STUDYING THE AFFECTTED FACTORS TO FREEZE DRYING PROCESS OF NATTO PREPARATION

NGHIÊN CỨU CÁC YẾU TỐ ẢNH HƯỞNG ĐẾN QUÁ TRÌNH SẤY THĂNG HOA CHẾ PHẨM NATTO

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ABSTRACT

Freeze drying process is the drying method which preserves the original biological characteristics of products with high quality compared to other drying methods, providing high efficiency during usage. Natto is known as a product extracted from soybeans fermented by Bacillus Natto bacteria. It is used as a functional food to support stroke people and prevent from disease. Therefore, the freezing drying process was used to study the impacts of factors such as drying temperature, freezing water temperature, pressure, material thickness for preservation of the nutritional composition and biological characteristics of Natto composition. Besides it determines the technological parameters during freeze drying process to increase the velocity of the drying process and shorten the drying time, thereby reducing the energy costs of drying.

Keywords: *bacillus Natto; energy; freeze drying process; freeze drying; functional food.*

TÓM TẮT

Sấy thăng hoa là phương pháp sấy ôn hòa, sản phẩm bảo toàn được hoạt tính sinh học ban đầu, chất lượng thực phẩm cao so với các phương pháp sấy khác, mang lại hiệu quả cao trong quá trình sử dụng. Natto là sản phẩm chiết xuất từ đậu nành lên men bằng vi khuẩn Bacillus Natto, *được ứng dụng như một thực phẩm chức năng giúp hỗ trợ cho người bị tai biến mạch máu, và sử dụng để phòng ngừa căn bệnh này. Do vậy, tác giả tiến hành nghiên cứu các yếu tố như: nhiệt độ sấy, nhiệt độ nước đóng băng, áp suất, chiều dày nguyên liệu sấy… ảnh hưởng đến quá trình sấy thăng hoa đối với chế phẩm Natto. Mục đích của nghiên cứu là nhằm bảo toàn thành phần dinh dưỡng và hoạt tính sinh học của chế phẩm Natto. Bên cạnh đó,các thông số công nghệ trong quá trình sấy thăng hoa được xác định nhằm tăng vận tốc của quá trình sấy, rút ngắn thời gian sấy, từ đó giảm chi phí năng lượng của quá trình sấy.*

Từ khóa: *Bacillus Natto; năng lượng; quá trình sấy thăng hoa; sấy thăng hoa; thực phẩm chức năng.*

1. INTRODUCTION

Currently, there are many common methods which are used to dry the industrial food, such as convection drying, radiation drying, frozen drying, or drying by high-frequency electric current, etc... In which, freeze drying process is considered as the best method for the final product with the highest quality compared to other food drying methods. Freeze drying is the process of extracting moisture from the material by the

sublimation of water in low temperature conditions. In the decade of 1950's of the last century, this method can only be applied in the pharmaceutical industry. However, since 1970, it has been applied on food in developed countries [1, 2].

Natto is a product extracted from soybeans, fermented with *Bacillus Natto* bacteria. This food contains enzymes *Nattokinase* works, which helps to destroy blood clot and effects on *Fibrin* 4 times stronger in comparison with *Plasmin*. *Plasmin* is the only enzyme in the body having the ability to destroy clot [3, 4]. Therefore, Natto could be used as a functional food to support people with stroke. Also, it could help to prevent the stroke disease [5].

Applied freeze drying technology in manufacturing Natto products could help to produce biological products and functional foods, creating opportunities for patients to reach the expected product at lower price in comparison with import products. Hence, the authors decided to implement the research in drying Natto products by freeze drying technology [6 – 10].

2. MATERIALS AND METHODS

2.1 Materials

Bacillus Natto seed was collected by and supplied from Department of Biotechnology, HCM City University of Technology. This kind of Natto is belonging to Vietnam Type Culture Collection (VTCC), Vietnam National University. The seed was lyophilized and stored at -70°C temperature. This seed was activated by using the original environment to grow and proliferate, and observation cell morphology was continuously conducted. Then, this strain was used to ferment the soy substrates for producing Natto products [11-12].

2.2 Methods

2.2.1 Method to determine the rate of frozen water by freezing temperature

The sample was frozen to explore the temperature and weighed by electronic scale Satorius basic Type BA310S (accuracy to ± 0.001 gram) to determine W_1 gram. Then it was applied freeze drying process until the temperature of the Natto sample reached 0°C. The dried sample was weighted the second time by electronic scale Satorius basic Type BA310S to determine W_2 gram [2, 13-14].

The volume of water W(g) present in 100 grams raw material of Natto products was determined. According to the analysis, the presence of water content in the composition of raw material Natto is 79.16% [12,15].

$$W = 100*79.16\% = 79.16 \text{grams} \quad (1)$$

To find the IR proportion (%) of frozen water, Eq. (2) is applied. The same formula was used for 6 samples in freezing mode at different temperatures [-15°C, -20°C, - 25°C, -30°C, -35°C, -40°C]. Each sample was tested 3 times.

$$IR\ (\%) = \frac{\Delta W}{W} = \frac{W_1 - W_2}{W} * 100\ \% \quad (2)$$

Whereas, $t_{cđ}$ (°C) is the freezing temperature of the Natto material composition; W_1 (g) is the volume of raw materials Natto products at the end of phase 1, Freezing; W_2 (g) is the volume of raw materials Natto products at the end of Phase 2, Freeze drying, with temperature of dried material 0°C; W (g) is the amount of water present in the material [2, 16].

2.2.2 Microbiological method

This method was used for checking morphological characteristics of micro-organisms, Gram stain method [11, 17].

2.2.3 Physical method

Method of determining moisture by drying method and Method of determining the total ash content were used in this study [1- 3].

2.2.4 Biochemical method

This method was used to determine the reduction of sugar content by means of Bertrand and the quantity of lipids by Soxhlet method; quantify total nitrogen by Kjeldal method; determine the potency of enzyme protease by improvement Anson method; inspect activity thrombolytic of enzyme Nattokinase (check the activity of the enzyme Nattokinase thrombolytic by ethanol experiment tests, which had been carried out in the Department of Hematology transfusion, Hue Medical and Pharmaceutical University); determine vitamin K (Analytical methods to determine the amount of vitamin K in the composition Natto by high-pressure liquid chromatography UV detector), [13-15,17].

2.2.5 Statistical processing method

A statistical processing method was applied to optimize the experiments. The analysis of variance (ANOVA) for the quadratic equation was used to understand the significant effect of variables and their interactions on the response of the adsorption system [2-3].

3. RESULTS AND DISCUSSIONS

3.1 Analyzing, identifying ingredients within fermented Natto

After creating Natto composition by fermented soybeans based on Bacillus Natto bacteria, the chemical compositions of Natto product were examined to evaluate nutritional value and biological ingredients of preparations. The analytical results are shown in Table 1.

Table 1. *The percentage of ingredients in Natto fermentation*

No.	Ingredient	Unit	Percentage
1	Water	% weigh	79.160 $^{\pm 0.0001}$
2	Sugar (reducing sugar)	% dry measure	14.660 $^{\pm 0.0013}$
3	Protein	% dry measure	29.950 $^{\pm 0.0001}$
4	Lipid	% dry measure	11.700 $^{\pm 0.0009}$
5	Glucide	% dry measure	2.970 $^{\pm 0.0001}$
6	Vitamin K	µg	134.240

The results of Table 1 indicates that the majority components of fermented Natto are water (accounting for 79.16%), protein (accounting for nearly 29.95%) and the majority components of fermented Natto are glucide, lipid, accounting for 11.7% and 2.97%, respectively. Especially, the amount of vitamin K in fermented Natto is very high, around 123.61µg/100g. Based on obtained products, the biological activity was examined by determining the activity of the protease enzyme in fermentation Natto. We conducted 3 replicated examinations and obtained result at 0.054 (HP/g) (Table 2).

3.2 Determining influencing factors to freeze drying process of Natto preparation

3.2.1 Determining the rate frozen water under freezing temperatures

According to the method of determining the ratio of frozen water IR (%), 6 samples were performed. Each test was conducted three times, the experimental data obtained as Figure 1. It shows that the rate of frozen water increases from 61.550 to 92.847% when freezing temperature reduces from -15°C to -35°C. It also indicates that if the temperature

drops down to -40°C, the rate of frozen water increases no significantly.

Table 2. *The activity of enzyme protease in fermented Natto*

Product	Activity (HP/g)
Fermented Natto	0.054 ± 0.00002

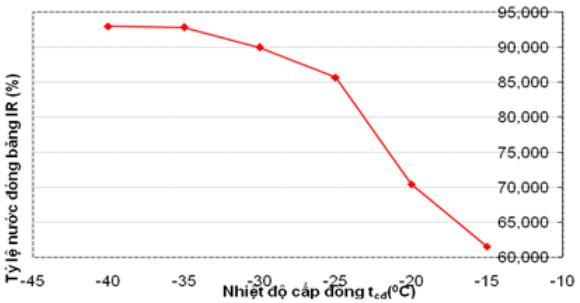

Figure 1*. The rate of frozen water under freezing temperatures*

3.2.2 Determining the influence of drying temperature on the drying time

Six samples were dried in the range of temperature T_{say} (°C) = [30°C, 32°C, 34°C, 36°C, 38°C, 40°C], with 8% humidity. We chose the parameters for the drying process as follows: freezing temperature -25°C, thickness 15mm, pressure 0,1mmHg. Each sample was examined 3 times. The results are shown on the graph in Figure 2.

From the above results, it indicates that if drying temperature increases from 30°C to 38°C, the drying time reduces from 12.43h down to 9.71h. The necessary heat transferring to the material would increase the drying temperature. Within constant pressure, when the ambient temperature in the sublimation chamber increase from 30 ÷ 38°C, it would lead the increase of the evaporation intensity. Therefore, the drying time is shortened. However, the research results show that if the temperature rises above 40°C, the drying time will increases to 10.27h. Rising temperatures will melt the solid ice, which affects the efficiency of freeze drying process. It means that the time for drying will prolong than usual. To determine if the above drying temperature ranges affected to the biological activity or not, we conducted the experiment to identify the active force of enzyme protease.

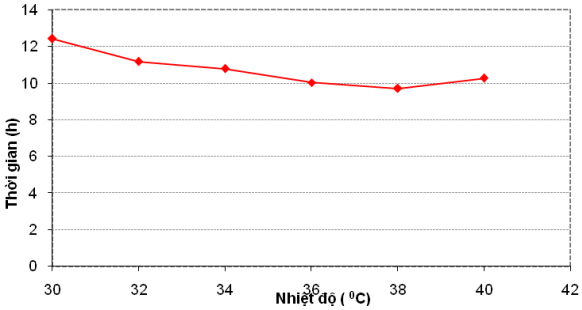

Figure 2*. The influence of drying temperature on the drying time*

3.2.3 Determining the influence of drying temperature on the activity of protease enzymes

The activity of the protease enzyme in Natto products was determined as Anson improved method. The obtained results are as follows:

Table 3. *The influence of drying temperature to the activity of protease enzyme*

No.	Drying temperature T (^{0}C)	Activity (HP/g)
1	30	0.047 ± 0.00002
2	32	0.044 ± 0.00002
3	34	0.046 ± 0.00001
4	36	0.047 ± 0.00003
5	38	0.045 ± 0.00002
6	40	0.046 ± 0.00001

From the above results, it shows that the drying temperature does not affect the composition of Natto, particularly the biological activity of the protease enzyme.

3.2.4 Determining the influence of material thickness on the drying time

Surveyed Natto preparations were freeze dried under 5 samples with different thickness

δ (mm) = [10, 15, 20, 25, 30]. Each dried sample was conducted repeated 3 times and the results are presented as Figure 3.

Results of experiment in monitoring the relationship between drying time and material thickness showed that 10mm thickness of materials must require 9.82h drying time. If the thickness of Natto preparation increases from 15 ÷ 30 mm, the drying time will also increase in the range of 10.27 ÷ 14.73h. Consequently, the more thickness of the composition increase, the more time for drying require. Due to the thicker of material layer, ice sublimation process is more difficult.

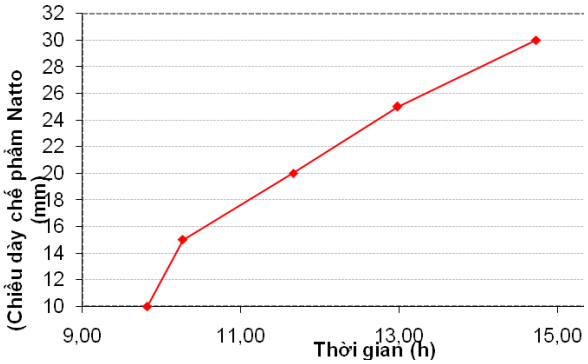

***Figure 3**. The influence of material thickness on the drying time*

3.2.5 Determining the influence of drying material thickness to protease enzyme activity

In freeze drying, the heat transferring in sublimation process is performed by mean of exposure through the stainless steel tray containing materials. In high vacuum environment and without turning, the heat transferring to material during of drying process is uneven in surface of Natto products. Usually, the materials that contact directly with the surface of the tray will have temperature higher than the rest. Therefore, we analyzed the activity of Natto products after drying to check if the thickness of the material may affect the drying capacity or not. Results after comparing on colorimetric spectrophotometer are shown in Table 4.

As can be seen from table 4, the activity of protease enzyme in Natto preparation does not differ so much. It proves that the thickness of material does not significantly affect to the quality of the dried products.

***Table 4**. The influence of material thickness to activity of protease enzyme*

No.	Thickness of Natto, δ (mm)	Activity (HP/g)
1	10	0.046 ± 0.00002
2	15	0.047 ± 0.00001
3	20	0.044 ± 0.00002
4	25	0.047 ± 0.00001
5	30	0.046 ± 0.00003

3.2.6 Determining the influence of vacuum pressure to the drying time

In freeze drying technology, the pressure is one of the factors that greatly affect the drying process, specifically the drying time. An experiment was conducted to clarify this issue by drying 6 samples with values of pressure P (mmHg) = [0.1; 0.2; 0.3; 0.4; 0.5; 0.6] mm Hg within selected conditions. Each experiment is repeated 3 times. The results are presented as following Figure 4.

The analytical results show that drying Natto preparations in pressure 0,1mmHg helps shortening drying time (around 9.97h) in comparison with remaining pressure.

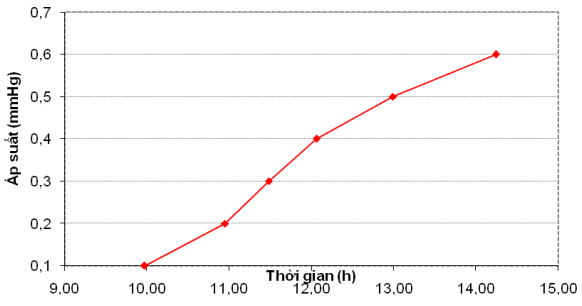

***Figure 4.** The influence of vacuum pressure to the drying time*

3.3 Optimizing the elements by method Box Wilson

In this study, empirical model within 4 elements was chosen: TYT 2^4, and performed in the following equation:

$$Y^{\wedge} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4$$

In which:

4 elements, including: the thickness of Natto product, freezing temperature, sublimation pressure, sublimation temperature.

Z1: the thickness of Natto product; range of thickness from 10mm to 20mm; low level: (-) 10mm; high level: (+): 20mm; standard level: 15mm

Z2: freezing temperature; range of temperature from -25°C to -35°C; low level: (-) -25°C; high level: (+): -35°C; standard level: -30°C;

Z3: sublimation pressure; range of pressure from 0,1mmHg to 0,5mmHg; low level: (-) 0,1mmHg; high level (+): 0,5mmHg; standard level: 0,3mmHg;

Z4: sublimation temperature; range of temperature from 30°C đến 38°C; low level: (-) 30°C; high level: (+): 38°C; standard level: 34°C;

Standard level: $Z1^0$ = 15mm; $Z2^0$ = -30°C; $Z3^0$ = 0,3mmHg; $Z4^0$ = 34°C

Y(h): time for drying

a. Experimental plan of full elements TYT 24 was encrypted and presented as a matrix, results shown in the table 5 [1, 2, 4].

b. Calculating regression coefficients by the formula:

$$b_j = \frac{1}{N}\sum_{i=1}^{N} x_{ji}y_i \qquad (3)$$

b_0 = 12.12; b_1 = 0.241; b_2 = -0.316;

b_3 = 0.647; b_4 = -0.310; b_{12} = -0.650;

b_{13} = -0.491; b_{14} = -0.634; b_{23} = -0.344;

b_{24} = 0.0012; b_{34} = -0.245

In planning matrix, there is no parallel experiment. In this case, to determine the variance, 3 experiments were checked and received three experimental values as follows:

Y^0_1 = 10.38; Y^0_2 = 10.21; Y^0_3 = 10.35

$$\overline{Y^0} = \frac{\sum_{u=1}^{3} Y_u^0}{3} = 10.31333 \qquad (4)$$

$$S_{th}^2 = \frac{\sum_{u=1}^{3}\left(Y_u^0 - \overline{Y^0}\right)^2}{3-1} = 0.016467 \qquad (5)$$

$$\Rightarrow S_{th} = 0.12832$$

$$Sbj = \frac{S_{th}}{\sqrt{N}} = \frac{0.12832}{\sqrt{16}} = 0.032081 \qquad (6)$$

c. Testing the significance of regression coefficients as Student Standard:

$$t_j = \frac{|b_j|}{S_{bj}} \qquad (7)$$

$$t_0 = \frac{12.12}{0.032081} = 377.79$$

t_1 = 7.52; t_2= 9.86; t_3 = 20.18 ; t_4 = 9.66;

t_{12} = 20.3; t_{13} = 15.31; t_{14} = 19.75;

t_{23} = 10.71; t_{24} = 0.039 ; t_{34} = 7.64

According to the Student distribution, t0.05(3)= 7.45. Due to t_{24} = 0.039 < 7.45 therefore coefficient b_{24} is was excluded from the regression equation. The regression equation is presented as formula:

$$Y^{\wedge} = 12.12 + 0.241x_1 - 0.316x_2 + 0.647x_3 - 0.310x_4 - 0.650x_1x_2 - 0.491x_1x_3 - 0.634x_1x_4 - 0.344x_2x_3 - 0.245x_3x_4$$

d. Testing the compatibility of empirical regression equation:

The compatibility of the regression equation with experiment was tested with the standard Fisher:

$$F = \frac{S_{du}^2}{S_{th}^2} \quad (8)$$

In which:

$$S_{du}^2 = \frac{\sum_{i=1}^{N}(y_i - y_i\wedge)^2}{N-1} \quad (9)$$

N: the number of experiment = 16;

l: mean coefficient: l = 10;

$S^2_{du} = 0.240763$

$$\Rightarrow F = \frac{0.240763}{0.016467} = 14.6212 \quad (10)$$

Looking up table $F_{1\text{-}P}$ $(f_1 - f_2)$ with p = 0.05, f1 = 4, f2 = 2 ; $F_{0.95}(3.2) = 19.3$

$F < F_{1\text{-}P}$ $(f_1 - f_2)$ It means that the founded regression equation is compatible with the experiments.

The founded regression equation is:

$$Y\wedge = 12.12 + 0.241x_1 - 0.316x_2 + 0.647x_3 - 0.310x_4 - 0.650x_1x_2 - 0.491x_1x_3 - 0.634x_1x_4 - 0.344x_2x_3 - 0.245x_3x_4$$

e. Optimization as method Box Wilson:

To achieve the optimization, the first step is to select the basic movements, thereby calculating the remaining steps are as follows:

$$\delta_j = \delta_i . \frac{b_j.\Delta_j}{b_i.\Delta_i} \quad (11)$$

We chose the basic step as factor X_1 with δ_1= 1mm, from which the jump of other factors could be calculated as:

$$\delta_2 = \delta_1 . \frac{b_2.\Delta_2}{b_1.\Delta_1} = -1.3108$$

$$\delta_3 = \delta_1 . \frac{b_3.\Delta_3}{b_1.\Delta_1} = 0.1074$$

$$\delta_4 = \delta_1 . \frac{b_4.\Delta_4}{b_1.\Delta_1} = 1.0279$$

Conduct experiments on the slopes according to the calculated parameters. Obtained results are shown in Table 5.

Experiment 4 indicates that the drying time is shortest at 8.25h when all elements reach at Z_1=11mm, Z_2=-26°C, Z_3= 0.008mmHg, Z_4 = 38°C. We have the shortest drying time is reached 8.25h. When continue in experiment 5 and 6, the drying time is longer. It means that the obtained results in experiment 4 are the optimal parameters.

3.4 Surveying the variation of moisture over the time of freeze drying

To study the variation of moisture over time, we dried Natto preparations according to optimal parameters (table 5), the drying product reaches moisture at 8%. The result is performed as the following Figure 5.

Table 5. *Experimental results*

Name	Z1	Z2	Z3	Z4	Y(h)
Basic	15	-30	0.3	34	-
Coefficient bj	0.24125	-0.31625	0.6475	-0.31	-
Variability	5	5	0.2	4	-
bj.Δj	1.20625	-1.58125	0.1295	-1.24	-
δj	1	-1.3108	0.1074	1.0279	-
Rounding	1	1	0.1	1	-
Experiment 1	14	-29	0.2	35	10.26
Experiment 2	13	-28	0.1	36	9.77
Experiment 3	12	-27	0.008	37	9.54
Experiment 4	11	-26	0.008	38	8.25
Experiment 5	10	-25	0.008	39	10.57
Experiment 6	9	-24	0.008	40	10.78

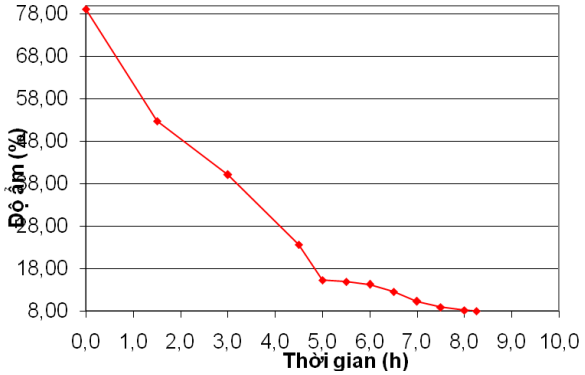

Figure 5. *The variation of humidity over time of freeze drying*

3.4 Assessing the quality of Natto preparation after freeze drying

3.4.1 Assessing the quality of Natto products via analysis of nutrients and bioactive

After studying the factors that affect the process of freeze drying, and the optimal conditions for drying Natto products, we assess the quality of the composition after drying based on the following criteria: composition nutritional, biological activity of Natto products after freeze drying in comparison with Natto products before drying. The results are presented as following figure:

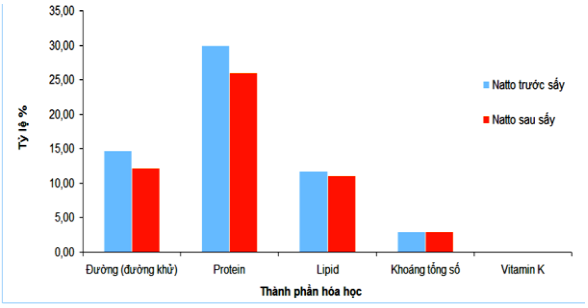

Figure 6. *The chemical components of Natto products before and after freeze drying*

Besides analyzing and determining the nutritional components in the composition of Natto, the biological activity of both compositions before and after freeze drying were also examined. The results were as shown in Table 6.

At the same time, we tested the thrombolytic activity of the enzyme Nattokinase in dried Natto preparation at Department of Hematology-transfusion, Hue Medical and Pharmaceutical University. The experiment was done as following steps:

Table 6. *The activity of enzyme protease in Natto preparation before and after freeze drying*

Product	Activity (HP/g)
Natto before drying	0.054 $^{\pm 0.00002}$
Natto after drying	0.047 $^{\pm 0.00003}$

- The original blood sample without antifreeze was put in vitro. After 10 minutes, blood sample was being clotted. After 1h, blood completely clotted (components of blood and fibrin).

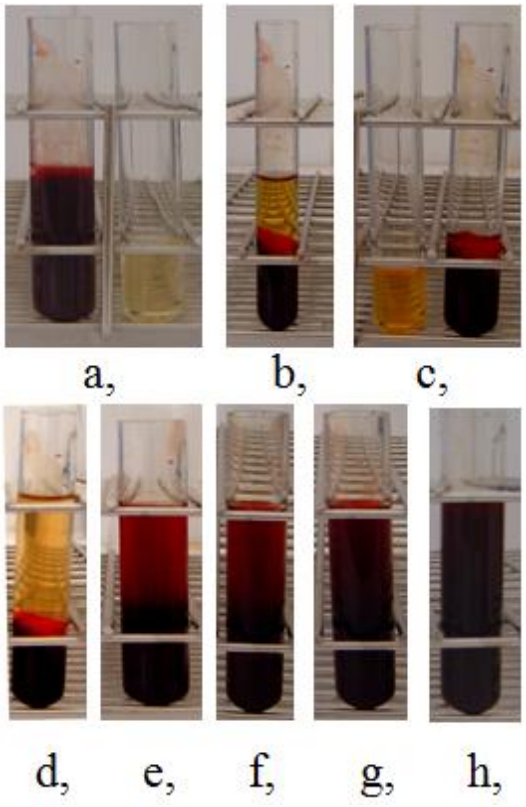

Figure 7. *Natto preparations dissolve blood clots: a, clot blood; b, Centrifugal separation of blood; c, Separated clot from blood; d, Initial time; e, after 5'; f, after 10'; g, after 30'; h, after 60'.*

- Separating serum and doing experiment by ethanol without flocculation phenomenon (negative result).
- Putting Natto preparations on tube containing blood clots, blood clots were being melted after a few minutes. Normally, the melting of blood clots appears after 72 hours. After that, doing centrifugal separation tests the experimental tube and remaining as a second time with ethanol method, there was flocculation phenomenon (positive result). It proves that Natto preparation could help melting thrombolytic. The results are presented as in Figure 7.

3.4.2 Assessing the quality of Natto products through sensory evaluation

- About state of product: Before drying, Natto preparation is in high viscosity condition. After freeze drying, preparations Natto is dry and in high porosity condition. After reverting, Natto preparation is back to its original state.
- About color: The color of fermented Natto is satisfactory compared to the composition after freeze drying. The color of Natto products changed a little bit (from brown to dark brown).
- About the smell: During freezing, the smell of the dried product has been changed but not too much. The smell was diminished after freeze drying. It is pleased to smell than original fermented Natto products.

In summary, through the above analytical results on organoleptic and nutritional value, it shows that freeze drying process has many advantages, which could maintain the nutritional and organoleptic value of original Natto preparations.

4. CONCLUSION

Assessing the quality of Natto products during freeze drying plays an important role not only in the food industry but also in the pharmaceutical industry. Based on the obtained results from experiments, we got a number of key conclusions:

- A recommended freeze drying process for Natto preparation with the optimal technological parameters, namely: thickness of product: 11mm, the temperature of the product before freeze drying: -26°C; the pressure in the chamber sublimation: 0.008mmHg, sublimation chamber temperature: 38°C.
- Several identified nutritional components of Natto products before and after freeze drying. Fermented Natto contains 29.95% protein, 134.24μg/100g vitamin K and a number of glucide, lipid, and ash. Similarly, Natto preparation after freeze drying contains 26.01% protein, 123.61μg/100g vitamin K and a number of glucide, lipid, and ash.
- Identified potency of protease enzyme in Natto products before and after freeze drying: protease enzyme in fermented Natto is 0.054HP/g. After freeze drying, protease enzyme in Natto product active force is 0.047HP/g.
- By analyzing the nutritional content and the value of organoleptic and biological activity of the composition after freeze drying, it shows that freeze drying method is one of the modern methods which keep the original properties of materials.

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REFERENCES

[1] Tran Duc Ba, Pham Van Bon, *Cold technology for tropical food*, HCMC University of Technology, 1993.
[2] Tran Duc Ba, Nguyen Tan Dung, Tran Ngoc Ha, *Studying freeze drying*, Journal of Science and Technology, 2007.
[3] Nguyen Bin, *The process equipment in chemical engineering and food*, Hanoi Science and Technology Publishing, Vol 2.
[4] Nguyen Canh, *Experimental planning*, HCMC University of Technology, 1993.
[5] Nguyen Huynh Diem Chi, *Research freeze drying crab*, Master Thesis, HCM Nong Lam University, 1996.
[6] Nguyen Tan Dung, *Research freeze drying prawn*, Master Thesis, HCM Nong Lam University, 2005.
[7] Nguyen Ngoc Minh, Nguyen Dinh Ai, *Hemostasis and coagulation technique in clinical applications*, Medical Publishing, 1999.
[8] Nguyen Van Mui, *Determination of enzyme activity*, Hanoi Science and Technology Publishing, 2002.
[9] Nguyen Xuan Phuong, *Cold food engineering*, Hanoi Science and Technology Publishing, 2004.
[10] Le Ngoc Tu, *Industrial Biochemistry*, Hanoi Science and Technology Publishing, 1998.
[11] Sumi H., H. Hamada, K. Nakanishi, H. Hiratani, *Enhancement of the fibrinolytic activity in plasma by oral administration of Nattokinase*, Acta Haematol 84(3), pp. 139-43;
[12] Sumi H., H. Hamada, H. Mihara, *A novel strong fibrinolytic enzyme (Nattokinase) in the vegetable cheese Natto*, International Journal of Fibronolysis and Thrombolysis,Abstracts of the ninth international congress on fibrinolysis, Amsterdam, Vol.2, Sup.1, pp. 67, 1998.
[13] Ha Duyen Tu, *Technical analysis of food sensory*, Hanoi Science and Technology Publishing, 2006.
[14] Achanta S., M.R. Okos, *Impact of drying on bilogical product quality*, India, 1995.
[15] Bell.G.A. and J.D. Mellor, *Further developments in adsorption freeze-drying*, Canada, 1990.
[16] Iyniec Z., J. Klimczak and S. Michalowski, *Freeze-drying of the black currant juice*, Drying Technology, 1995.
[17] Jankovies M., *Physical properties of convectively dried and freeze-dried berrylike fruits*, Australia, 1993.

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