MINISTRY OF EDUCATION AND TRAINING
NHA TRANG UNIVERSITY

TRAN THANH GIANG

RESEARCH ON DRYING, EXTRACTION, AND EVALUATION OF BIOLOGICAL ACTIVITY OF TWO MEDICINAL PLANTS, *SALVIA MILTIORRHIZA* BUNGE AND *PARIS POLYPHYLLA* SMITH FOR APPLICATION IN FUNCTIONAL FOODS DEVELOPMENT

Major: Food Technology

Code: 9540101

SUMMARY OF THE DOCTORAL DISSERTATION

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The project was completed at Nha Trang University.

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Counter-argument 1:

Counter-argument 2:

Counter-argument 3:

The dissertation was defended before the University-level Doctoral Dissertation Evaluation Council meeting at Nha Trang University at ... o'clock ... on ... day ... month ... year .....

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

INTRODUCTION

Research on and application of natural medicinal herbs in healthcare have attracted increasing attention, particularly products derived from plants rich in bioactive compounds.

Salvia miltiorrhiza Bunge and *Paris polyphylla* Smith are two valuable medicinal herbs known for their outstanding bioactive properties such as antioxidant, cardioprotective, antimicrobial, and cancer cell inhibitory effects. However, research on standardizing drying and extraction processes to preserve and enhance the bioactive content of these two species remains limited.

Drying and extraction processes play a crucial role in determining the raw material quality and extraction efficiency of bioactive compounds. Studying the influence of drying and extraction conditions on the content and biological activity of *Salvia miltiorrhiza* Bunge and *Paris polyphylla* Smith is essential to provide a scientific basis for the development of functional food products with high biological value and safety.

RESEARCH OBJECTIVES

- To determine the most suitable drying conditions for two medicinal plants, *Salvia miltiorrhiza* Bunge and *Paris polyphylla* Smith.

- To determine the optimal extraction conditions for bioactive compounds from two medicinal plants, *Salvia miltiorrhiza* Bunge and *Paris polyphylla* Smith.

- To test the application of bioactive-rich extracts from two medicinal plants, *Salvia miltiorrhiza* Bunge and *Paris polyphylla* Smith, in the production of microencapsulated powder, with the aim of developing functional food products.

RESEARCH CONTENT

- Investigation of suitable drying conditions for two medicinal plants: *Salvia miltiorrhiza* Bunge and *Paris polyphylla* Smith.

- Investigation of optimal extraction conditions for bioactive compounds from two medicinal plants, *Salvia miltiorrhiza* Bunge and *Paris polyphylla* Smith.

- Testing the application of bioactive-rich extracts from two medicinal plants, *Salvia miltiorrhiza* Bunge and *Paris polyphylla* Smith, in the production of microencapsulated powder for functional food development.

CHAPTER 1. OVERVIEW

1.1. *Salvia miltiorrhiza* Bunge

Dan sam (DS - *Salvia miltiorrhiza* Bunge) is a widely used medicinal herb in traditional Chinese and Vietnamese medicine, notable for its antioxidant, cardioprotective, and anti-inflammatory properties. According to a report by Mei et al. (2019), 201 chemical compounds had been identified in *Salvia miltiorrhiza*, including 81 hydrophobic compounds (mainly diterpenoids and related quinone derivatives), 43 hydrophilic compounds (mainly phenolic acids), and 77 compounds belonging to various structural groups.

1.2. *Paris polyphylla* Smith

Bay la mot hoa (BLMH - *Paris polyphylla* Smith*)* is a rare medicinal plant with antimicrobial, cancer cell inhibitory, and immune-boosting effects. According to Ding et al., more than 320 compounds had been isolated and identified from *Paris polyphylla*, primarily steroid saponins, cholestanol saponins, C-21 steroids, phytosterols, insect allergens, triterpenes, and other secondary metabolites.

1.3. Drying

Drying is a common preservation method for raw materials, based on the principle of removing water through heat and mass transfer. The result is a reduction in moisture content and water activity, inhibition of enzymes and microorganisms that cause spoilage, extended shelf life, reduced weight and volume, and convenience for packaging and transportation. Several drying methods were studied in this dissertation to determine the appropriate drying methods and conditions for *Salvia miltiorrhiza* roots and *Paris polyphylla* rhizomes, including: natural drying and artificial drying (hot air drying, microwave drying, infrared drying, drying with dry air at low relative humidity and low temperature - often called freeze-drying and freeze-drying).

1.4. Extraction

Extraction is a crucial step in obtaining bioactive compounds from raw materials. Essentially, it's a process using selectively soluble solvents to separate and recover target components. This dissertation investigates several influencing factors to determine the optimal extraction conditions for two types of raw materials: *Salvia miltiorrhiza* roots and *Paris polyphylla* rhizomes, including: extraction solvent and method;

material/solvent ratio; extraction time and temperature; and irradiation time and microwave power.

1.5. Microencapsulated from plants

Microencapsulation is a technology that encapsulates bioactive compounds (polyphenols, flavonoids, essential oils, etc.) in protective membranes with ultrafine particle sizes (usually under 1,000 nm), to increase stability, control release, reduce losses caused by oxidation, light, temperature, and humidity, and improve solubility/bioavailability. This study used a coating mixture including maltodextrin (MD), gum arabic (GA), modified corn starch (MCS), and pectin (PEC) to create microencapsulated particles from concentrated extracts of DS roots and BLMH rhizomes.

CHAPTER 2. MATERIALS AND METHODS OF RESEARCH

2.1. Materials and chemicals

The roots of *S. miltiorrhiza* Bunge were collected in Sin Ho district, Lai Chau province (latitude 22.359586°N, longitude 103.250646°E) on February 18, 2022. The rhizomes of *P. polyphylla* Smith were collected in Mu Cang Chai district, Yen Bai province (latitude 21.838733°N, longitude 104.108827°E) on February 20, 2022.

2.2. Research methods

2.2.1. General research flowchart

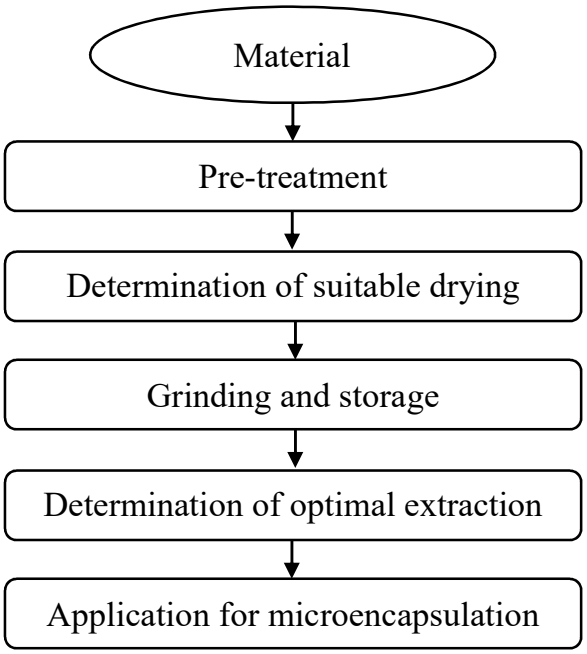


Figure 2.3. General experimental setup diagram

2.2.2. The study determined the appropriate drying conditions (Figure 2.4).

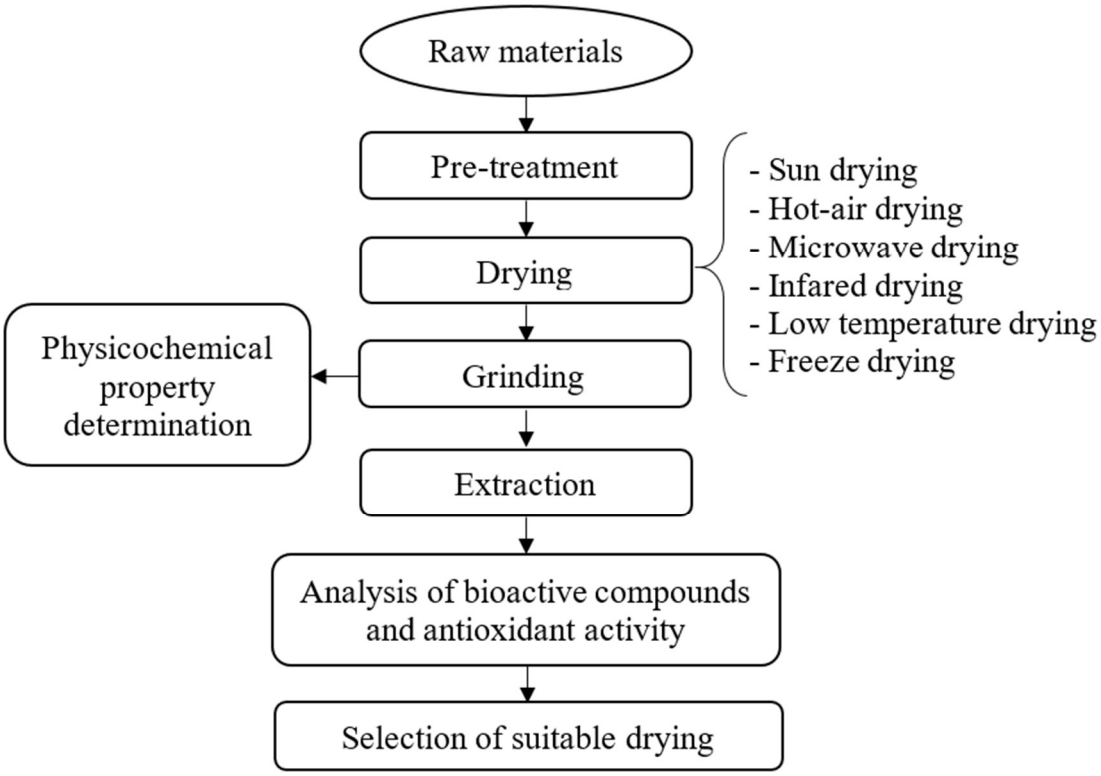


Figure 2.4. Experimental setup diagram for determining drying conditions

2.2.3. Study of extraction conditions (Figure 2.5)

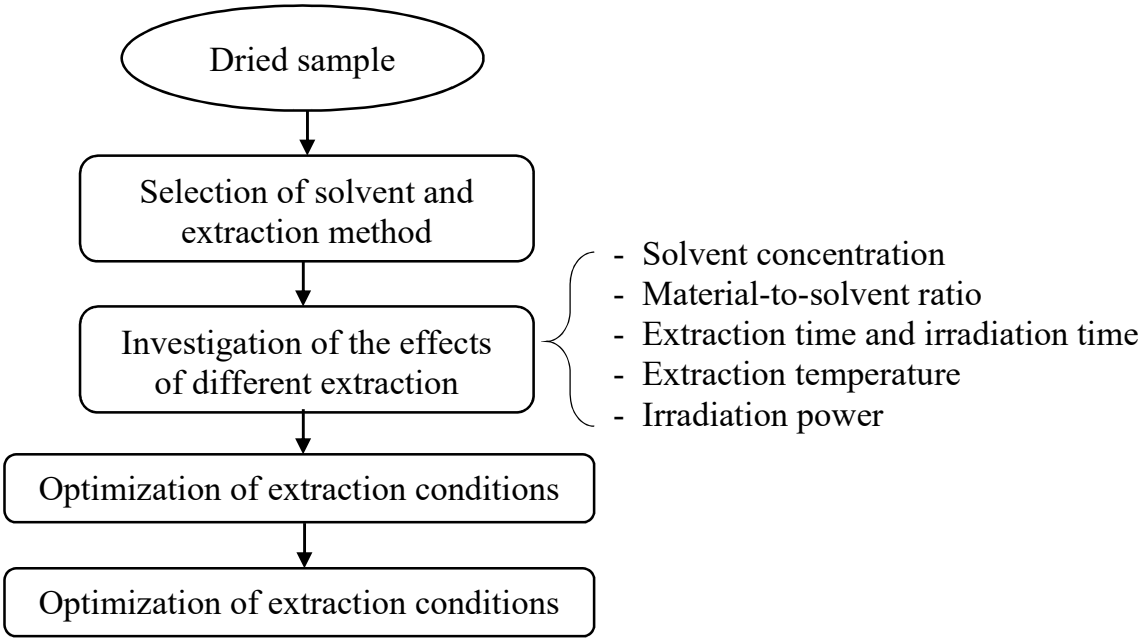


Figure 2.5. Experimental setup diagram for determining extraction conditions

2.2.4. Experimental study on the creation of microencapsulated powder from the DS roots and the BLMH rhizomes (Figure 2.6)

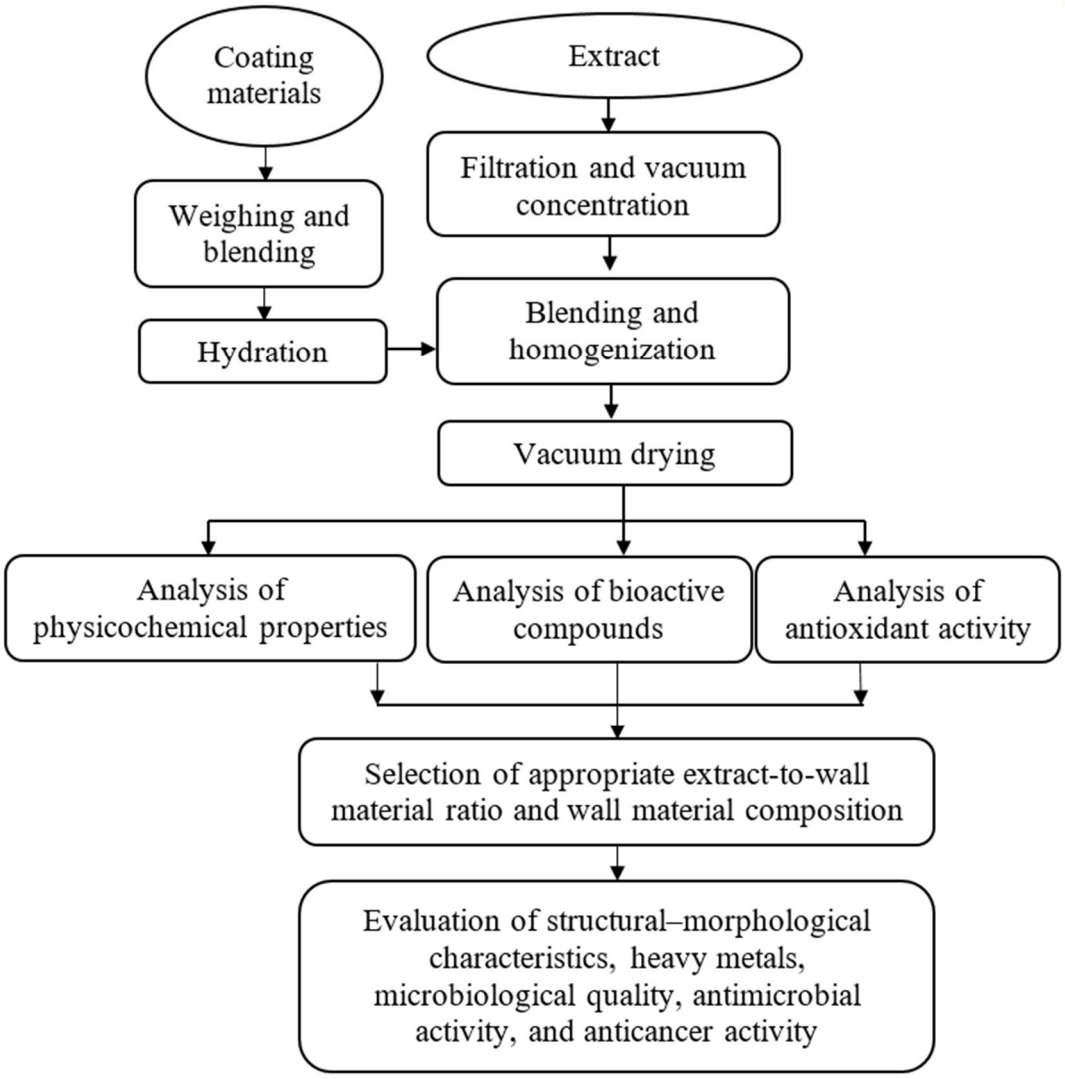


Figure 2.6. Experimental diagram for the production of microencapsulated powders

2.2.5. Analytical methods

Methods for determining physicochemical properties: drying time, energy consumption, drying efficiency, extraction efficiency, color, moisture content, water activity, extraction efficiency, solubility, particle size, zeta potential, surface structure, differential thermal analysis, X-ray diffraction, thermogravimetric.

Methods for determining bioactive compounds: total saponins, total phenolics, total flavonoids, total alkaloids.

Methods for determining *in vitro* antioxidant activity: ABTS and DPPH free radical scavenging ability, antioxidant activity by iron reduction.

Methods for analyzing heavy metals Pb, Hg, Cd, and As.

Microbiological analysis methods: total aerobic microorganisms, *Coliforms*, *Escherichia coli, Staphylococcus aureus,* total yeast and mold spores, *Clostridium perfringens*. Methods for evaluating antimicrobial activity by diffusion through agar wells and by dilution in liquid medium.

Methods for evaluating the ability to inhibit *in vitro* cancer cell growth by the MTT test.

Statistical analysis: IBM SPSS Statistics 22 software was used to process the data with a significance level of less than 0.05 ($p < 0.05$).

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Study of drying conditions

3.1.1. Study of drying conditions for DS roots

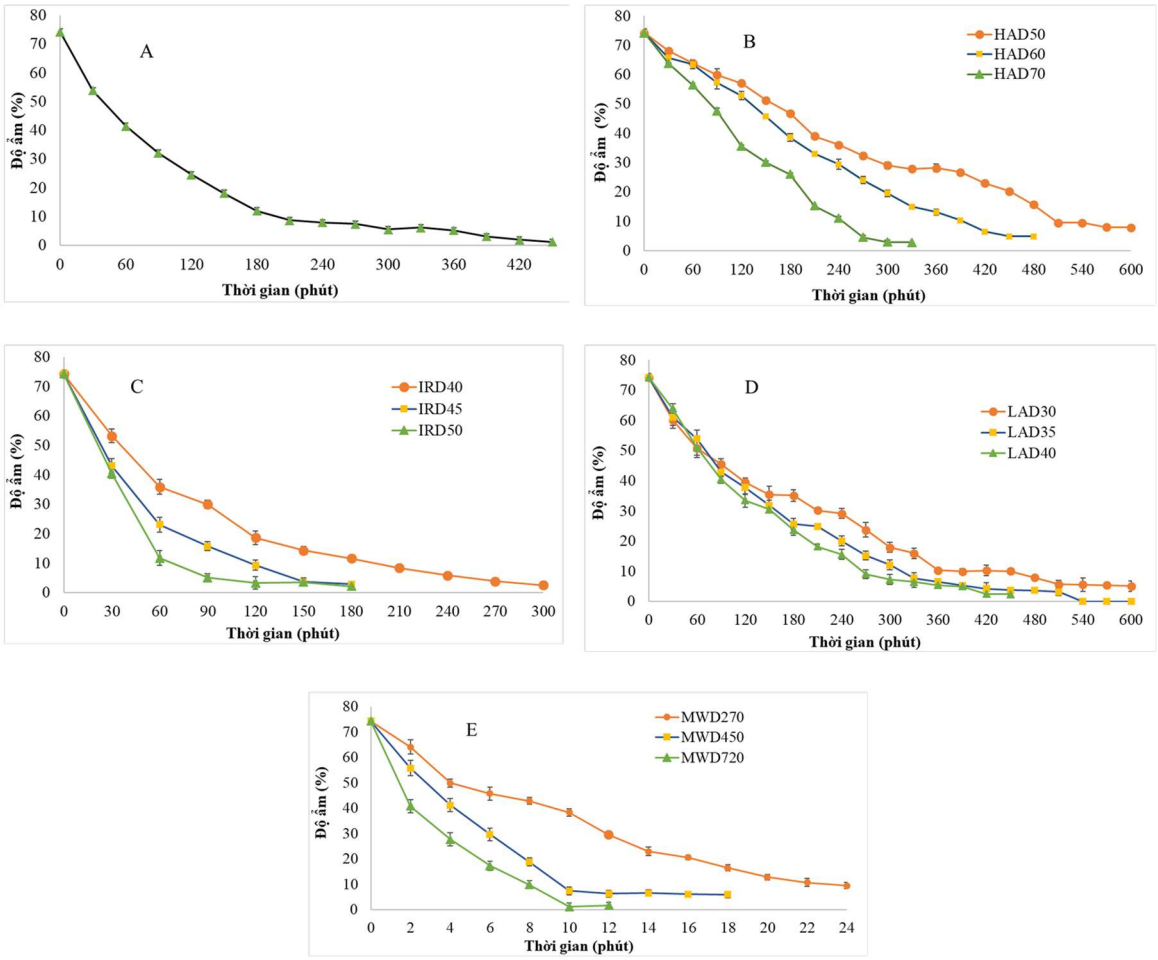


Figure 3.1. Drying curve of DS roots under different drying conditions

A: Drying, B: Hot air drying, C: Infrared drying, D: Low-temperature drying, E: Microwave drying

Microwave drying (especially at 720 W) significantly shortens drying time, achieves lower moisture content, and reduces the risk of oxidation of bioactive compounds.

3.1.1.2. Influence of drying methods and conditions on the physicochemical properties of DS root

a. The influence of drying methods and conditions on the color of the DS roots.

Overall, the dried samples tended to be brighter in color compared to the fresh samples. The FRD samples had the highest brightness. The MWD samples had lower brightness than FRD, but were still brighter than most convection drying methods .

b. Influence of drying methods and conditions on the physicochemical properties of the DS roots

The MWD720 sample showed the most outstanding advantages among the dried samples.

Table 3.2. Effect of drying conditions on physicochemical properties of DS roots

Drying conditions	Drying time (h)	Moisture (%)	Water activity (a_w)	Consume energy (kWh)
SD	8.3	7.53 ± 0.11^{c}	0.63 ± 0.03^{b}	**0.00**
HAD50	9.0	11.35 ± 0.74^{b}	0.64 ± 0.02^{b}	12.60
HAD60	7.5	7.30 ± 0.18^{c}	0.56 ± 0.01^{d}	10.50
HAD70	5.0	6.33 ± 0.35^{c}	0.51 ± 0.03^{fg}	7.00
IRD40	5.0	7.63 ± 0.47^{c}	0.53 ± 0.02^{ef}	9.00
IRD45	3.0	7.33 ± 1.96^{c}	0.52 ± 0.02^{efg}	5.40
IRD50	2.3	6.73 ± 0.58^{c}	0.51 ± 0.01^{fg}	4.14
MWD270	0.95	10.07 ± 0.50^{b}	0.59 ± 0.02^{cd}	0.26
MWD450	0.43	6.04 ± 1.96^{c}	0.51 ± 0.02^{fg}	0.19
MWD720	**0.3**	**3.77 ± 1.55^{d}**	**0.48 ± 0.04^{g}**	**0.22**
LAD30	10.0	9.64 ± 0.29^{b}	0.59 ± 0.01^{cd}	19.00
LAD35	9.0	10.33 ± 0.40^{b}	0.61 ± 0.02^{bc}	17.10
LAD40	8.3	10.34 ± 1.12^{b}	0.55 ± 0.01^{de}	15.77
FRD	**48**	**4.38 ± 0.00^{d}**	**0.42 ± 0.00^{h}**	**105.60**
FS	0	74.25 ± 1.25^{a}	0.93 ± 0.00^{a}	ND

Sun drying: SD; hot-air drying at 50°C, 60°C, 70°C: HAD50/60/70; infrared drying at 40°C, 45°C, 50°C: IRD40/45/50; microwave drying at 270W, 450W, 720W: MWD270/450/720; low-temperature drying at 30°C, 35°C, 40°C: LAD30/35/40; freeze-drying: FRD; undetermined: ND

c. Influence of drying methods and conditions on the content of bioactive compounds in the DS roots.

Table 3.3. Effect of drying conditions on bioactive content of DS roots

Drying conditions	TSC (mg EE/g DM)	TPC (mg GAE/g DM)	TFC (mg CE/g DM)	TAC (mg AE/g DM)
SD	20.68±0.86^{d}	2.98±0.13^{k}	0.75±0.12^{d}	3.95±0.72^{hi}
HAD50	21.27±1.16^{d}	4.28±0.02^{ghi}	0.58±0.04^{def}	3.39±0.11^{i}
HAD60	24.85±0.74^{c}	5.73±0.30^{f}	0.68±0.03^{de}	3.62±0.58^{hi}
HAD70	27.00±2.43^{c}	8.16±0.41^{e}	0.52±0.03^{efg}	4.22±0.60^{ghi}
IRD40	19.17±1.32^{de}	2.87±0.28^{k}	0.37±0.01^{g}	4.33±0.53^{fghi}
IRD45	19.60±1.00^{de}	3.52±0.30^{hik}	0.58±0.03^{def}	5.21±0.70^{fg}
IRD50	27.22±3.34^{c}	4.67±0.06^{fgh}	1.93±0.16^{b}	5.37±0.48^{f}
MWD270	12.89±1.17^{f}	4.98±0.40^{fg}	0.68±0.07^{de}	11.63±0.71^{d}
MWD450	18.01±1.68^{de}	13.05±1.68^{c}	1.99±0.23^{b}	12.21±0.55^{cd}
MWD720	**32.00±3.05^{b}**	**9.33±0.14^{d}**	**1.26±0.02^{c}**	**13.16±0.31^{c}**
LAD30	16.68±0.48^{e}	3.09±0.53^{ik}	0.40±0.04^{fg}	4.52±0.83^{fgh}
LAD35	21.34±1.56^{d}	3.78±0.27^{ghik}	0.44± 0.03^{fg}	5.22±0.71^{fg}
LAD40	19.28±1.91^{de}	3.88±0.08^{ghik}	0.57± 0.02^{def}	6.58±0.54^{e}
FRD	**33.66±3.49^{b}**	**16.68±0.27^{b}**	**2.32± 0.12^{a}**	**16.52±0.23^{b}**
FS	**48.76±1.16^{a}**	**21.24±0.86^{a}**	**2.35± 0.24^{a}**	**26.10±0.74^{a}**

d. Influence of drying methods and conditions on antioxidant activity of DS roots

Table 3.5. Effect of drying conditions on antioxidant activity of DS roots

Drying conditions	DRSC (mg TE/g DM)	ARSC (mg TE/g DM)	FRAP (mg TE/g DM)
SD	3.97 ± 0.69^{hi}	10.56 ± 1.10^{gh}	7.29 ± 0.60^{f}
HAD50	9.64 ± 0.59^{ef}	13.51 ± 1.04^{ef}	12.14 ± 0.99^{d}
HAD60	12.87 ± 0.28^{d}	**18.14 ± 0.79^{bc}**	12.58 ± 0.84^{cd}
HAD70	8.54 ± 0.25^{fg}	14.10 ± 1.74^{e}	9.24 ± 0.26^{e}
IRD40	3.63 ± 0.31^{i}	12.22 ± 1.30^{efg}	5.29 ±0.33^{h}
IRD45	5.07 ± 0.58^{hi}	13.92 ± 1.28^{e}	7.29 ± 0.60^{f}
IRD50	9.04 ± 0.56^{f}	15.95 ± 1.35^{d}	13.46 ± 1.71^{cd}
MWD270	9.32 ± 0.49^{f}	13.53 ± 0.58^{ef}	8.96 ± 0.31^{e}
MWD450	11.15 ± 1.75^{e}	**19.61 ± 0.55^{b}**	20.91 ± 1.76^{b}
MWD720	**17.72 ± 1.32^{c}**	**17.89 ± 1.17^{bc}**	**22.76 ± 0.41^{a}**

LAD30	5.34 ± 0.76^{h}	6.44 ± 0.25^{i}	5.44 ± 0.32^{gh}
LAD35	9.52 ± 0.41^{f}	9.64 ± 1.27^{h}	6.35 ± 0.60^{fgh}
LAD40	7.33 ± 0.52^{g}	11.85 ± 0.11^{fg}	7.01 ± 0.85^{fg}
FRD	**22.58 ± 0.16^{b}**	**21.70 ± 0.79^{a}**	**21.83 ± 0.90^{ab}**
FS	**26.15 ± 1.10^{a}**	17.54 ± 1.05^{cd}	14.13 ± 1.30^{c}

The results show that MWD720 offers only slightly lower antioxidant efficiency than FRD but has distinct advantages in terms of economics, energy consumption, and technological feasibility, thanks to its short processing time and simple equipment design. Therefore, MWD720 is a more suitable choice for practical applications and commercialization of dried rice products.

3.1.2. Study of drying conditions for BLMH rhizomes

3.1.2.1. Drying curves of BLMH rhizomes using different drying methods and conditions

The results showed a similar transformation trend as in the DS roots. Compared to DS roots, the drying time of BLMH rhizomes was significantly shorter.

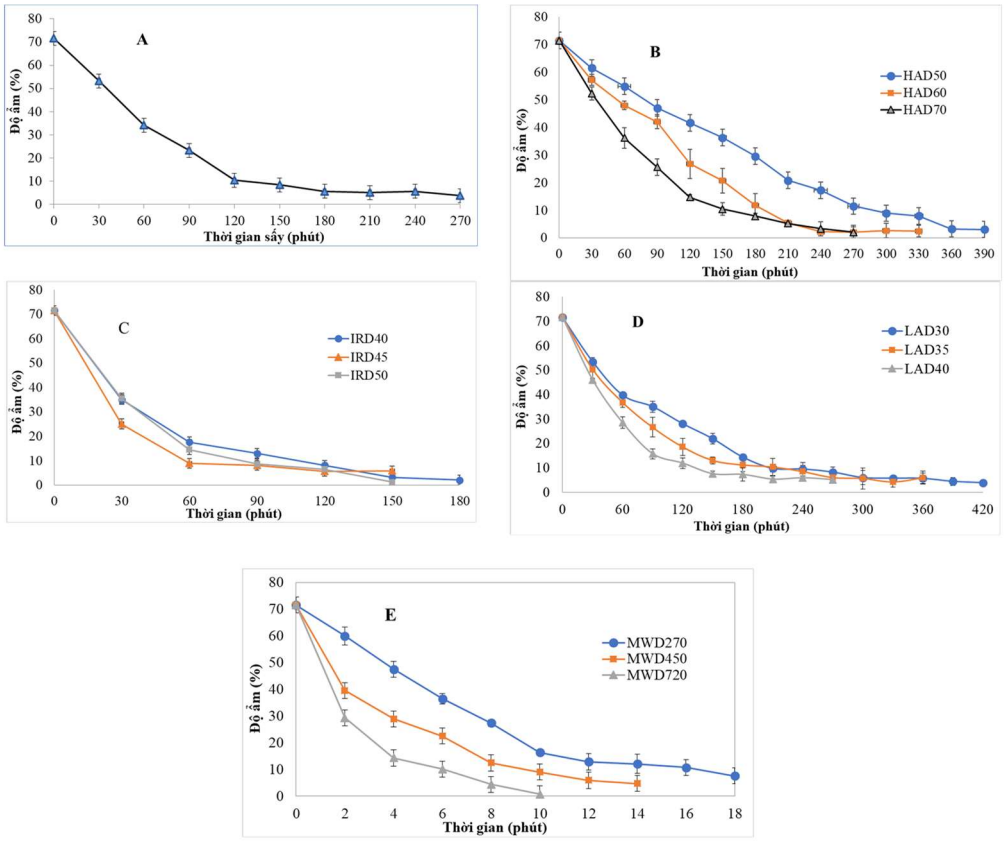


Figure 3.4. Drying curve of BLMH rhizomes under different drying conditions

A: Drying, B: Hot air drying, C: Infrared drying, D: Low-temperature drying, E: Microwave drying

3.1.2.2. Influence of drying methods and conditions on the physicochemical properties of BLMH rhizomes

a. The effect of drying methods and conditions on the color of BLMH rhizomes

In general, freeze-drying maintains color stability. Conversely, convection or radiation drying methods, which typically take longer or at high heat outputs, lead to increased browning rates and greater color changes.

b. Influence of drying methods and conditions on physicochemical properties of BLMH rhizomes

Table 3.7. Effect of drying conditions on physicochemical properties of BLMH rhizomes

Drying conditions	Drying time (h)	Energy consumption (kWh)	Moisture (%)	Water activity (a_w)
SD	4.5	0.0	10.04 ± 0.22^{b}	0.58 ± 0.03^{b}
HAD50	6.5	9.1	8.17 ± 0.24^{def}	0.47 ± 0.02^{d}
HAD60	5.5	7.7	5.28 ± 0.55^{cdef}	0.45 ± 0.07^{de}
HAD70	4.5	6.3	6.49 ± 1.01^{gh}	0.40 ± 0.02^{fg}
IRD40	3.5	6.3	7.99 ± 0.35^{fg}	0.43 ± 0.01^{def}
IRD45	3.0	5.4	6.66 ± 1.06^{efg}	0.41 ± 0.02^{efg}
IRD50	2.5	4.5	6.43 ± 1.31^{hi}	0.38 ± 0.02^{gh}
MWD270	0.73	0.19	9.90 ± 0.77^{cd}	0.54 ± 0.01^{c}
MWD450	0.47	0.21	5.19 ± 0.65^{fgh}	0.45 ± 0.04^{de}
MWD720	0.17	0.12	4.62 ± 0.69^{k}	0.34 ± 0.02^{gh}
LAD30	7.0	13.3	8.79 ± 0.86^{bc}	0.56 ± 0.03^{bc}
LAD35	6.0	11.4	8.92 ± 0.40^{cde}	0.52 ± 0.03^{bcd}
LAD40	5.0	9.5	6.74 ± 1.23^{cdef}	0.47 ± 0.01^{cde}
FRD	**48.0**	**105.6**	**5.37 ± 0.50^{ik}**	**0.32 ± 0.12^{h}**
FS	ND	ND	71.54 ± 0.99^{a}	0.93 ± 0.00^{a}

c. The effect of drying methods and conditions on the content of bioactive compounds in BLMH rhizomes

Table 3.8. Effect of drying conditions on bioactive content of BLMH rhizomes

Drying conditions	TSC (mg EE/g DM)	TPC (mg GAE/g DM)	TAC (mg AE/g DM)
SD	25.52 ± 0.55^{cd}	0.58 ± 0.06^{de}	0.12 ± 0.03^{fgh}

HAD50	17.83 ± 2.02^{f}	0.54 ± 0.10^{e}	0.13 ± 0.02^{fg}
HAD60	**27.11 ± 0.89^{c}**	**0.78 ± 0.07^{bc}**	0.21 ± 0.03^{e}
HAD70	20.60 ± 1.75^{ef}	0.59 ± 0.03^{de}	0.34 ± 0.03^{b}
IRD40	13.83 ± 1.66^{g}	0.40 ± 0.02^{f}	0.08 ± 0.01^{hi}
IRD45	23.27 ± 2.58^{de}	0.65 ± 0.04^{de}	0.11 ± 0.01^{ghi}
IRD50	27.67 ± 1.41^{c}	0.69 ± 0.11^{cd}	0.21 ± 0.01^{e}
MWD270	24.77 ± 0.96^{cd}	0.44 ± 0.04^{f}	0.14 ± 0.01^{fg}
MWD450	19.26 ± 2.00^{f}	0.62 ± 0.01^{de}	0.30 ± 0.03^{c}
MWD720	14.61 ± 2.11^{g}	0.45 ± 0.04^{f}	0.12 ± 0.01^{fgh}
LAD30	14.67 ± 1.91^{g}	0.39 ± 0.06^{f}	0.15 ± 0.01^{f}
LAD35	19.29 ± 1.34^{f}	0.65 ± 0.02^{de}	0.10 ± 0.01^{ghi}
LAD40	13.35 ± 0.76^{g}	0.39 ± 0.08^{f}	0.07 ± 0.01^{i}
FRD	**42.29 ± 2.72^{b}**	**0.88 ± 0.04^{b}**	**0.25 ± 0.01^{d}**
FS	60.61 ± 2.26^{a}	1.95 ± 0.10^{a}	0.38 ± 0.05^{a}

d. The effect of drying method and conditions on antioxidant activity of BLMH rhizomes

Hot-air drying at 60°C (HAD60) achieves a balance between quality and efficiency: cell structure is preserved, moisture removal is effective, active ingredient content is maintained at a high level, and antioxidant values (ARSC, DRSC, FRAP) are stable. Therefore, HAD60 is a suitable drying condition for drying BLMH rhizomes.

Table 3.10. Effect of drying conditions on antioxidant activity of BLMH rhizomes

Drying conditions	ARSC (mg TE/g DM)	DRSC (mg TE/g DM)	FRAP (mg TE/g DM)
SD	1.53 ± 0.13^{h}	1.19 ± 0.07^{e}	0.58 ± 0.05^{i}
HAD50	1.71 ± 0.09^{fgh}	0.28 ± 0.07^{h}	0.94 ± 0.05^{ef}
HAD60	**2.23 ± 0.08^{c}**	**1.47 ± 0.01^{c}**	**1.27 ± 0.05^{c}**
HAD70	1.89 ± 0.06^{def}	1.31 ± 0.02^{de}	0.67 ± 0.03^{hi}
IRD40	1.76 ± 0.16^{efg}	0.42 ± 0.03^{g}	0.74 ± 0.08^{gh}
IRD45	2.03 ± 0.19^{d}	1.23 ± 0.05^{de}	0.87 ± 0.15^{fg}
IRD50	2.28 ± 0.19^{c}	1.26 ± 0.08^{de}	0.96 ± 0.16^{ef}
MWD270	1.78 ± 0.03^{efg}	0.40 ± 0.02^{g}	0.88 ± 0.04^{fg}
MWD450	1.95 ± 0.17^{de}	1.32 ± 0.13^{d}	1.08 ± 0.12^{de}
MWD720	1.23 ± 0.04^{i}	0.76 ± 0.05^{f}	1.18 ± 0.09^{cd}
LAD30	1.58 ± 0.08^{gh}	0.42 ± 0.05^{g}	0.60 ± 0.02^{hi}

LAD35	1.65 ± 0.01^{gh}	0.49 ± 0.04^{g}	0.61 ± 0.09^{hi}
LAD40	1.65 ± 0.09^{gh}	0.25 ± 0.02^{h}	0.76 ± 0.04^{gh}
FRD	**2.66 ± 0.11^{b}**	**2.01 ± 0.01^{b}**	**1.49 ± 0.06^{b}**
FS	2.94 ± 0.04^{a}	3.61 ± 0.14^{a}	1.72 ± 0.13^{a}

3.2. Results of the extraction conditions study

3.2.1. Research results on the conditions for DS root extraction

3.2.1.1. Results of selecting solvent type and extraction method for the DS roots

Using 50% ethanol as a solvent and the UAE extraction method resulted in higher yield of active compounds and the highest antioxidant activity.

3.2.1.2. Research results on the influence of DS root extraction conditions

The suitable parameters for inclusion in the optimization experiment were selected as follows: using the UAE extraction method; ethanol concentration of 60-80%; extraction temperature of 40-60°C; extraction time of 10-30 minutes; and material/solvent ratio of 1/60-1/100 (g/mL).

3.2.1.3. Results of optimizing the DS root extraction conditions

The ANOVA analysis results show that the quadratic regression equation fits the experimental data well (high R^2) and the *p* -values *of lack of fit* are all greater than the statistical significance level.

The optimal conditions obtained from the response surface model were ethanol concentration of 80%, extraction temperature of 40° C, extraction time of 27 minutes, and solvent/material ratio of 88 (mL/g).

Table 3.13. Coefficients of regression equation and results of analysis of variance

Regression coefficient	**TSC (Y_1)**		**SEE (Y_2)**		**TPC (Y_3)**		**ARSC (Y_4)**		**FRAP (Y_5)**	
	Fore-cast	*p* - *value*	Fore-cast	*p* - *value*	Fore-cast	*p* - *value*	Fore-cast	*p* - *value*	Fore-cast	*p* - *value*
β_0	**50.05**	**<0.0001**	**68.33**	**<0.0001**	**48.03**	**<0.0001**	**93.66**	**<0.0001**	**85.26**	**<0.0001**
β_1	**7.66**	**<0.0001**	**10.47**	**<0.0001**	**-5.06**	**0.0014**	**-9.54**	**0.0010**	-3.53	0.2489
β_2	**-4.47**	**0.0032**	**-6.10**	**0.0032**	-2.22	0.947	**-6.28**	**0.0148**	0.48	0.8719
β_3	**4.82**	**0.0019**	**6.58**	**0.0019**	**6.96**	**0.0001**	**14.28**	**0.0001**	**9.98**	**0.0051**
β_4	**4.81**	**0.0020**	**6.56**	**0.0019**	**7.26**	**0.0001**	**13.26**	**0.0001**	**12.94**	**0.0008**
β_{12}	0.69	0.7497	0.945	0.7489	1.95	0.3745	2.81	0.4761	-0.92	0.8579
β_{13}	2.03	0.3551	2.77	0.3551	-0.40	0.8518	-1.79	0.6470	-1.61	0.7545
β_{23}	-3.06	0.1723	-4.18	0.1723	0.91	0.6740	2.36	0.5490	-2.74	0.5967

β_{14}	0.83	0.7005	1.14	0.6997	-0.83	0.7009	-0.06	0.9877	-2.11	0.6834
β_{24}	0.43	0.8395	0.6	0.8387	-0.53	0.8061	-3.92	0.3250	-4.55	0.3852
β_{34}	0.54	0.8000	0.74	0.8000	**-4.93**	**0.0386**	-7.75	0.0655	-7.97	0.1408
β_{11}	-3.46	0.0824	-4.73	0.0823	**-6.49**	**0.0041**	**-9.89**	**0.0114**	**-13.07**	**0.0113**
β_{22}	2.33	0.2255	3.19	0.2249	2.03	0.2908	5.70	0.1109	7.26	0.1227
β_{33}	**-6.22**	**0.0053**	**-8.49**	**0.0053**	-3.39	0.0892	-6.46	0.0748	-3.59	0.4276
β_{44}	**-7.04**	**0.0023**	**-9.62**	**0.0023**	-1.74	0.3623	-4.56	0.1934	-3.91	0.3886
R^2	0.91		0.91		0.91		0.92		0.82	
R^2 adjust	0.80		0.80		0.80		0.82		0.61	
p - value	0.0003		0.0003		0.0004		0.0002		0.0120	
p - values lack of fit	0.5644		0.5638		0.3931		0.5547		0.4413	

3.2.2. Research results on the conditions for extracting BLMH rhizomes

3.2.2.1. Results of solvent selection and extraction method for BLMH rhizomes

Using 50% ethanol as a solvent and the MAE method yielded the highest extraction efficiency, both in terms of active ingredient content and antioxidant capacity.

3.2.2.2. Research results on the influence of conditions for extracting BLMH rhizomes.

The selected boundary ranges for the optimized experimental design included: MAE extraction method, microwave power 600 W, material/solvent ratio 1/30 g/mL, ethanol concentration 60-80%, extraction time 10-30 minutes, and irradiation time 6-8 seconds/minute.

3.2.2.3. Results of optimizing the extraction conditions for BLMH rhizomes

The ANOVA analysis results show that the quadratic regression equation fits the experimental data well (high R^2), p - values *of lack of fit* are all greater than the statistical significance level). The optimal conditions obtained from the response surface model were an ethanol solvent concentration of 80%; an extraction time of 27 minutes, and an irradiation time of 8 seconds/minute.

Table 3.17. The coefficients of regression equation and the results of analysis of variance

Regression coefficient	**TSC (Y_1)**		**SEE (Y_2)**		**TPC (Y_3)**		**ARSC (Y_4)**		**FRAP (Y_5)**	
	Fore-cast	*p - value*	Fore-cast	*p - value*	Fore-cast	*p - value*	Fore-cast	*p - value*	Fore-cast	*p - value*
β_0	**50.23**	**<,0001**	**62.98**	**<,0001**	**0.84**	**<,0001**	**5,10**	**<,0001**	**1.23**	**<,0001**

β_1	**8.55**	**<,0001**	**10.71**	**<,0001**	-0.005	0.4896	**-0.37**	**<,0001**	**0.06**	**0.0051**
β_2	**3.39**	**0.0007**	**4.25**	**0.0007**	**0.04**	**0.0019**	**0.09**	**0.0056**	**0.04**	**0.0250**
β_3	0.41	0.4061	0.52	0.4045	**0.035**	**0.0034**	**0.13**	**0.0013**	0.01	0.3532
β_{12}	-0.26	0.7047	-0.32	0.7049	**0.03**	**0.0250**	0.06	0.0891	0.01	0.7094
β_{13}	**3.33**	**0.0035**	**4.18**	**0.0035**	**0.025**	**0.0462**	0.06	0.0798	-0.05	0.0641
β_{23}	0.45	0.5187	0.56	0.5172	-0.01	0.3401	**0.08**	**0.0340**	0.02	0.2897
β_{11}	**3.10**	**0.0056**	**3.88**	**0.0056**	**-0.03**	**0.0217**	**-0.65**	**<,0001**	**-0.06**	**0.0389**
β_{22}	**-3.17**	**0.0051**	**-3.98**	**0.0051**	-0.02	0.0717	-0.07	0.0694	-0.04	0.1165
β_{33}	**2.07**	**0.0268**	**2.60**	**0.0268**	0.01	0.2613	0.05	0.1583	-0.03	0.1898
R^2	0,990		0,990		0.952		0.995		0.913	
R^2 adjust	0.972		0.972		0.866		0.985		0.757	
p - value	0.0002		0.0002		0.0083		< 0.0001		0.0330	
P - values Lack of fit	0.0677		0.0670		0.8952		0.5474		0.1219	

3.3. Results of evaluating the properties of microencapsulated powder of DS root and BLMH rhizome

3.3.1. Results of the analysis of the DS root microencapsulation powder

3.3.1.1. Results of physicochemical properties, active ingredients, and antioxidant activity of DS microencapsulated powder

Table 3.19. Results of analysis of properties of DS microencapsulated powder

No.	Extract/ Coating material	MD/GA/ MCS/PEC	Moisture (%)	Water activity (a_w)	TSC (mg EE/g DM)	FRAP (mg TE/g DM)
1	**60/40**	**4/1/4/1**	6.64±0.25^{abc}	0.516±0.003^{ab}	140.10±4.69^{f}	4.45±0.65^{c}
2		**3/1/5/1**	6.82±0.24^{ab}	0.515±0.003^{abc}	150.30±1.26^{e}	6.98±0.93^{a}
3		**5/1/3/1**	6.51±0.24^{bc}	0.512±0.003^{abcd}	127.20±2.28^{g}	**7,61±0, 86^{b}**
4	**50/50**	**4/1/4/1**	6.51±0.28^{bc}	0.514±0.003^{abc}	241.00±1.72^{b}	4.56±0.47^{c}
5		**3/1/5/1**	6.54±0.34^{bc}	0.508±0.003^{bcde}	237.40±1.14^{b}	4.65±0.10^{c}
6		**5/1/3/1**	6.23±0.29^{cd}	0.502±0.003^{de}	216.60± 2.21^{d}	4.81±0.76^{c}
7	**40/60**	**4/1/4/1**	6.36±0.25^{bc}	0.505±0.004^{cde}	237.40±1.97^{b}	4.58±0.17^{c}
8		**3/1/5/1**	6.25±0.15^{cd}	0.502±0.003^{de}	262.80± 3.58^{a}	4,24±0,50^{c}
9		**5/1/3/1**	**5.79±0.3^{d}**	**0.499±0.004^{e}**	**264.70±2.9^{a}**	**7.30±0.96^{c}**
0*	**No coating**	**0**	7.11±0.5^{a}	0.521±0.005^{a}	267.40±0.28^{a}	8.05±0.06^{a}

Samples with a 40/60 ratio and MD/GA/MCS/PEC ratio of 5/1/3/1 showed outstanding physicochemical results, with the highest TSC and FRAP values, indicating almost complete protection of the active ingredient.

3.3.1.2. Results of structural and morphological measurements

a. Zeta potential, particle size, and surface structure of SEM

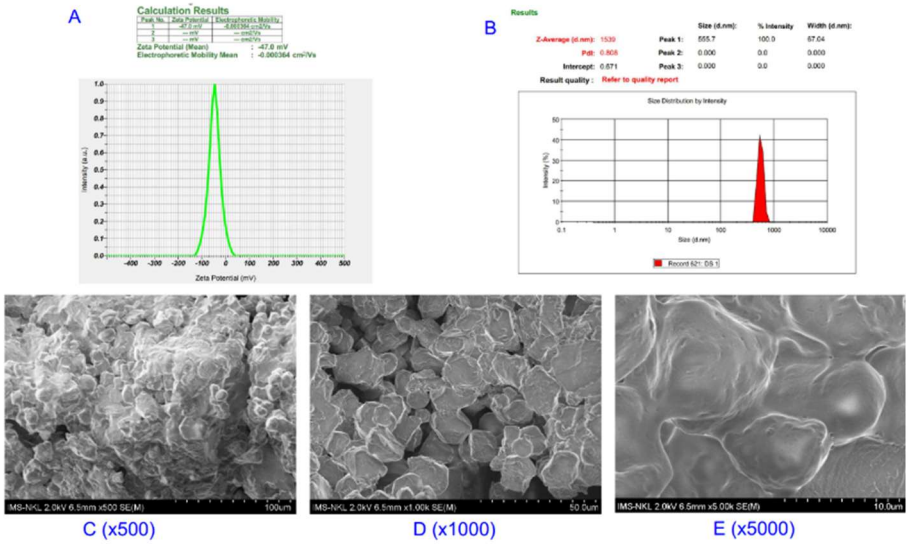


Figure 3.11. Zeta potential spectrum (A), particle size distribution (B), SEM images (C, D, E) of DS microencapsulated powder

b. DSC, TGA, and XRD spectra of DS microencapsulated powder

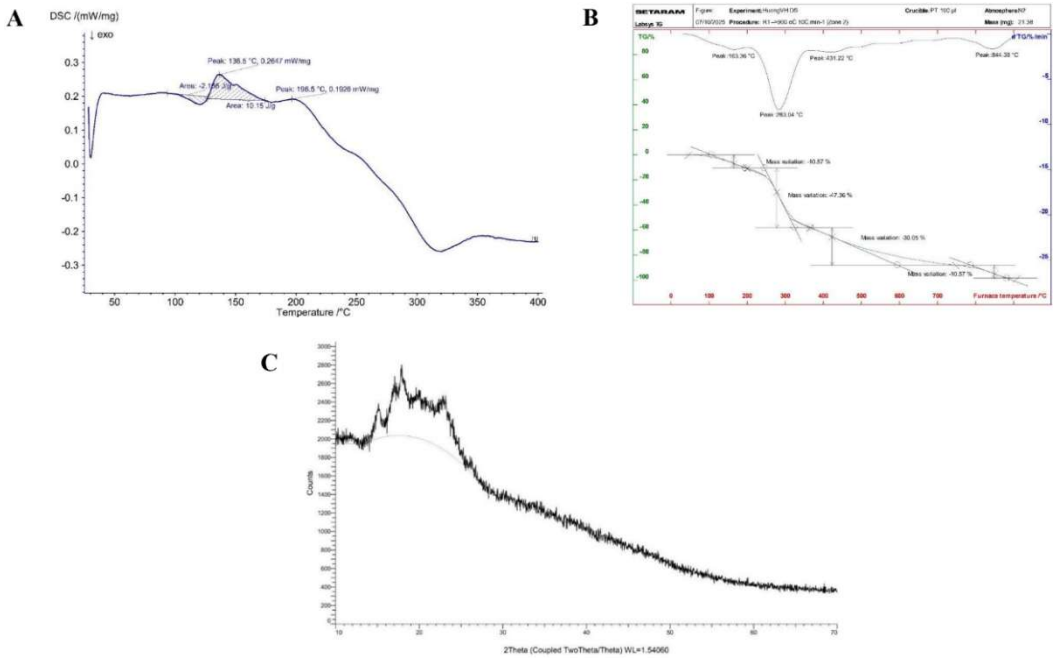


Figure 3.12. DSC (A), TGA (B), and XRD (C) spectra of DS microencapsulated powder

3.3.1.3. Results of analysis of some active ingredients, microbiological testing, heavy metal testing, and evaluation of the antimicrobial and anticancer potential of DS microencapsulated powder.

a. Results of the active ingredient content test

The active ingredients Cryptotanshinone and Tanshinone I are preserved after the microencapsulation process.

b. Microbiological test results

The results of the microbiological analysis of the DS microencapsulated powder (Table 3.21) show that the microbiological quality meets safety requirements.

Table 3.21. Results of microbiological analysis of DS microencapsulated powder

No.	Analytical parameters	Analysis results	Analysis methods
1	Total number of aerobic	4.98×10^{-1} CFU/g	TCVN 4884-1:2015
2	*Coliforms*	KPH	TCVN 6848:2007
3	*Escherichia coli*	KPH	TCVN 7924-2:2008
4	*Staphylococcus aureus*	KPH	TCVN 4830:2005
5	Total number of yeast/mold spores	0.21×10^{-1} CFU/g	TCVN 8275:2010
6	*Clostridium perfringens*	KPH	TCVN 4991:2005

c. Results of heavy metal testing

The results of heavy metal testing of DS microencapsulated powder (Table 3.22) show that the main indicators are all "KPH" (not detected) with low detection limits.

Table 3.22. Results of heavy metal analysis of DS microencapsulated powder

No.	Analytical parameters	Analysis results	Analysis methods
1	Lead (Pb) content	KPH	TCVN 8126 – 1:2009
2	Mercury (Hg) content	KPH	TCVN 7604:2007
3	Cadmium (Cd) content	KPH	TCVN 7603:2007
4	Arsenic (As) content	KPH	TCVN 9521:2012

d. Results of antimicrobial activity assessment

DS microencapsulated powder exhibits a pronounced antimicrobial effect on Gram-positive and Gram-negative bacteria and pathogenic yeasts. Notably, its MIC value indicates a relatively broad spectrum of antimicrobial activity, with less sensitivity to yeasts.

Table 3.23. Results of antimicrobial activity evaluation of DS microencapsulated powder

Concen-tration (mg/mL)	Inhibition ring diameter (mm)				
	E. coli	***S. aureus***	***Salmonella***	***B. anthracis***	***C. albicans***
70	-	-	-	24;24;24	23;23;23
50	21;21;21	22;22;22.5	23;23.5;23.5	18;18;19	17.5;17.5;18
30	19;18.5;19	18;18;18	17;16.5;17	15;14;14.5	12.5;13;13
10	11;11;11	12;11;12	14;14;14	-	-
Positive symptoms	29;29;29	29;30;29.5	29; 28; 29	30;30;30	29;28.5; 28.5

Table 3.24. Results of MIC determination of DS microencapsulated powder

Microorganisms	MIC (mg/mL)
Staphylococcus aureus	12.5
Escherichia coli	12.5
Salmonella	10
Bacillus anthracis	25
Candida albicans	27.5

e. Results of *in vitro* cancer cell growth inhibition

Table 3.25. Results of evaluating *in vitro* cancer cell growth inhibition of DS microencapsulated powder

Cancer cell line (cell type)	% inhibition of cell growth at a concentration of 100 µg/mL	GI_{50} (µg/mL)
MCF-7 (Breast, ER positive)	>100	17.2 ± 2.6
MDAMB231 (Breast, TNBC)	>100	44 ± 6.8
T47D (Breast, ER positive)	>100	16.5 ± 1.8
ZR-75-1 (Breast, ER positive)	>100	23 ± 6.6
SKBR3 (Breast, HER2 positive)	>100	14.7 ± 2.0
BT474 (Breast, HER2 positive)	>100	36 ± 0.3
BT20 (Breast, ER negative)	>100	25 ± 6.1
MDAMB468 (Breast, TNBC)	>100	44 ± 3.2
MCF10A (Normal breast)	97 ± 1	36 ± 2.1
BxPC3 (Pancreas)	68 ± 2	74 ± 2.2

3.3.2. Results of the analysis of BLMH rhizome microencapsulated powder

3.3.2.1. Results of physicochemical properties, active ingredients, and antioxidant activity of BLMH microencapsulated powder

The ratio of concentrate to coating mixture of 40/60, and the ratio of coating materials MD/GA/MCS/PEC 3/1/5/1, yielded outstanding physicochemical results, achieving the highest TSC and TPC.

Table 3.26. Results of analysis of properties of BLMH microencapsulated powder

No.	Extract/ wall material	MD/GA/ MCS/PEC	Moisture (%)	Water activity (a_w)	TSC (mg EE/g DM)	FRAP (mg TE/g DM)
1	60/40	4/1/4/1	6.11±1.23^{ab}	0.446±0.004^{f}	149.23±3.83^{bcd}	4.45±0.65^{c}
2		5/1/3/1	6,78±2.08^{ab}	0.498±0.004^{d}	138.18±18.01^{cde}	6.98±0.93^{a}
3		3/1/5/1	**7.45±0.09^{a}**	0.493±0.003^{d}	147.01±2.55^{bcd}	**7.61±0, 86^{b}**
4	50/50	4/1/4/1	6,60±0.54^{ab}	0.500±0.003^{c}	143,21±15.6^{c}	4.56±0.47^{c}
5		5/1/3/1	4.53±0.36^{c}	0.509±0.003^{c}	140.72±2.48^{bcde}	4.65±0.10^{c}
6		3/1/5/1	6.64±2.18^{ab}	0.504±0.003^{c}	123,11±20.91^{e}	4.81±0.76^{c}
7	40/60	4/1/4/1	5.70±1.02^{bc}	0.560±0.011^{a}	153.73±7.72^{bc}	4.58±0.17^{c}
8		5/1/3/1	6.97±0.02^{ab}	0.464±0.00 2^{e}	132.45±9.51^{de}	4,24±0,50^{c}
9		**3/1/5/1**	**7.51±0.35^{a}**	**0.517±0.001^{b}**	**158.90±8.08^{bc}**	**7.30±0.96^{c}**
10	No coating	0	6.52±0.07^{ab}	0.491±0.004^{d}	207.69±1.14^{a}	8.05±0.06^{a}

3.3.2.2. Results of structural-morphological measurements

a. Zeta potential, particle size, and SEM image

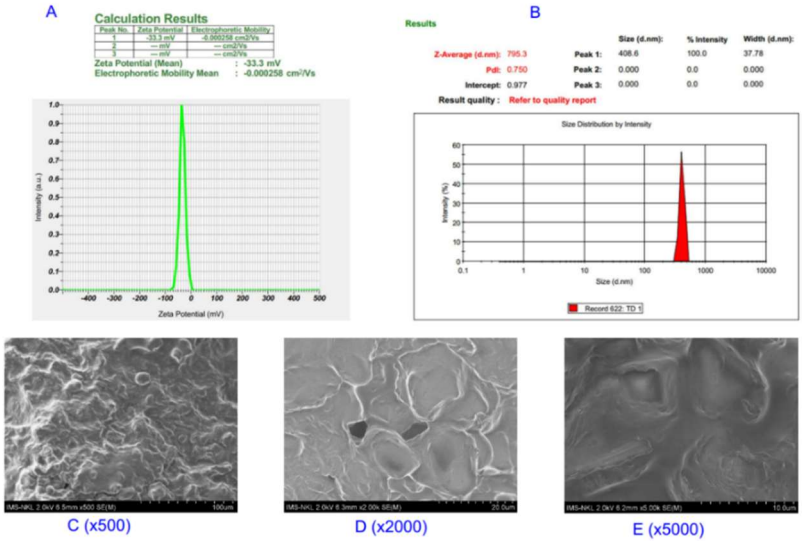


Figure 3.13. Zeta potential spectrum (A), particle size distribution (B), and SEM images (C, D, E) of BLMH microencapsulated powder

b. DSC, TGA, and XRD spectra of BLMH microencapsulated powder

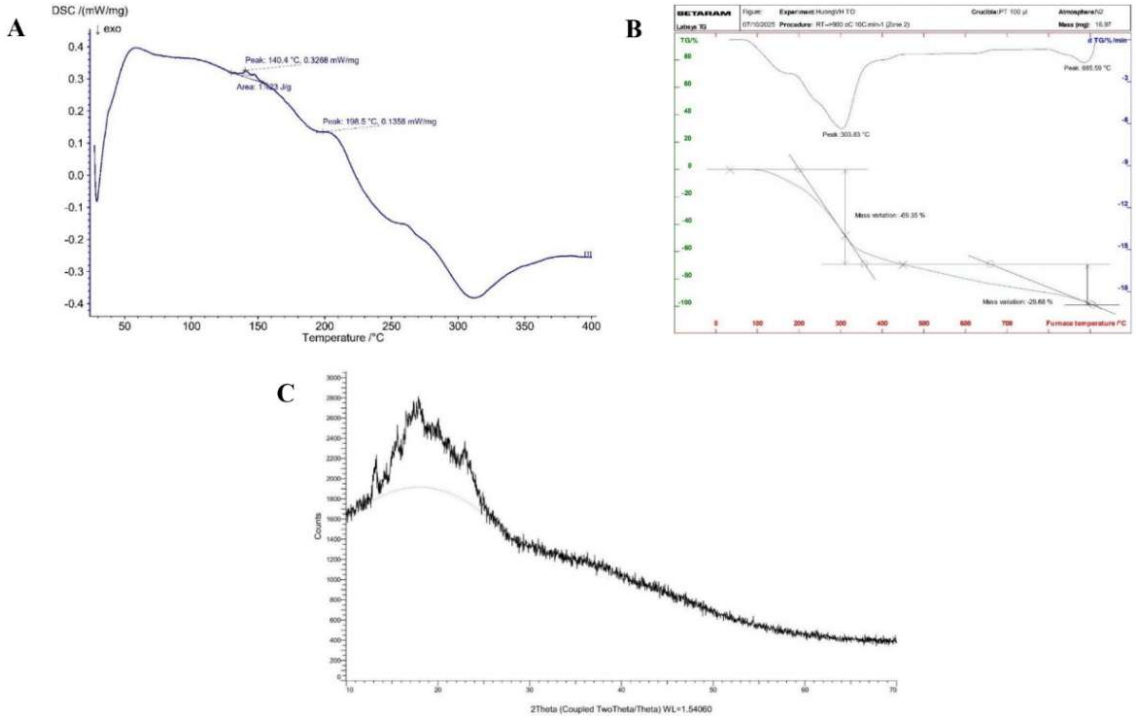


Figure 3.14. DSC (A), TGA (B), and XRD (C) spectra of BLMH microencapsulated powder

3.3.2.3. Results of microbiological and heavy metal testing and antimicrobial and anticancer activity of BLMH microencapsulated powder.

a. Results of microbiological testing

Table 3.27 shows that the total number of aerobic microorganisms is very low, indicating that the raw materials and production process are well-controlled in terms of hygiene.

Table 3.27. Results of microbiological analysis of BLMH microencapsulated powder

No.	Analytical parameters	Analysis results	Analysis methods
1	Total number of aerobic microorganisms	$3.85x10^1$ CFU/g	TCVN 4884-1:2015
2	*Coliform*	Not detected (ND)	TCVN 6848:2007
3	*Escherichia coli*	ND	TCVN 924-2:2008
4	*Staphylococcus aureus*	ND	TCVN 4830-2005
5	Total number of yeast and mold spores	$0.12x10^1$ CFU/g	TCVN 8275-2010
6	*Clostridium perfringens*	ND	TCVN 4991-2005

b. Results of heavy metal testing

The results of heavy metal testing of BLMH microencapsulated powder (Table 3.28) show that the main indicators are all "Not detected" with low detection limits.

Table 3.28. Results of heavy metal testing of BLMH microencapsulated powder

No.	Analytical parameters	Analysis results	Analytical methods
1	Lead (Pb) content	ND	TCVN 8126-1:2009
2	Mercury (Hg) content	ND	TCVN 7604:2007
3	Cadmium (Cd) content	ND	TCVN 7603:2007
4	Arsenic (As) content	ND	TCVN 9521:2012

c. Results of antimicrobial activity assessment

Table 3.29. Results of antimicrobial activity evaluation of BLMH microencapsulated powder

Concen-tration (mg/mL)	Inhibition ring diameter (mm)				
	E. coli	***S. aureus***	***Salmonella***	***B. anthracis***	***C. albicans***
70	-	-	-	24;24;24	25;25;25
50	24;24;24.5	25;25;25	26;26;26	22;22;22	22.5;22.5;22.5
30	22;21.5;21.5	23;23;23	21;21;21	13.5;14;14	16;16;16
10	18;18;18	18;17.5;18	15;15;15	-	-
Positive symptoms	30;30;30	31;31;30.5	31;31;31	28;28;28	30;30;30

Table 3.30. Results of MIC determination of BLMH microencapsulated powder

Microorganisms	MIC (mg/mL)
Staphylococcus aureus	10
Escherichia coli	10
Salmonella	10
Bacillus anthracis	25
Candida albicans	22.5

The BLMH microencapsulated powder exhibited relatively strong antimicrobial activity with a broad spectrum of action. MIC values indicated that the BLMH microencapsulated powder showed a broader activity spectrum and greater sensitivity toward Gram-negative bacteria than toward yeast.

d. Results of *in vitro* cancer cell growth inhibition

Table 3.31. Results of evaluating *in vitro* cancer cell growth inhibition of BLMH microencapsulated powder

Cancer cell line (cell type)	% inhibition of cell growth at a concentration of 100 µg/mL	GI_{50} (µg/mL)
MCF-7 (Breast, ER positive)	>100	7.32 ± 1.93
MDAMB231 (Breast, TNBC)	>100	12.7 ± 0.4
T47D (Breast, ER positive)	>100	7.67 ± 0.44
ZR-75-1 (Breast, ER positive)	>100	12.0 ± 2.0
SKBR3 (Breast, HER2 positive)	>100	12.5 ± 1.0
BT474 (Breast, HER2 positive)	>100	12.0 ± 0.5
BT20 (Breast, ER negative)	>100	10.7 ± 2.7
MDAMB468 (Breast, TNBC)	>100	6.33 ± 0.93
MCF10A (Normal breast)	>100	12.3 ± 1.1
BxPC3 (Pancreas)	117 ± 3	11.7 ± 0.3

The BLMH microencapsulated powder exhibited pronounced *in vitro* inhibitory activity against the proliferation of all tested cancer cell lines. However, the sample did not demonstrate high selectivity between cancerous and normal cells. Notably, it showed strong inhibitory effects on breast cancer cell lines MCF-7 and T47D, with GI_{50} values of 7.32 and 7.67 µg/mL, respectively.

CHAPTER 4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusion

4.1.1. Drying conditions for DS roots and BLMH rhizomes

The recommended drying method for DS roots is microwave drying at 720 W.

The recommended drying method for BLMH rhizomes is hot air drying at 60ºC.

4.1.2. Extraction conditions for DS roots and BLMH rhizomes

Extraction conditions for the DS roots were: Ultrasonic-assisted extraction (UAE) using 80% v/v ethanol solvent, extraction temperature of 40°C, extraction time of 27 minutes, and material/solvent ratio of 1/88 (g/mL). Under these optimal conditions, the TSC, SEE, TPC, ARSC, and FRAP values were 68.64 mg EE/g DM, 86.07%, 42.19 mg GAE/g DM, 91.24%, and 85.67 mg TE/g DM, respectively.

Conditions for extracting BLMH rhizomes were: Microwave-assisted extraction (MAE) with 80% v/v ethanol solvent, extraction time of 27 minutes, and irradiation time of 8 seconds/minute. Under these optimal conditions, TSC, SEE, TPC, ARSC, and FRAP reached 65.74 mg EE/g DM, 82.44%, 0.91 mg GAE/g DM, 4.45%, and 1.21 mg TE/g DM, respectively.

4.1.3. Conditions for producing microencapsulated powders of DS roots and BLMH rhizomes

The microencapsulation system for DS roots uses a concentrate-to-wall material ratio of 40/60, with a wall material blend of MD/GA/MCS/PEC at a ratio of 5/1/3/1.

The BLMH microencapsulation system uses a a concentrate-to-wall material ratio of 40/60, with a wall material blend of MD/GA/MCS/PEC at a ratio of 3/1/5/1.

The obtained microencapsulated powder exhibited good structural stability and physicochemical properties, as evidenced by SEM, DLS, DSC, TGA, and XRD analyses; it also met the requirements for microbiological safety and heavy metal limits. In addition, the product demonstrated notable biological activities, including antioxidant capacity, antibacterial activity, and *in vitro* cancer cell growth inhibition.

4.2. Recommendations

Evaluate the techno-economic efficiency of the process, including energy consumption across unit operations (drying, extraction, and microencapsulation), and develop mass and energy balance models at the pilot scale to estimate production costs and assess commercialization potential.

Extend investigations on the stability and bioavailability of the product in specific food systems by evaluating the stability of bioactive compounds during storage, their release behavior, and bioavailability, thereby providing a more comprehensive scientific basis for the development of functional food products.

Further elucidate the relationship between bioactive composition and the mechanisms underlying biological activities, particularly the inhibition of cancer cell proliferation, to enhance the scientific reliability in evaluating the application potential of the product.

KEY FINDINGS

Dissertation topic:	**Research on drying , extraction and evaluating the biological activity of two medicinal plants, *Salvia miltiorrhiza* Bunge and *Paris polyphylla* Smith for application in functional foods development.**
Major:	Food Technology
Code:	9540101
Course:	2021
Instructor:	Assoc. Prof. Dr. Nguyen Van Tang Assoc. Prof. Dr. Nguyen Van Hoa
Training facilities:	Nha Trang University

Key findings:

1. The dissertation identified the optimal drying method for *Salvia miltiorrhiza* roots as microwave drying at 720 W and for *Paris polyphylla* rhizomes as hot air drying at 60°C. The resulting dry powder has moisture < 6.0% , water activity < 0.5 , with total saponin content and DPPH reaching 32.00-27.11 mg escin/g dry matter, respectively and 17.72-1.47 mg trolox/g dry matter, respectively.

2. The dissertation determined the optimal extraction conditions for Salvia miltiorrhiza roots using ultrasound-assisted extraction with 80% (v/v) ethanol, 40°C, 27 minutes, and a material/solvent ratio of 1/88 (g/mL); and for *Paris polyphylla* rhizomes using microwave-assisted extraction with 80% (v/v) ethanol, 27 minutes, and a irradiation time of 8 seconds/minute. Under these conditions, the total saponin content and extraction yield reached 68.64 and 65.74 mg escin/g dry matter, respectively, corresponding to 86.07 and 82.44%.

3. The dissertation determined the optimal microencapsulation conditions for the concentrated extract of *Salvia miltiorrhiza* root with a maltodextrin/gum arabic/modified corn starch/pectin coating system at a ratio of 5/1/3/1 and for *Paris polyphylla* rhizome at a ratio of 3/1/5/1, with the same extract/coating system ratio of 40/60 (based on dry matter). The resulting microencapsulated powder exhibited high thermochemical stability, a uniform structure, good protection of the active ingredient, strong antimicrobial activity (MIC of 10-27.5 mg/mL for *Salvia miltiorrhiza*; of 10-25 mg/mL for *Paris polyphylla*) and strong inhibition of cancer cell growth, especially on the SKBR3 strain with *Salvia miltiorrhiza* (GI_{50} of 14.7 µg/mL) and MDAMB468 with *Paris polyphylla* (GI_{50} of 6.33 µg/mL).

Supervisors **Doctoral students**

Assoc. Prof. Dr. Nguyen Van Tang

Tran Thanh Giang

Assoc. Prof. Dr. Nguyen Van Hoa

LIST OF PUBLISHED WORKS

1. Tran TG, Nguyen VH & Nguyen VT (2024), "Physicochemical, phytochemical and antioxidant properties of medicinal plant roots Dan sam (*Salvia miltiorrhiza* Bunge) prepared under different drying conditions", *Cogent Food & Agriculture*, 10(1), 2420843, DOI: 10.1080/23311932.2024.2420843

2. Tran TG, Nguyen VH, Nguyen TV & Nguyen VT (2025), "Preparation of medicinal plant rhizome Bay la mot hoa (*Paris polyphylla* Smith) under various drying conditions", *Cogent Food & Agriculture*, 11(1), 2518217, DOI: 10.1080/23311932.2025.2518217

3. Trần T. G., Nguyễn V.H. & Nguyễn V. T. (2026), "Optimization of extraction conditions for phytochemical compounds and antioxidant capacity from Dan sam root (*Salvia miltiorrhiza* Bunge)", *Journal of Science and Technology Hanoi University of Industry*, 62(3), 244-252.