and Rf of the standard are equal, respectively 0.411 and 0.415.

3.4.2. Proposing inulin purification process from naturally grown Dangshen root

*** Inulin purification process**

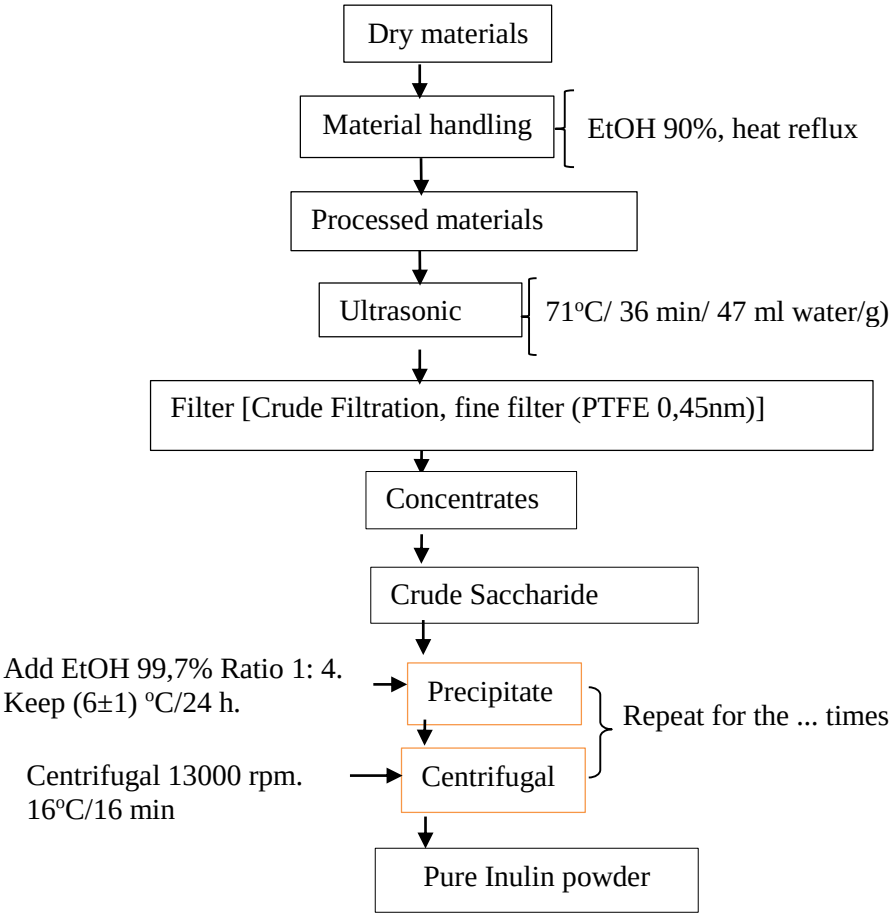


Fig3.26. The process of purifying insulin from Dangshen extract

*** Process explanation**

Raw material treatment: Fresh ingredients are processed, cleaned, and dried to<60°C until the humidity is <10%.The dry sample is free from fat, free sugars, colorants, amino acids, and soluble in 90% EtOH. Solvents in alcohol solvents (belonging to another study). The dried medicinal residue is used as raw material for inulin and fructan extraction.

Ultrasonic extraction: The medicinal residue is extracted with the help of ultrasonic waves with a water solvent, temperature 71°C, the ratio of material/solve 1g/47 ml for 36 minutes.

Filtration: Coarse filtration with cloth, then filter and finally filter through an ultrafiltration membrane (PTFE 0.45nm) to collect the filtrate.

Concentration: The solution is concentrated at 55°C, up to 16°Bx to remove a part of water, save solvent costs, increase dry matter content.

Precipitation of crude saccharides: Add EtOH 90%. Keep at (6 ±1)°C/24 h. Centrifuge 16°C / 13000 rpm /16 minutes to collect coarse saccharide precipitate and remove colorants, free sugar. Dry the precipitate.

Precipitation-centrifugation: Dissolve the precipitate with distilled water 2 times and inulin precipitates at 90% ethanol concentration. Refrigerate at (6 ±1)°C/24 h. Centrifuge 16°C/13000 rpm/min/16 minutes clean crystallization many times. Determination of inulin content after each purification. Check the purity in the final purified sample with TLC thin sheet and use this purified sample to perform a molecular structure study in the next experiment.

3.4.3. Characterization of inulin molecular structure

3.4.3.1. Molecular weight of inulin

The analytical results showed that the pure starch extracted from Dangshen contained two types of polysaccharide: 96.448% compounds with a molecular weight of about 3.193 Da and 3.552% of compounds with a molecular weight of about 1.112.892 Da (Table 3.15). Experimental and analytical results (Fig 3.27) conclude that Inulin in Lam Dong roots have a DP of 19-23 Fructose units, a molecular weight of about 3193 Da, and the other Polysacchaite has a molecular weight of 1.112.892 Da.

Table 3.15. Results of molecular w,eight analysis of polysaccharide components in Dangshen

No	Rt (min)	Pic area	Rate of pic area (%)	Molecular mass (Da)
1	4,919	247,161	3,552	1.112.892
2	8,673	6711, 200	96,448	3.193

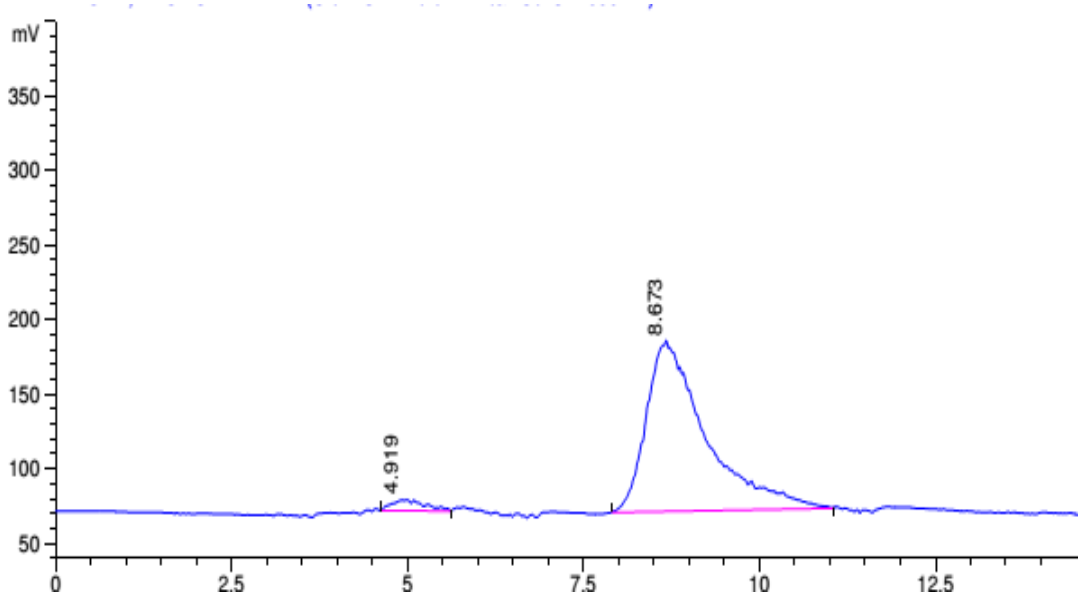
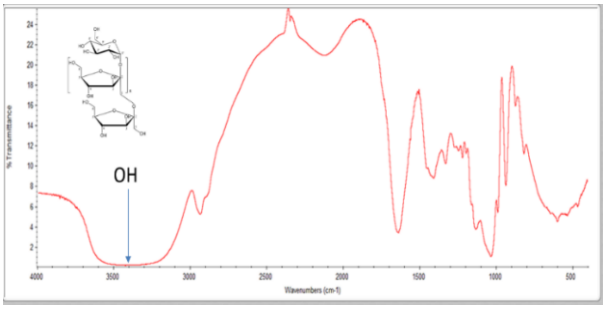


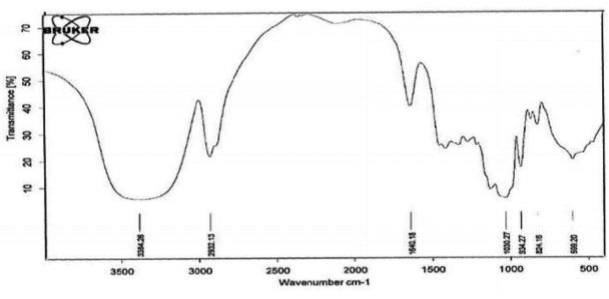
Figure 3.27. Gel chromatography of Dangshen polysaccharide sample

3.3.3.2. Determination of inulin molecular structure

Analysis results of IR, NMR, DEPT, HSQC, HMBC spectra of pure Dangshen powder. (Fig3.28 ÷ 3.34) and table 3.16. Polysaccharide in Dangshen is identified as a fructose – polysaccharide, with a DP of about 19-23 (Fig 3.35).



(a).



(b)

Figure 3.28- 3.29. IR spectra of purified Dangshen powder (a) and commercial Inulin (b)

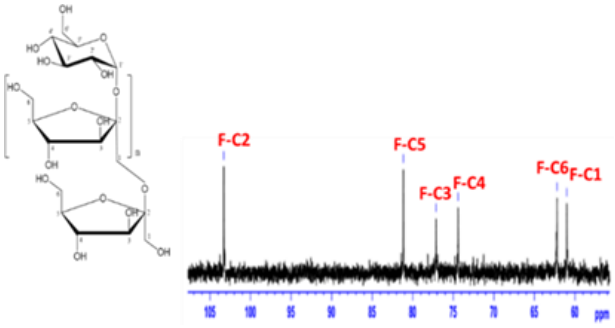


Fig 3.30. Spectrum ^{13}C-NMR (D_2O, 125 MHz)

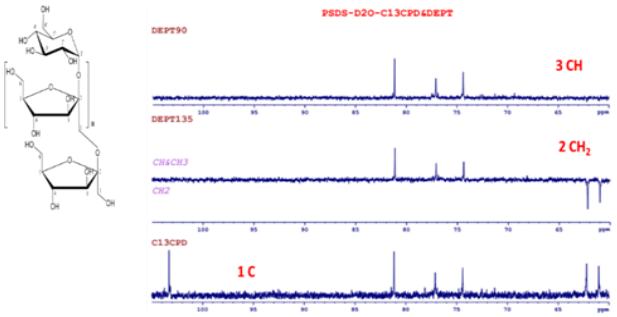


Fig 3.31. Spectrum DEPT

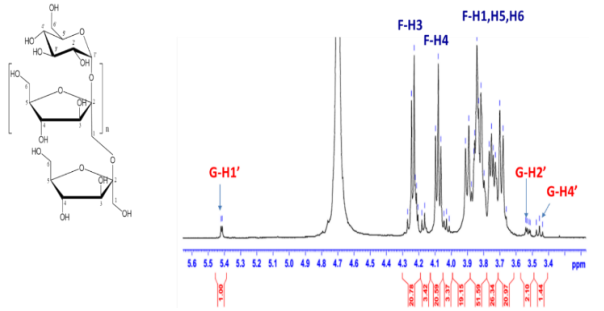


Fig 3.32. Spectrum ^{1}H-NMR (D_2O, 500 MHz)

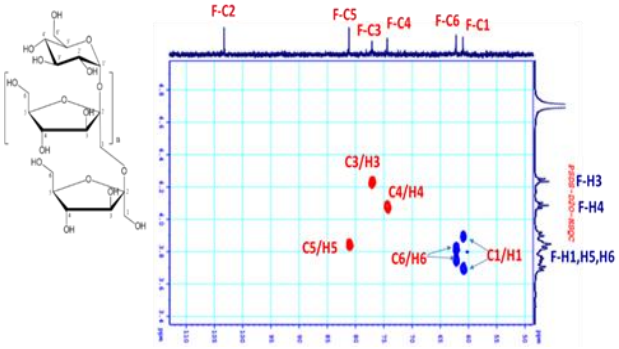


Fig 3.33. Spectrum HSQC

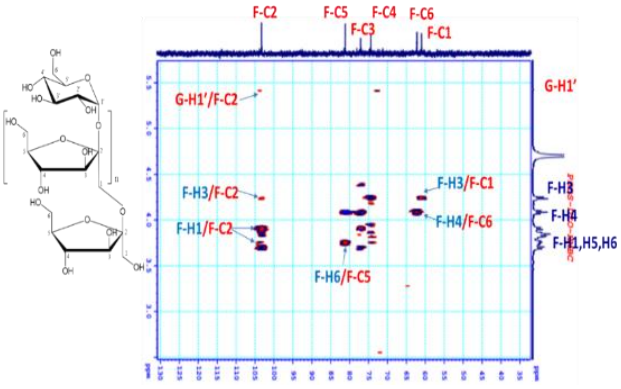


Fig 3.34. Spectrum HMBC

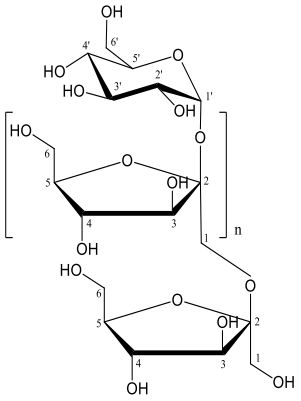


Fig 3.35. Chemical structure of Inulin

Table 3.16. NMR spectral data and molecular structure of Inulin

C	*δ_C (ppm)	[a,b]δ_C (ppm)	DEPT	[a,c]δ_H (ppm) (*J*, Hz)	Important HMBC interactions (H→C)
→1)-β-D-Fructofuranosyl-(2→					
1	61,42	61,01	CH_2	3,90 (d, *J* = 10,0 Hz, 1H) 3,69 (d, *J* = 10,0 Hz, 1H)	C-2, C-3
2	103,39	103,31	C	-	
3	77,58	77,11	CH	4,23 (m, 1H)	C-1, C-2, C-4
4	74,93	74,41	CH	4,08 (m, 1H)	C-3, C-5, C-6
5	81,43	81,16	CH	3,85 (m, 1H)	C-4, C-3
6	62,48	62,21	CH_2	3,83 (m, 1H) 3,75 (m, 1H)	C-4, C-5

α-D-Glucopyranosyl-(1→					
1’	92,73	kth		5,41 (d, *J* = 4,0 Hz)	C-2
2’	70,75	kth		3,53 (dd, *J* = 4,0; 10,0 Hz)	
3’	73,04	kth		3,82 (m)	
4’	69,78	kth		3,46 (t, *J* = 9,5 Hz)	
5’	71,64	kth		3,82 (m)	
6’	60,75	60,5		3,79 (m)	

*Notes: a Measured in D_2O, b 125MHz, c 500MHz; kth- signal unknown, * according to De Oliveira et al.*

The research results show that: Inulin in natural lam Dong province is in the form of GFn. There are two types of polysaccharide, one is fructose-polysaccharide inulin, accounting for a large amount of 96,448% with a molecular weight of about 3.193 Da, and the other accounts for 3.552%, has a molecular weight of about 1.112.892 Da, and is a branched-chain levan. A specific feature is that high molecular weight polysaccharide is very rare in natural plants, but it is discoverred in Dangshen roots of Lam Đong province. Inulin with high Molecular weight from synthetic sources is not readily available in the market on a large scale, most likely due to its high production costs. Thus, inulin with a high molecular weight in the Dangshen party of Lam Dong province, if isolated, could be a potential source of FOS.

3.5. Study on inulin powder spray spray

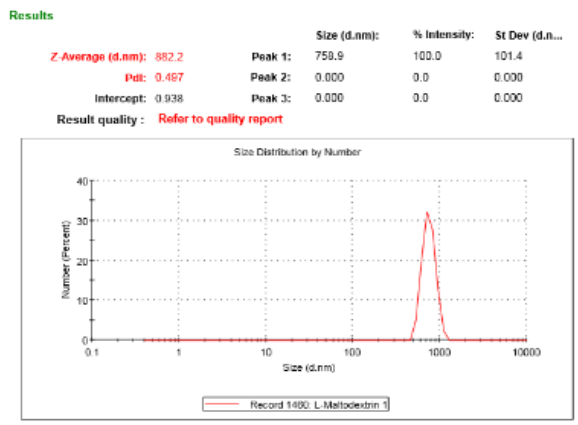
3.5.1. Determine carrier and carrier ratio

3.5.1.1. Determination of carrier

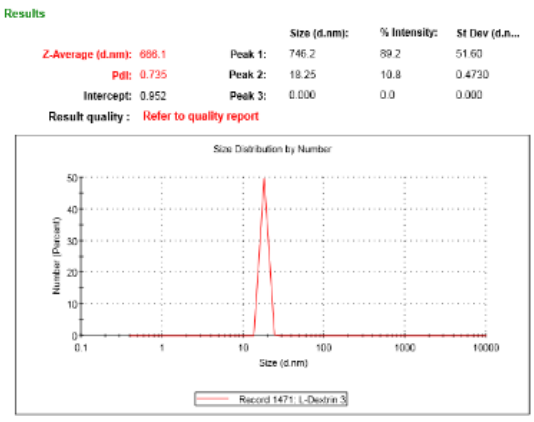
Drying parameters: Spray drying solution 16^{o}Bx; Carrier concentration 10%; Inlet/outlet air temperature 180/85^{o}C; compressed air pressure 3 atm; The speed of the injection disc (RRD) is 16000 rpm; Peristaltic pump speed (RRD) 10 rpm.

a. Consider physical properties

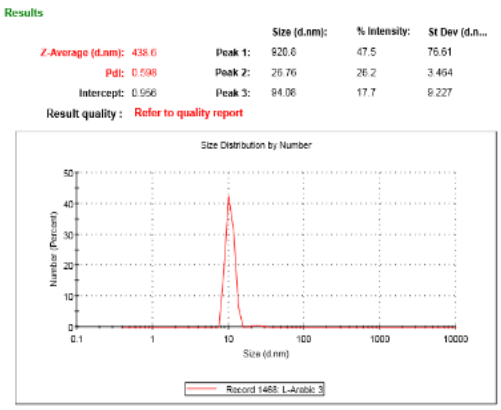
*** Particle size and size distribution index**



a. Particle size spectrum of Dangshen samples with Maltodextrin carrier



b. Particle size spectrum of Dangshen samples with Dextrin carrier



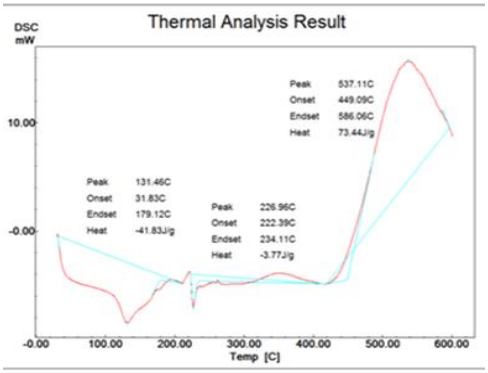
c. Particle size spectrum of Dangshen samples with Gum Arabic carrier

Fig 3.36. Analysis diagram of particle size and dispersion spectral analysis

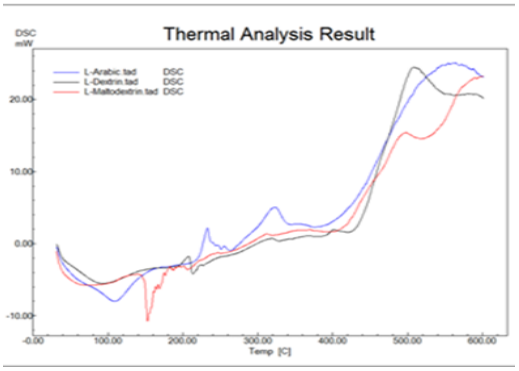
Tesults (Fig 3.36 a,b,c) showed that the drying powder with maltodextrin carrier had the smallest Dpi 0.497 compared to the two of Dextrin and Gum Arabic.

* **Regarding thermal properties**

Results present in Fig 3.37a and 3.37b.



a) Pure Dangshen



b) Dried Dangshen powder sprayed with carriers

Fig 3.37. Diagram of differential thermal analysis of pure powder (a) and spray drying (b)

The analysis results of the differential thermal analysis diagram (Fig 3.37 a, b) show that the maltodextrincarrier makes the drying powder more heat stable than the carrier dextrin and Gum arabic.

b. About Inulin content

Table 3.19. Inulin content and sensory properties of spray-dried powders

Type of carrier	Solubility	Inulin content (mg/g)	Fructan content (mg/g)	Total mineral content (%)	pH	Sensory
Maltodex	Dissolve easily	$446,60^{a}$ ±4,75	$472,56^{a}$ ± 3,22	$4,82^{b}$ ± 0,07	5,46	Slightly sweet and sour, characteristic Dangshen smell
Dextrin	Dissolve easily	$404,14^{b}$ ±5,99	$431,16^{b}$ ±7,59	$1,91^{c}$ ± 0,08	5,15	Slightly sweet and sour, characteristic Dangshen smell
Gum Arabic	Dissolve easily	$442,39^{a}$± 5,19	$469,75^{a}$± 2,19	$5,09^{a}$ ± 0,05	5,84	Slightly sour, slightly bitter taste, characteristic Dangshen smell

Note: Different letters in the same column are different (p <0.05).

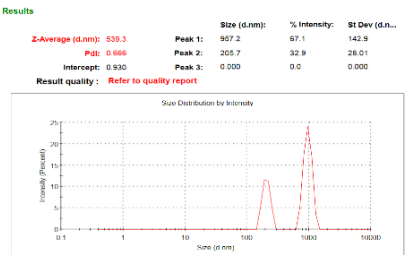
Research results (Table 3.19) showed that: Maltodextrin is the suitable carrier selected for use as a carrier for the study of spray drying powder production.

3.5.1.2. Determination of carrier concentration

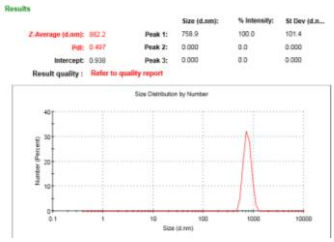
∗About particle size

Maltodextrin carrier at concentrations of 5,10,20% (w/v) is added to the extract. The drying temperature set up at 185 / 85ºC inlet/outlet heat corresponding to the ĐS1 spray drying powder; ĐS2; ĐS3.

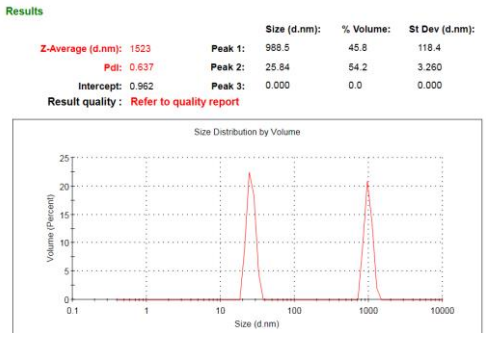
The analytical results of the size spectrum (Fig 3.38) showed that: The ĐS3 sample did not meet the requirements of the criteria, so it was rejected. The sample ĐS1 was dominant in the particle size but weak in the Dpi index. Therefore, the thermal properties and inulin content need to be analyzed directly to compare and evaluate these two samples and compare them with the pure ĐS sample.



a. Powder Dangshen 1 (ĐS1)



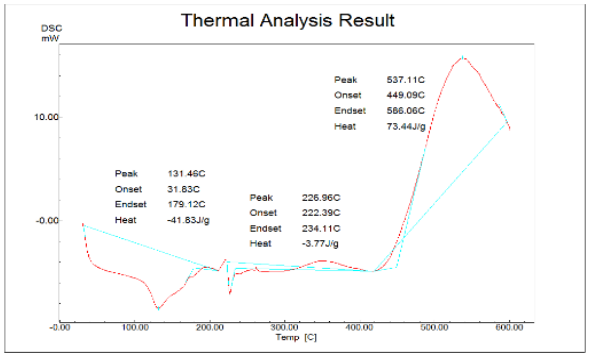
b. Powder Dangshen (ĐS2)



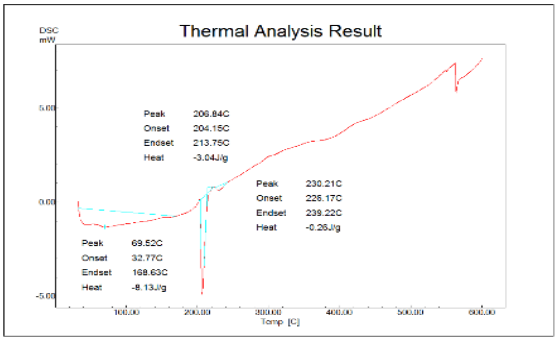
c. Powder Dangshen 3 (ĐS3)

Fig. 3.38 (a, b, c). The particle size of the drying powders depends on the carrier concentration

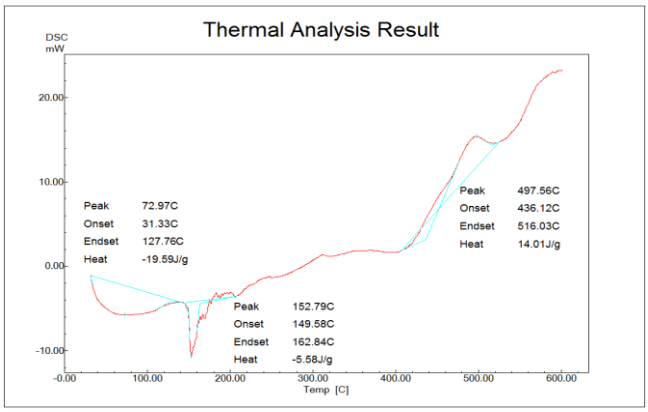
∗ About thermal properties



a) Pure Dangshen powder



b) Party Dangshen spray drying powder 1 (ĐS1)



c) Party Dangshen spray drying powder 2 (ĐS2)

Fig 3.39. Thermal analysis diagram of Dangshen spray (a) ĐS purification, b) drying DS1, c) drying ĐS2

The analysis results of the thermal diagram (Fig 3.39.a, b, c) show that the sample ĐS2 is more heat stable than that of the ĐS1 sample. Besides, the Pdi of the ĐS2 model is 0.497, very close to 0.4.

In terms of thermal properties, sample ĐS2 was selected

Research results showed that: Inulin content in sample ĐS1 reached 431,16^{b} mg/g powder, and sample ĐS2 get at 459,90^{a} mg/g powder. Meanwhile, the fructan content of samples ĐS1 and ĐS2 were 452,56^{b}, 469,40^{a} powder, respectively. Thus, the ĐS2 has higher contents of Inulin and fructans than the ĐS1. Combined with research results on thermal properties (Fig 3.39.a, b, c), the concentration of 10% carrier is for supplementation. add to the extract.

3.5.2.Determine the inlet gas temperature

The carrier was fixed at 10%, the inlet drying temperature was at 130,150,185^{o}C, the output drying temperature was 85^{o}C, corresponding to the drying samples ĐS3, ĐS5, ĐS2, ĐS6. Particle size analysis results are shown in the charts (Fig 3.41) and Table 3.21.

Table 3.21. The particle size and Pdi according to the drying temperature

Drying chamber temperature - in / out (^{o}C)	Pattern	Average particle size (d.nm)	Particle size and density		Pdi	Humidity (%)
			Particle size (d.nm)	Density (%)		
130/85	ĐS4	1224	876,4±110,7	8,4	0,734	6,25±0,4
			85,71±10,89	0,7		
			13,51±1,466	90,9		
150/85	ĐS5	1070	1054±132,2	18,2	0,806	5,40±0,05
			117,3±14,57	0,8		
			14,55±1,851	80,9		
185/85	ĐS2	884,2	758,9±101,4	100	0,497	6,06±0,27
210/85	ĐS6	996,2	442,2±54,28	100	0,686	4,27±0,33

When drying in /out 185/85^{o}C, the ĐS2 drying sample has the smallest size, reaching 884.2 nm and PDI 0.497, particle density 100%. This result shows that sample ĐS2 has uniformity in particle size. The rest of the drying samples were big size. Specifically, samples ĐS4 and ĐS 5 size were a larger size than 1000 nm, Pdi> 0.7, and up to three types of seeds in the drying sample. This result shows that, when drying at different temperatures than 185/85, the patterns will have bigger and not the same particle size. Thus, the

appropriate parameter for the powder spray drying process is the carrier 10%, the drying temperature in /out 185 /85^{o}C; 3 atm compressed air pressure, 20 rpm peristaltic pump speed; Spray disc speed 16000 rpm.

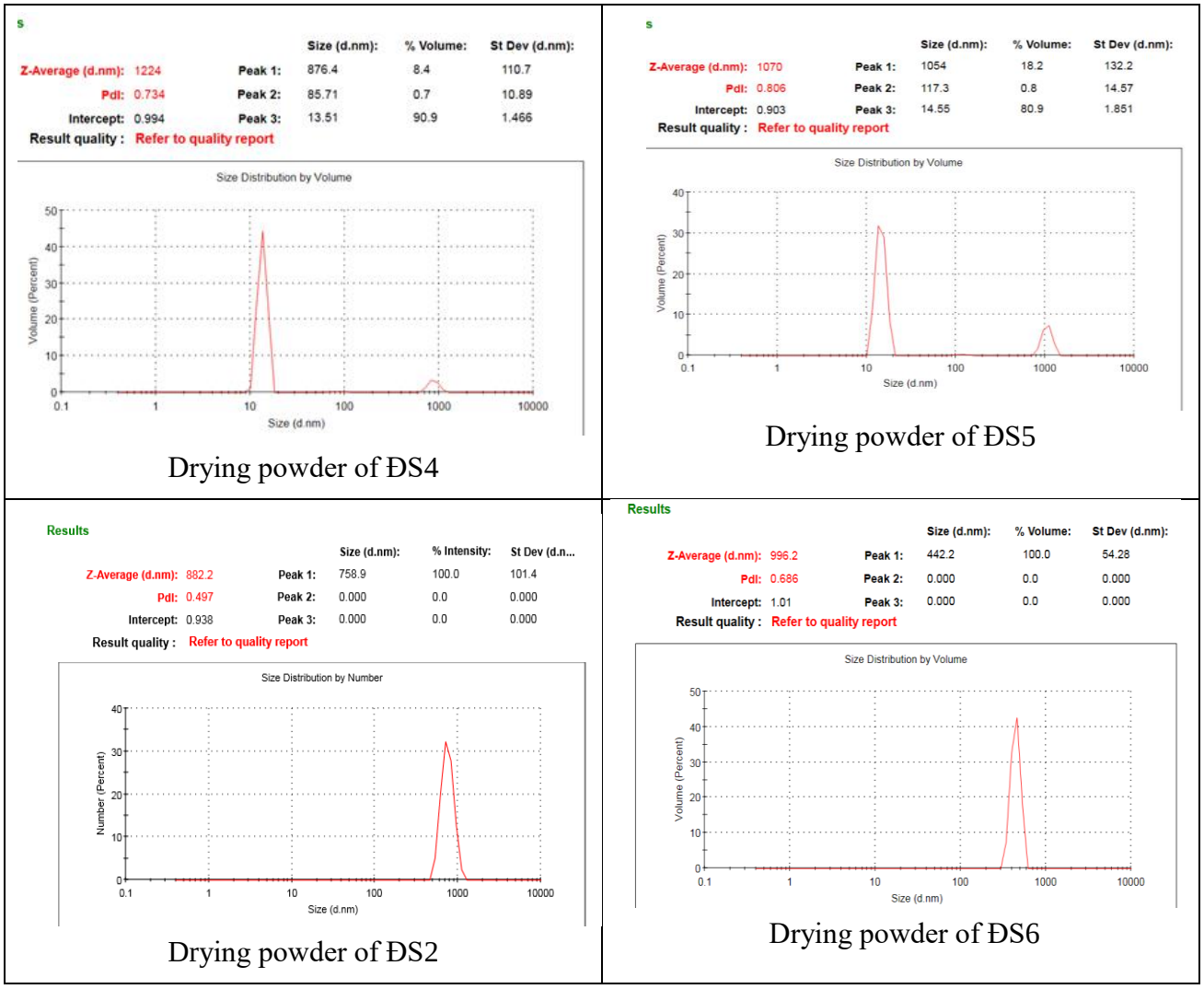


Figure 3.41. The particle size of the powders is heat-dried

3.5.3. Proposing a spray drying process to create inulin powder from Dangshen root

3.5.3.1. Process proposal

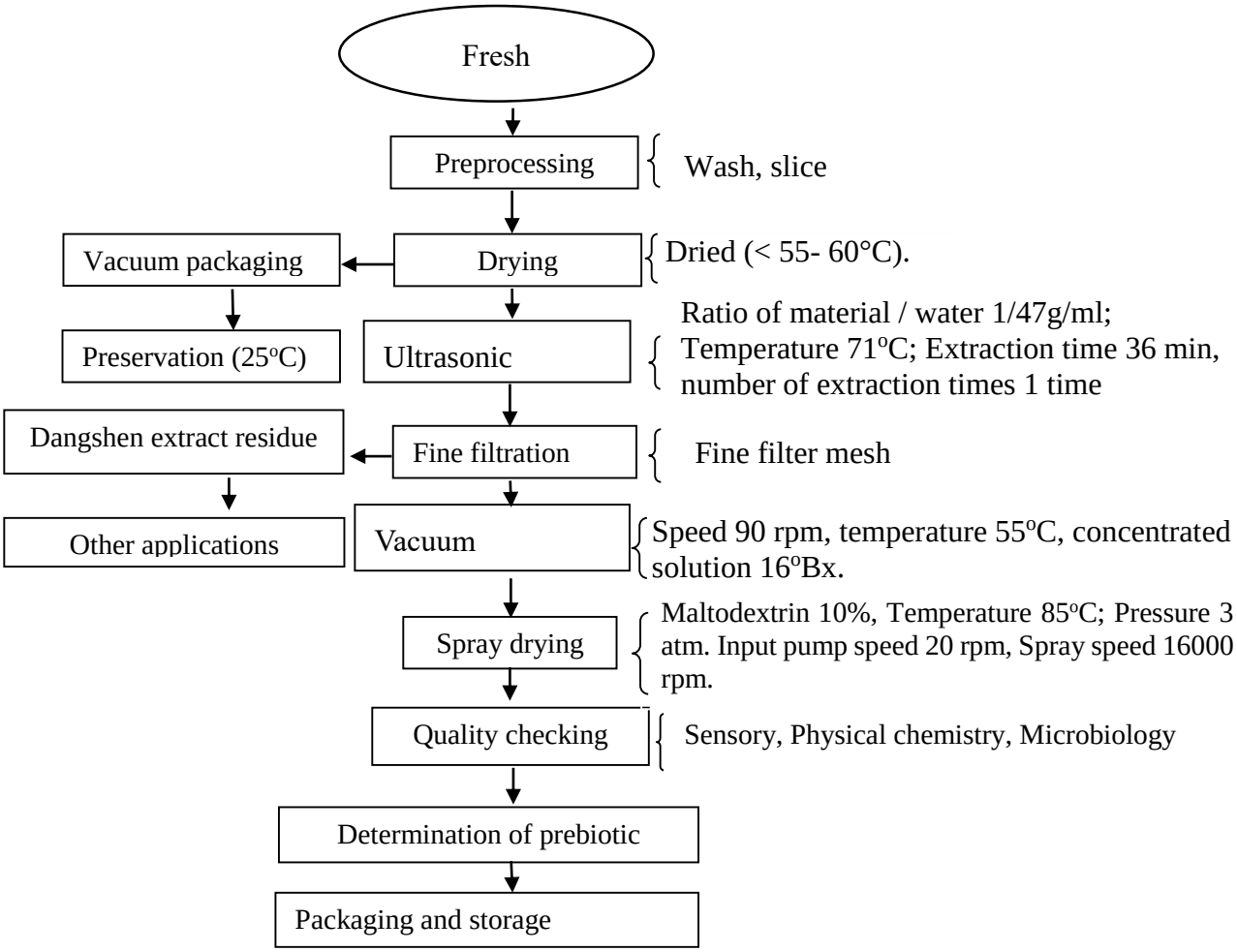


Figure 3.42. The process of creating powder spray

***Process explanation**

1. Fresh railway, meeting the standard of pharmaceutical material, is washed, sliced thinly with a knife, heated (<60 °C) to dry (humidity <10%), vacuum-packed, and preserved at normal temperature.

2. Extraction ultrasonic extract at temperature 71°C/36 min/47 ml/g. Extract one time.

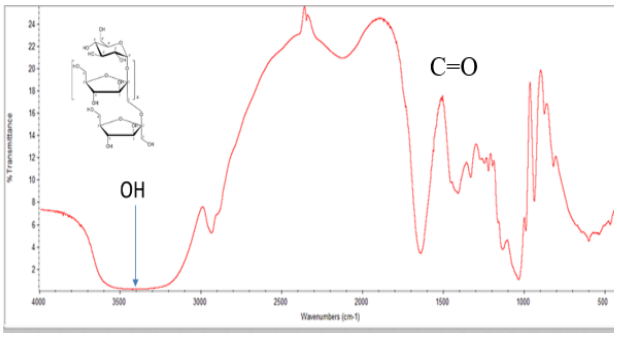
3. Concentrate the extract under vacuum (Speed 90 rpm, temperature 55°C until the concentrate at 16°Bx.

4. Spray-drying: Add 10% Maltodextrin to the concentrate, homogenize the solution. Spray drying (temperature 85°C; Compressed air pressure 3 atm, Peristaltic pump speed 20 rpm, Spray speed 16000 rpm) to collect finished powder.

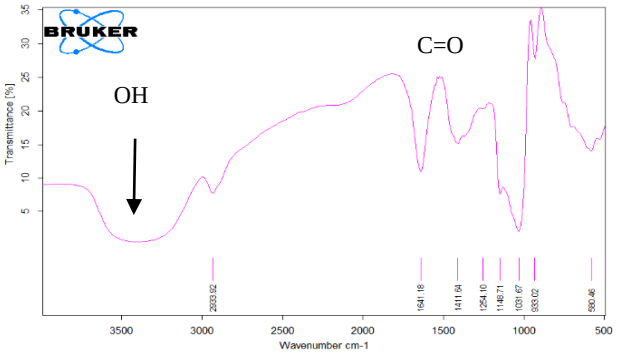
5. Check the quality of finished powder through the evaluation of sensory properties, physical chemistry, microbiology, prebiotic activity. Compare with Vietnamese Standards about quality for functional food products.

6. The finished Dangshen spray-drying powder is packed in vacuum-sealed nylon, preserved at room temperature (25°C).

3.5.3.2. Test production and quality assessment of inulin powder from Dangshen root



a)FT-IR spectrum of pure Dangshen powder



b) FT-IR spectrum of spray-dried Dangshen powder

Figure 3.43. FT-IR spectrum of pure Dangshen powder (a) and spray-drying (b)

FT-IR spectra will show the structural stability of the inulin in spray-dried powder. The study results show (Fig 3.43): Inulin chemical structure is similar between pure railway sample and spray-dried sample. Besides, pic in the 943 cm^{-1} regions indicated the presence of Fructose-furanoryl unchanged.

Therefore, in the above drying mode, it does not transform the molecular structure of the inulin in spray drying powder. It is the basis to confirm the biological properties of spray-dried Dangshen powder in the next reaseach.

Table 3.22. The properties results of the finished spray-dried Dangshen powder

No	Characteristics	Unit	Results	Limit allowed
1. Sensory Characteristics				
1.1.	Color		Pale brown	
1.2	Odor		The aroma of typical Dangshen	
1.3	Taste		Lightly sweet	
2. Physical and chemical properties				
2.1	Humidity	%	6.06±0.27	
2.2	The temperature that completely destroys the molecules	^oC	>600	
2.3	Particle size	d.nm	884.2	
2.4	Density	%	100	
2.5	Dpi		0.497	
2.7	pH		5.18±0.01	
2.8	Inulin content	(mg/g)	445.90 ± 2.79	
2.9	Fructan content	(mg/g)	469.40 ±1.61	
2.10	Solubility	g/ml/mim	1/9.5±0.5/15	
2.10	Total mineral	%	4.82 ± 0.07	
3. Heavy metal (By QĐ No:6/2007/QĐ-BYT. Date 19/12/2007*)				
3.1	Pb	ppm	Not detected	10

3.2	Cd	ppm	Not detected	0.3
3.3	Hg	ppm	Not detected	0.5
3.4	As	ppm	Not detected	5.0
3.5	Inorganic impurity	ppm	Not detected	
4. Microbiological safety (By QĐ No: 6/2007/QĐ-BYT. Date 19/12/2007*)				
4.1	Total aerobic microorganisms	CFU/g	1.0 x10^2	10^4
4.2	Total *Eschirichia coli*	CFU/g	Not detected	Negative
4.3	Total *Clostridium perfringens*	CFU/g	Not detected	10
4.4	Detect *Salmonella spp*	Yes or Not detected in 25 g	Not detected	Negative
4.5	Total number of yeast-mold spores	CFU/g	Not detected	10^2

Research results (Table 3.22) show:

∗ **About sensory quality**

Spray-drying powder products are obtained from powdered Dangshen extract, yellow-brown, fragrant with Dangshen aroma.

∗ **Regarding physical and chemical criteria**

The obtained drying powder has moisture (6.06±0.27) %, total mineral (4.82b ± 0.07) %, and completely water-soluble Dangshen powder (1g/10 ml), time dissolve quickly (15 minutes). The pH is in the range (5.18 ± 0.00).

The finished spray-dried powder product had relatively high inulin content, averaging (445.90 ± 2.79) mg/g. Average fructan content (469.40 ±1.61) mg/g powder. No detected heavy metal.

∗ **About microbiological criteria**

Railway drying powder meets the requirements of microbiological criteria according to the regulations of the Ministry of Health.

*From the above analysis, it can be seen that spray-dried Dangshen powder products by the process of the thesis meet the sensory quality, chemistry, and microbiological requirements (*Ministry of Health *Standards). Therefore, it can use for food production and functional foods*.

3.6. Testing of inulin use from synbiotic powder cream and orientation of application in food

3.6.1. Effect of Dangshen inulin powder on the growth of certain types of bacteria used in the production of probiotic preparations

Table 3.23. The prebiotic activity of spray drying powder

No	Name of the bacteria	Bacterial density (CFU g)			
		in the primary biomass	After raising	After mixing with Dangshen powder	Density fluctuation (times)
1	*E. faecalis (M1)*	1.7 x10^{10}	2 x10^9	5 x10^9	↑2.5
2	*L. acidophillus (M2)*	8 x10^9	3.1 x10^9	9 x10^9	↑ 2.9
3	*L. rhamnosus (M3)*	6.3 x10^9	3 x10^9	8 x10^9	↑2.6

4	*E. faecalis (M4)*	2 x10^{10}	5 x10^{6}	7 x10^{6}	↑1.4
5	*L. plantarum (M6)*	2 x10^{9}	3 x 10^{9}	15.9 x10^{9}	↑5.3
6	*L. acidophillus (M7)*	4 x10^{10}	2 x10^{10}	2.3 x10^{11}	↑11.5
7	*B. longum (M8)*	10^{8}	8.2 x 10^{9}	8 x10^{10}	↑9.75
8	*B. lactics (M9)*	10^{8}	1.3x 10^{9}	7x10^{9}	↑5.38

Note: CFU Colony Forming Unit; ↑ Increase times.

When added at a concentration of 4% to the culture medium to create prebiotics, it can stimulate growth and increase the biomass of 6 individual tested bacterial strains from 1.4 ÷ 11.5 times. The research results also showed that spray-dried inulin powder when added with 4% concentration to the culture medium could strongly increase the biomass of L. plantarum, L. acidophilus (M7), B. longum, B. lactic from 5.3 ÷ 11.5 times. Therefore, these strains were selected to be mixed with Dangshen powder to form synbiotic products in subsequent experiments.

3.6.2. Optimizing the mixing process of Dangshen powder with probiotic biomass to produce synbiotic products

*** Define the regression model**

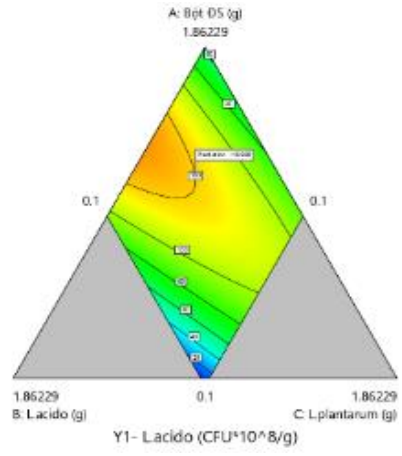
The Yi regression equations are as follows:

$\mathbf{Y_1}$= 18,75X_1-751,35X_2 +199,10X_3-264,81X_4 – 671,62X_5 + 1603,55X_1X_2 + 136,11X_1X_3 + 265,78X_1X_4 + 1111,23X_1X_5 + 753,29X_2X_3 + 1110,64X_2X_4 + 2489,48X_2X_5 + 85,47X_3X_4 + 545,84X_3X_5 + 1694,67X_4X_5.

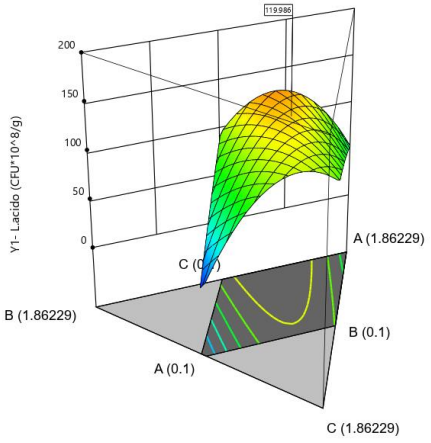
$\mathbf{Y_2}$= -1,17X_1 – 233,92X_2 – 502,14X_3 + 451,98X_4 + 275,61X_5 + 486,06X_1X_2 + 1895,58X_1X_3 -719,92X_1X_4 – 179,97X_1X_5 + 1296,71X_2X_3 + 79,59X_2X_4 – 358,28X_2X_5 + 258,43X_3X_4 + 535,89X_3X_5 – 1280,18X_4X_5.

$\mathbf{Y_3}$= -2,34X_1 + 1278,78X_2 – 539,63X_3 + 465,39X_4 – 452,61X_5 – 1555,93X_1X_2 + 974.59X_1X_3 -375.52X_1X_4 + 643X_1X_5 – 1409,43X_2X_3 – 3231,67X_2X_4 – 1069,47X_2X_5 + 789,65X_3X_4 + 1362,59 X_3X_5 + 936,64X_4X_5.

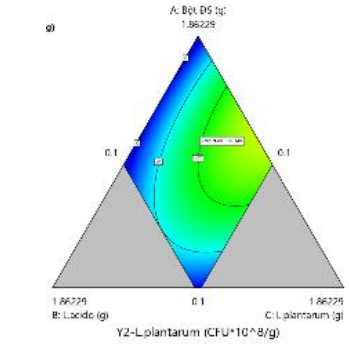
$\mathbf{Y_4}$= - 0,467X_1 + 1470,62X_2 – 882,72X_3 + 865,77X_4 – 291,82X_5 – 2032,82X_1X_2 + 1643.38X_1X_3 - 1141,00X_1X_4 + 1489,03X_1X_5 – 956,27X_2X_3 – 3637,44X_2X_4 – 1485,51X_2X_5 + 436,49X_3X_4 + 1836,43X_3X_5 – 590,01X_4X_5.



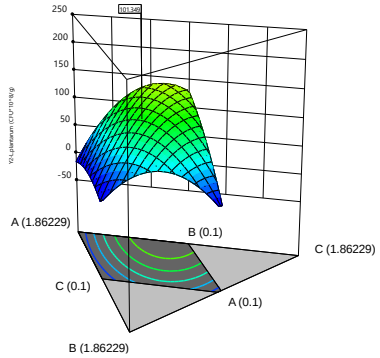
A1. Contour lines show the effect of the rate of Dangshen powder on the bacterial density *L. acidophillus*



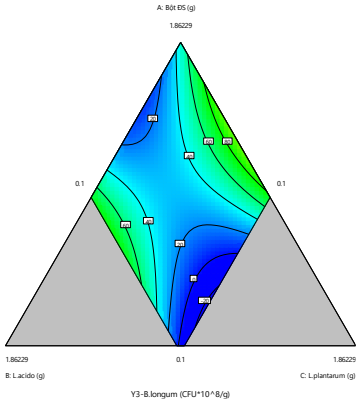
B1. Contour lines show the effect of the rate of Dangshen powder on the bacterial density *L. acidophillus.*



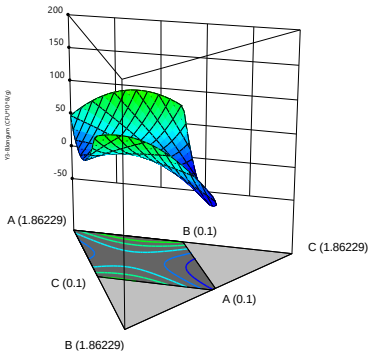
A2. Contour lines show the effect of the rate of Dangshen powder on the bacterial density *L. plantarum*



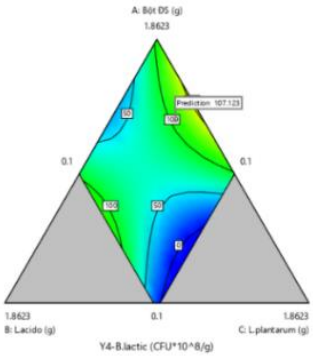
B2. Contour lines show the effect of the rate of Dangshen powder on the bacterial density *L. plantarum.*



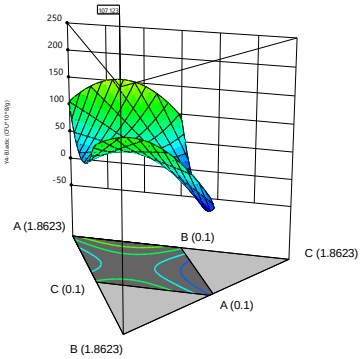
A3 Contour lines show the effect of the rate of Dangshen powder on the bacterial density *B. longum.*



B3. Contour lines show the effect of the rate of Dangshen powder on the bacterial density *B. longum.*



A4. Contour lines show the effect of the rate of đang sâm powder on the bacterial density *B. lactic.*



B4. Contour lines show the effect of the rate of đang sâm powder on the bacterial density *B. lactic.*

Figure 3.44. 2D (A), 3D (B) models of the response of target Yi target function under the effect of supplementary Dangshen drying powder rate

*** Determining the optimal parameters for the mixing process to create synbiotic**

Check again at the suggested optimum and compare with the combinations with the highest biomass. The results are presented in Table 3.28.

From the research results, the optimal formula for mixing to create synbiotics (g/g) was determined: the content of ginseng inulin powder 0.514 g was mixed with the biomass of L. acidophillus 0.22 g (Special biomass 5x10^{10} CFU/g); 0.128 g of L. plantarum biomass (specific biomass 2 x 10^{11} CFU/g); 0.033 g of B. longum (specific biomass 8 x10^{10} CFU/g) and 0.1 g of B. lactics biomass (specific biomass 10^{11} CFU/g) were selected to make synbiotic products.

Table 3.28. Results of retesting some combinations with the highest density

Ingredient	Mass in synbiotic (g/3g)				Own biomass CFU/g	Mass in synbiotic (CFU/g)			
	TH Optimal	TH 12	TH 3	TH 11		TH Optimal	TH 12	TH 3	TH 11
Dangshen powder	1.746	1.543	1.35	1.254		-	-	-	-
L. acidophillus	0.10	0.66	0.268	0.166	$5x10^{10}$	$2.6x10^{11}$	$15.5x10^{10}$	$9\ x10^{10}$	$8\ x10^{7}$
L. plantarum	0.614	0.385	1.00	0.522	$2\ x10^{11}$	$5.6\ 10^{11}$	$8\ x10^{11}$	$2.81x10^{11}$	$4\ x10^{11}$
B. longum	0.10	0.1	0.275	0.1	$8\ x10^{10}$	$8.8\ 10^{10}$	$9\ x10^{10}$	$6\ x10^{10}$	$7.5x10^{9}$
B. lactics	0.440	0.31	0.1	0.956	10^{11}	$4.1x10^{11}$	$3.9\ x10^{11}$	$1.1\ x10^{11}$	10^{9}
						Density fluctuations in synbiotics after 24 - 48 h of culture (Number of times increases or decreases)			
L. acidophillus						↑5.2	↑5.1	↑1.8	Not increase
L. plantarum						↑2.8	↑4	1.4	↑2
B. longum						↑1.1	↑1.1	Not increase	Not increase
B. lactics						↑4.1	↑3.9	↑1.1	Not increase

3.7.Testing synbiotic preparations on laboratory mice

The results of testing the product on laboratory mice are as follows:

Acute toxicity test: From the test results, it was found that the LD_{50} value of the synbiotic product could not be determined, i.e. the product did not show acute oral toxicity at the maximum possible dose for rats (Dmax) is 36.5 g/kg.

Effects on treatment of acute diarrhea: Synbiotic preparations have the ability to treat diarrhea in laboratory rats.

Immune-stimulating effects: A synbiotic preparation has shown immunostimulating effects in immunocompromised mice by a single peritoneal injection of cyclophosphamide 150 mg/kg.

Semi-permanent toxicity test: Mice taking a synbiotic preparation at a dose of 1.2 g/kg for 14 or 28 consecutive days did not affect renal function.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

From the above research results, it shows that the dissertation has completed all the set content, shown through the following conclusions:

1) The thesis has studied, and the results show that it is possible to base on the morphological and structural characteristics of the Dangshen naturally roots in Lac Duong Dist, Lam Dong province for

distinguishing age and determine the harvesting time of Dangshen. On the other hand, the thesis also confirmed that three-year-old roots have the composition of substances as the standards of Vietnamese Pharmacopoeia, 2017. Besides, the presence of almost all groups of 3-year-old rhizome. The active ingredient is soluble in various solvents (*n*-hexane, ethanol, water).

2) The thesis has optimized the process of extracting inulin from natural Dangshen grown in Lac Duong ditst, Lam Dong province.

Research results have identified the suitable factors for inulin collection precipitation: Ethanol solvent at 80% concentration for inulin precipitation and 90% ethanol concentration for fructan; The temperatura of concentrate the extract in a vacuum the was 55°C and the appropriate storage of inulin precipitate was (6 ±1)°C. An average precipitation of 95.53%.

The thesis has identified the appropriate parameters for the extraction of inulin from the Dangshen : extraction temperature 71^{o}C, extraction time 36 minutes and the ratio of water/material is 47ml/g. Inulin extraction efficiency in Dangshen tubers was 23.93%, Fructan extraction efficiency 26.96% and total dissolved extracts reached 61.35%.

3) The thesis has purified and characterized the inulin molecular structure obtained. The study results showed that inulin purified from the raw liquid by precipitation many times (6 times), the inulin purity reached (97.85^{a} ± 0.84)%. The two Fructose-polysaccharide inulin in Dangshen root in Lac Duong Dist, Lam Dong province, accounts for 96.448%, has a molecular mass of about 3.193 Da, and another polysaccharide accounts for 3.552%, has a molecular weight of about 1.112.892 Da.

4) The thesis studied the inulin powder spray drying: the drying aid was maltodextrin with the addition of 10%, the air temperature of the drying chamber was 85^{o}C, the compressed air pressure of 3 atm, the Input pump speed 20 rpm, Spray speed 16000 rpm. Recovered spray drying powder with inulin content (445.90 ±2.79)/g powder; Fructan content reached (469.40 ±1.61) mg /g powder. The obtained drying powder had a pH (5.18 ± 0.01); Mineral content of (4.82 ± 0.07) %. Good solubility (g /ml/min) 1/9.5 ± 0.5 /15; Humidity (6.06 ± 0.27)% ; No inorganic and heavy metal impurities were detected. Dangshen powder has particle size (882.2 ± 101.4) nm, density 100%, dispersion DPI 0.497. The temperature that completely destroys the molecules is above 600^{o}C. Spray-drying powder meets food safety and hygiene standards according to the Health Ministry's regulations for industrial products.

5) Dangshen spray- powder containing Inulin has strong prebiotic properties with a dose of 4% (w/w- equivalent to 1.8-2% based on inulin content) can stimulate cell proliferation (CFU) of 8 strains (The two of *L. acidophilus, L. plantarum, L. rhamnosus, B. longum, B. lactic,* the two of *Enterococcus faecalis)* from 1,4 ÷11.5 times, in which, *L. acidophillus* (M7) had the highest fertility rate of 2.3 x10^{11}CFU/ g and *Enterococcus faecalis (M3)* had the lowest fertility rate of 7x10^{6} CFU/g.

From the research results, the optimal formula for mixing to create synbiotics (g/g) was determined: the content of Dangshen powder 0.514 g/g was mixed with the biomass of *L. acidophillus* 0.22 g/g (own biomass 5x10^{10}CFU/g); 0.128 g/g of *L.plantarum* biomass (own biomass 2x10^{11}CFU/g); 0.033 g/g of *B. longum* biomass (own biomass 8 x10^{10}CFU/g) and 0.1 g/g of *B. lactics* biomass (own biomass 10^{11}CFU/g) were selected to make synbiotic products.

6) Synbiotic preparations tested in mice with doses of 1.2 g/kg and 2.4 g/kg for 14 or 28 days were non-toxic, did not affect the function of liver and kidney in rats and Synbiotic products have the ability to stimulate immunity, the ability to treat diarrhea in mice.

RECOMMENDATIONS

From the above research results, it is allowed to propose the following ideas:

- Propagate and plant iso ginseng to preserve genes and ensure raw materials for the production process.

- Continue to trial production of Dangshen powder type prebiotic and synbiotic drying powder on a large scale as a basis for pricing and commercialization of products.