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Research Article

Study Technological Factors Effect on the Loss of Protein, Carbohydrate and Lipid inside Royal Jelly in the Freeze Drying Process

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Abstract: This study published the mathematical models that were built by experiment to describe relationships between the loss of nutritional substances such as protein, carbohydrate and lipid of Royal jelly with technological factors in the freeze drying process such as temperature and pressure of freeze drying chamber; time of freeze drying process. These relationships were applied to determine the optimal technological factors. The results were found out the optimal technological mode as follow: the optimal temperature of freeze drying chamber was 24.35°C, the optimal pressure of freeze drying chamber was 0.368 mmHg and the optimal time of the freeze drying process was 19.225 h. Corresponding to these factors, the minimum value of the loss of protein, carbohydrate and lipid of Royal jelly were 1.968, 1.839 and 1.799% and the residual water content of final product after freeze drying were 3.51 under 4.5%. These optimal factors were really essential to establish the technological mode of the freeze drying process Royal jelly for preservation.

Keywords: Carbohydrate in royal jelly, lipid in royal jelly, protein in royal jelly, royal jelly, the loss of protein, lipid and carbohydrate of royal jelly, the technological factors of freeze drying process

INTRODUCTION

According to overview of some research results about nutritional substances of Royal jelly such as Lercker *et al*. (1986); Schmidt and Buchmann (1992); Hattori *et al*. (2007); Sabatini *et al*. (2009), it is obvious that the Royal jelly is not only rich in essential nutrients such as protein, carbohydrate and lipid but also rich in bioactive compounds (10-hydroxy-2-decenoic acid (10-HDA) and its isomer) and vitamins B_1, B_2, B_3, B_5, PP, B_c, H, A, D and E, … etc (Schmidt and Buchmann, 1992; Lercker *et al*., 1992; Hattori *et al*., 2007). In addition, the Royal jelly contains some minerals such as K, Ca, Na, Zn, Fe, Cu and Mn, with a strong prevalence of potassium (Stocker *et al*., 2005).

Protein of Royal jelly contains 29 amino acids, derivatives and contains hardly all essential amino acids for humans. But the most important is still aspartic acid, glutamic acid and the free amino acids as proline and lysine. Besides, protein of Royal jelly also contains some enzymes such as protease, glucose oxidase, phosphatase, cholinesterase and an insulin-like substance (Antinelli *et al*., 2003). Carbohydrate of Royal jelly is mainly sugars, including fructose and glucose. Fructose of Royal jelly is still more prevalent, fructose and glucose together account for 90% of the total sugars. The sucrose content varies considerably from one sample to another. Other sugars present in much lower quantities are maltose, trehalose, melibiose, ribose and erlose (Lercker *et al*., 1992; Daniele and Casabianca, 2012). Lipid of Royal jelly has low rate but it contains bioactive compounds (10-HDA, its isomer) rare in the nature (Morita *et al*., 2012; Dzung, 2013). Lipid fraction consists almost of free fatty acids with unusual and uncommon structures, with the rate of (80 ÷90) % by dry weight of lipid. They are mostly short chain (8 to 10 carbon atoms) hydroxy fatty acids or dicarboxylic acids, in contrast to the fatty acids with 14 to 20 carbon atoms which are commonly found in animal and plant material. These fatty acids are responsible for most of the recorded biological properties of Royal jelly (Schmidt and Buchmann, 1992). In addition to the free fatty acids, the lipid fraction contains some neutral lipids, sterols (including cholesterol, esgosterol) and an unsaponifiable fraction of hydrocarbons similar to beeswax extracts (Lercker *et al*., 1992; Hattori *et al*., 2007) (Fig. 1).

The research results of Ramadana and Al-Ghamdi (2012) shown that the most important nutritional compounds of Royal jelly including protein, carbohydrate and lipid have balanced rate between essential amino acids with different amino acids,

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Fig. 1: The royal jelly product in Viet Nam

Table 1: The composition of Royal jelly in Viet Nam

No	Substance	Value
1	Water	59.2%
2	Proteins	34.95% of dry weight
3	Carbohydrate (sugars)	39.09% of dry weight
4	Lipids	9.80% of dry weight
5	Minerals	2.70% of dry weight
6	10-HDA	7.60 mg/100 g of dry weight

between fructose, glucose with different sugars, between unsaturated fatty acids with saturated fatty acids. This is a product that is rare in nature. They are very good for growing up and development processing of human. Therefore, the Royal jelly was called as a natural product, a very perfect product for people's health and was also called as a natural pharmaceutical product (Sabatini *et al*., 2009; Isidorov *et al*., 2011). It has the ability of anti-aging; prevent the formation of free radicals from biochemical reactions in human body. It can help prevent the heart disease or heart disorder, psychophysiological disorder, nervous disorder, digestive disorder, cancer and many other diseases. In addition, it has the capable of restoring and protecting human's skin as well as increase energy and restores health to the body. Finally, it is able to cure women of gynaecological disease.

According to analytical results in Table 1 (Dzung and Oanh, 2013), the important chemical composition of Royal jelly in Viet Nam contains many different substances but their main constituents are water, protein, carbohydrate (sugars), lipids and mineral salts. Although they occur with notable variations the composition of Royal jelly remains relatively constant when comparing different colonies, bee races and time. The analytic data can be seen in Table 1.

The Royal jelly is a pharmaceutical product that has valuable and rare. As a result, the preservation Royal jelly is a problem that is considered. Currently, the Royal jelly is preserved from the freezing method or the freeze drying method. For the freezing method, the product after freezing process is put in freezing environment that has the optimal temperature. Therefore, when time of preservation process is prolonged over 3 month, the energy consumption for 1 kg final product increase and the quality product reduce. For the freeze drying method, the product after freeze drying process is preserved at the temperature room of 25°C, when time of preservation process is prolonged over 3 month, that quality product and the energy consumption for 1 kg product are constant (Ramadana and Al-Ghamdi, 2012).

However, the freeze drying is a complicated technique including three consecutive main stages. The first stage is freezing the material; the second and the third stage are respectively the sublimation drying and vacuum drying. Both these final stages determine the quality of the product. In Fig. 2, the constituent objective functions of the freeze drying process of Royal jelly were y_j, ($j = 1 \div 4$); the technological factors were Z_i, ($i = 1 \div 3$).

Therefore, the problem posed here is how to determine technological factors of freeze drying process of Royal jelly, including: temperature of freeze drying chamber (Z_1, °C), pressure of freeze drying chamber (Z_2, mmHg), time of freeze drying (Z_3, h) in order that the loss of nutritional compounds such as total protein (y_2, %), carbohydrate (y_3, %) and lipid (y_1, %) in final product after freeze drying reduce the minimum value, with the residual water content of the final product (y_1, %) under 4.5%. On the other hand, this product can also be prolonged time of using and export but their quality is still constant, (Dzung, *2012a, b). A problem is considered here is:* If the residual water content of the final product of Royal jlley after freeze drying is higher than 4.5%, the microorganisms will be capable to grow and develop and damage products. On the other hand, if residual water content of the final product is lower than 4.5%, activity of water inside the final product will be enough for existing and development of microorganisms (Wytrychowski *et al*., 2013).

As a result, the aim of this study is building and solving the multi-objective optimization problem of freeze drying process to determine technological factors in order that the loss of protein, carbohydrate and lipid

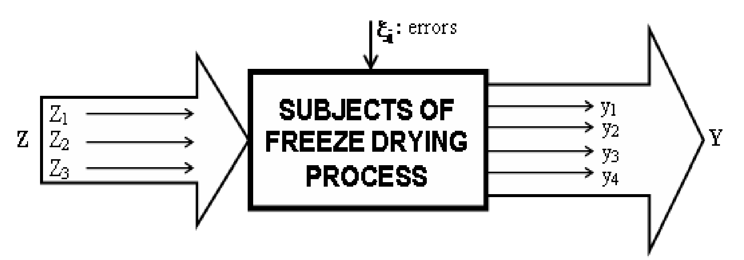

Fig. 2: Diagram of subjects of freeze drying process of Royal jelly

in final product of Royal jelly reach the minimum value and the residual water content of the final product of Royal jelly after freeze drying is under 4.5% (Dzung, *2012b).*

MATERIALS AND METHODS

Materials: The Royal jelly is harvested from bees's nest to grow up at Bao Loc area in Lam Dong province of Viet Nam. It is the pure natural product and does not mix any chemical composition. It is very thick solution. The basic composition of Royal jelly is presented in Table 1 (Dzung and Oanh, 2013).

Before the freeze drying, Royal jelly is frozen at the optimal freezing temperature in order that water in Royal jelly is completely crystallized. Acorrding to research result of Dzung (2014), it was obvious that when Royal jelly is frozen and reach temperature of-18.33°C, at the time, water inside Royal jelly was completely crystallized ω = 1 or ω = 100%.

Apparatus: Equipments used to research the loss of total protein; carbohydrate and lipid in final product of Royal jelly after freeze drying are listed as follow (Dzung, 2012a, b):

- Determining weigh of Royal jelly by Satoriusbasic Type BA310S: range scale (0÷350) g, error: ±0.1g = ±0.0001 kg.
- Determining temperature of Royal jelly Dual Digital Thermometer: range scale (-50÷70) °C, error ±0.05°C.
- The Freeze Drying System DS-3 (Fig. 3) that was controlled automatically by computer. It could reduce the temperature of freezing environment to (-50 ÷ -45) °C. The temperature, pressure and time profile of freeze drying process are measured by computer.
- Equipment used to analyse protein, carbohydrate and lipid and the loss of protein, carbohydrate and lipid in Royal jelly after freeze drying was Kjeldahl Equipment and Soxhlet Equipment. It was shown in Fig. 4a and b.

Methods: The method is used in this study as follow (Dzung, 2012a, b):

- Determining the temperature of environmental freeze drying (Z_1, °C); the pressure of environmental freeze drying (Z_2, mmHg); the time of freeze drying process (Z_3, h) of Royal jelly by the automatic measure and control system on computer of the Freeze Drying System DS-3.
- Determining the residual water content of the final product (y_1, %) by the mass sensor controlled by computer, (Dzung, 2012a, b):

$$y_1 = 100 - \frac{G_i}{G_e}(100 - W_i) \quad (1)$$

Fig. 3: The freeze drying system DS-3 with the auto-freezing (-50÷ - 45) °C

(a) (b)

Fig. 4: a) Kjeldahl Equipment; b) Soxhlet Equipment

where,

G_i (kg) = Weight of the initial material Royal jelly used for freeze drying

G_e (kg) = Weight of the final product of Royal jelly of freeze drying

W_i (%) = Initial water content of the material Royal jelly

- Determining the total protein in Royal jelly before and after freeze drying by the Kjeldahl method (FAO, 1986).
- Determining the total carbohydrate in Royal jelly before and after freeze drying by the hight-performance anion-exchange chromatography method (TCVN 4594: 1988).
- Determining the lipid in Royal jelly before and after freeze drying by the the Soxhlet method (FAO, 14/7, 1986).
- Determining the loss of total protein, total carbohydrate and total lipid of the final product (y_j, %) by Eq. (2), with j = 2, 3, 4:

$$y_j = \frac{m_1 - m_2}{m_1}100\% = \frac{\Delta m}{m_1}100\%; \quad (2)$$

With: $j = 2 \div 4$

where, the total protein, carbohydrate and lipid of the material initial and after freeze drying respectively m_1 (%) and m_2 (%) were calculated according to weight of dry matter. The fact that the product achieves the best quality means $y_{jmin} = 0$. In fact, $y_j > 0$.

- Using quadratic orthogonal experimental planning method (Dzung, 2012a, b) to build the mathematical models. Relationships between the loss of total protein, carbohydrate and lipid, the residual water content of the final product and

Table 2: The technological factors levels design

Parameters	Levels -α (-1.414)	Low-1	Central 0	High+1	+α (1.414)	Deviation ΔZ_i
Z_1,(°C)	17.93	20	25	30	32.07	5
Z_2, (mmHg)	0.008	0.137	0.447	0.758	0.886	0.3105
Z_3, (h)	17.172	18	20	22	22.828	2

technological factors (x_1, x_2, x_3) were written by the Eq. (3) as follow (Dzung, 2012a, b):

$$y = b_0 + \sum_{j=1}^{k} b_j x_j + \sum_{j\neq i;j=1}^{k} b_{ji} x_j x_i + \sum_{j=1}^{k} b_{jj}\left(x_j^2 - \lambda\right) \quad (3)$$

These variables x_1, x_2 and x_3 were coded by variables of Z_1, Z_2 and Z_3 presented as follow:

$$x_i = (Z_i - Z_i^0)/\Delta Z_j;\ Z_i = x_i.\Delta Z_i + Z_i^0 \quad (4)$$

where,

$Z_i^0 = (Z_i^{max} + Z_i^{min})/2$
$\Delta Z_i = (Z_i^{max} - Z_i^{min})/2$
$Z_i^{min} \leq Z_i \leq Z_i^{max}$; i = 1 to 3

The experimental number is determined:

$$N = n_k + n_* + n_0 = 2^k + 2k + n_0 = 18 \quad (5)$$

With: $k = 3$; $n_k = 2^k = 2^3 = 8$; $n_* = 2k = 2\times3 = 6$; $n_0 = 4$. The value of the star point:

$$\alpha = \sqrt{\sqrt{N2^{(k-2)}} - 2^{(k-1)}} = 1.414 \quad (6)$$

The condition of the orthogonal matrix:

$$\lambda = \frac{1}{N}\left(2^k + 2\alpha^2\right) = 2/3 \quad (7)$$

- Using the restricted area method (Dzung, 2012b) to solve the multi-objective objective optimization problem in the freeze drying process of Royal jelly.
- Using the mathematical tools to solve the optimization problem in freeze drying process Royal jelly and the mathematical models describing the relationships between the loss of total protein, carbohydrate and lipid inside the final product, the residual water content of the final product of Royal jelly after freeze drying.

RESULTS AND DISCUSSION

Building the mathematical models about relationships between the loss of total protein, carbohydrate and lipid, the residual water content of the final product with technological factors of the freeze drying process: The constituent objective functions of the freeze drying process including the residual water content of the final product of Royal jelly after freeze drying (y_1, %), the loss of protein (y_2, %), carbohydrate (y_3, %) and lipid (y_4, %) inside final product of Royal jelly of freeze drying process depended on the technological factors, including: temperature of freeze drying chamber (Z_1, °C), pressure of freeze drying chamber (Z_2, mmHg), time of freeze drying (Z_3, h). These constituent objective functions was determined by the experimental planning method with the quadratic orthogonal experimental matrix ($k = 3$, $n_0 = 4$). In addition, the experimental factors were established by conditions of the technological freeze drying (Dzung, 2012a, b and 2013), they were summarized in Table 2.

The experiments were carried out with all of the factor levels in Table 2 to determine the value of the objective functions that describe relationships between the loss of total protein, carbohydrate and lipid inside final product of Royal jelly after freeze drying, the residual water content of the final product of Royal jelly after freeze drying and technological factors. The results were summarized in Table 3 (Dzung, 2012a).

The mathematical models of regression equations below were obtained after processing the experimental data, calculating the coefficients, testing the significance of the coefficients by the Student test and testing the regression equations for the fitness of the experimental results by Fisher test (Dzung, 2012a, b and 2013) as follow:

- Methematical model of the residual water content of the final product:

$$y_1 = f_2(x_1, x_2, x_3) = 3.216 - 0.271x_1 + 0.116x_2 - 0.643x_3 - 0.111x_1x_3 + 0.139x_1^2 + 0.118x_2^2 + 0.237x_3^2 \quad (8)$$

- Methematical model of the loss of total protein inside the final product:

$$y_2 = f_1(x_1, x_2, x_3) = 2.245 + 0.274x_1 + 0.814x_3 + 0.303x_1^2 + 0.332x_2^2 + 0.311x_3^2 \quad (9)$$

- Methematical model of the loss of carbohydrate inside the final product:

$$y_3 = f_1(x_1, x_2, x_3) = 1.978 + 0.225x_1 + 0.228x_2 + 0.263x_3 + 0.144x_2x_3 + 0.239x_1^2 + 0.162x_2^2 + 0.169x_3^2 \quad (10)$$

Table 3: The experimental data of the loss of total protein, carbohydrate and lipid according to the degree-2 orthogonal experimental matrix with $k = 3$, $n_0 = 4$

N		x_0	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1^2-\lambda$	$x_2^2-\lambda$	$x_3^2-\lambda$	y_1	y_2	y_3	y_4
2^k	1	1	1	1	1	1	1	1	0.333	0.333	0.333	2.94	4.03	3.25	3.06
	2	1	-1	1	1	-1	-1	1	0.333	0.333	0.333	3.58	3.89	2.92	3.47
	3	1	1	-1	1	-1	1	-1	0.333	0.333	0.333	2.59	4.12	2.66	2.96
	4	1	-1	-1	1	1	-1	-1	0.333	0.333	0.333	3.49	3.72	2.12	2.65
	5	1	1	1	-1	1	-1	-1	0.333	0.333	0.333	4.11	2.83	2.58	2.45
	6	1	-1	1	-1	-1	1	-1	0.333	0.333	0.333	4.45	2.63	1.97	2.24
	7	1	1	-1	-1	-1	-1	1	0.333	0.333	0.333	4.19	2.98	2.35	2.14
	8	1	-1	-1	-1	1	1	1	0.333	0.333	0.333	4.51	1.69	1.97	2.04
2k	9	1	1.414	0	0	0	0	0	1.333	-0.667	-0.667	3.08	3.20	3.02	3.16
	10	1	-1.414	0	0	0	0	0	1.333	-0.667	-0.667	3.83	2.32	2.17	2.96
	11	1	0	1.414	0	0	0	0	-0.667	1.333	-0.667	3.80	3.00	2.84	2.65
	12	1	0	-1.414	0	0	0	0	-0.667	1.333	-0.667	3.02	2.63	2.05	2.55
	13	1	0	0	1.414	0	0	0	-0.667	-0.667	1.333	2.57	4.23	2.84	3.27
	14	1	0	0	-1.414	0	0	0	-0.667	-0.667	1.333	4.73	1.32	2.07	2.14
n_0	15	1	0	0	0	0	0	0	-0.667	-0.667	-0.667	3.34	2.09	1.87	1.73
	16	1	0	0	0	0	0	0	-0.667	-0.667	-0.667	3.18	2.32	2.00	1.84
	17	1	0	0	0	0	0	0	-0.667	-0.667	-0.667	3.25	2.23	1.82	1.63
	18	1	0	0	0	0	0	0	-0.667	-0.667	-0.667	3.17	2.52	1.94	1.94

Table 4: Roots of each one-objective optimization problem

Drying material	j	y_{jmin}	$x_1^{j\ opt}$	$x_2^{j\ opt}$	$x_3^{j\ opt}$
Royal jelly	1	2.43	1.414	-0.492	1.414
	2	1.65	-0.452	0.000	-1.309
	3	1.78	-0.533	-0.441	-0.590
	4	1.74	0.000	-0.352	-0.852

- Methematical model of the loss of lipid inside the final product:

$$y_4 = f_2(x_1, x_2, x_3) = 1.934+0.131x_2+0.404x_3 +0.417x_1^2+0.186x_2^2+0.237x_3^2 \quad (11)$$

Testing the fitness of the mathematical models (8), (9), (10) and (9) by Fisher criterion, it shown that these equations were completely compatible for the experimental data. They describe quite suitable relationships between the loss of total protein, carbohydrate and lipid inside final product of Royal jelly, the residual water content of the final product of Royal jelly and technological factors of the freeze drying process. Therefore, it can be used to determine the optimal technological mode of the freeze drying process.

Solving the one-objective optimization problems: Determining the technological mode of freeze drying or the optimal technological factors in order that the loss of total protein (y_2), carbohydrate (y_3) and lipid (y_4) inside final product of Royal jelly of the freeze drying process reach the minimum value. And the residual water content of the final product (y_1) reaches the minimum value but under 4.5%. It is easily obvious that this is the multi-objective optimization problem. However, the one-objective optimization problems must be solved to find the optimal utopian plan (or the utopian plan). If all the one-objective optimization problems have the same root, i.e., The utopian plan exist, the same root of all the one-objective optimization problems will be the optimal root of the utopian plan, this optimal root is the optimal of the multi-objective optimization problem and the optimal technological factors effect on the loss of total protein, carbohydrate and lipid inside final product of Royal jelly of the freeze drying process is found out by this optimal root. On the other hand, if all the one-objective optimization problems don't have the same root, i.e., The utopian plan does not exist, the multi-objective optimization problem will be established and solved to find the optimal Pareto root and the optimal Pareto plan. With this optimal Pareto root will determine the optimal technological factors of the freeze drying process of Royal jelly.

Solving the one-objective optimization problems or these one-objective optimization problems were found to achieve: $y_{1min} = \min f_1(x_1, x_2, x_3) \leq 4.5$; $y_{2min} = \min f_2(x_1, x_2, x_3)$; $y_{3min} = \min f_3(x_1, x_2, x_3)$; $y_{4min} = \min f_4(x_1, x_2, x_3)$; with the identified domain $\Omega_x = \{-1.414 \leq x_1, x_2, x_3 \leq 1.414\}$.

By using the meshing method programmed in Matlab R2008a software, the results of the optimal parameters of every objective function (8), (9), (10) and (11) limited in the experimental domain were summarized in Table 4 (Dzung, 2012a, b).

According to the Table 4, the utopian points were indentified: $f^{UT} = (f_{1min}, f_{2min}, f_{3min}, f_{4min}) = (2.43, 1.65, 1.78, 1.74)$. However, the utopian plan did not exist, because of $x^{jopt} = (x_1^{jopt}, x_2^{jopt}, x_3^{jopt}) \neq x^{kopt} = (x_1^{kopt}, x_2^{kopt}, x_3^{kopt})$ with $j, k = 1 \div 4$, $j \neq k$ (Dzung, 2012b).

Solving the multi-objective optimization problem by the restricted area method to determine the optimal technological factors of the freeze drying process: The experiment established 4 objective functions to describe the residual water content of the final product, the loss of total protein, carbohydrate and lipid in Royal jelly of the freeze drying process which were expressed by 4 regression Eq. (8), (9), (10) and (11), but the roots satisfying all function values (y_{1min}, y_{2min}, y_{3min}, y_{4min}) could not be found. Hence, the idea of the multi-objective optimization problem was to find the optimal Pareto root by the restricted area method for the optimal Pareto effect $y_P^R = (y_{1P}^R, y_{2P}^R, y_{3P}^R, y_{4P}^R)$ closest to the utopian point f^{UT} and the furthest from the restricted area. In addition, all objective functions $y_j = y_j(x) = f_j(x_1, x_2, x_3)$, ($j = 1 \div 4$) must satisfy technological conditions to aim at protecting quality of Royal jelly after the freeze drying, i.e. The loss of total protein, carbohydrate and lipid in Royal jelly after the freeze drying are under 3% (Dzung, 2014):

$$y_1<C_1 = 4.5;\ y_2<C_2 = 3.0;\ y_3<C_3 = 3.0$$
$$y_4<C_4 = 3.0 \qquad (12)$$

From (12), the multi-objective optimization problem was restated as follow (Dzung, 2012a, b): Finding root $x = (x_1^R, x_2^R, x_3^R) \in \Omega_x = \{-1.414 \le x_1, x_2, x_3 \le 1.414\}$ in order that:

$$\begin{cases} y_{jmin} = f_{jmin}\left(x_1^R, x_2^R, x_3^R\right) = \min f_j\left(x_1, x_2, x_3\right) \\ y_j \le C_j;\quad j = 1 \div 4; \\ \forall x \in \Omega_x = \{-1.414 \le x_1, x_2, x_3 \le 1.414\}; \end{cases} \qquad (13)$$

Therefore, the multi-objective optimization problem (13) was solved by the restricted area method with the R*-objective combiation function R*(y_1, y_2, y_3, y_4) = R*(x_1, x_2, x_3) = R*(x). The R*-objective combination function was established as follow (Dzung, 2012b):

$$\begin{cases} R^*(x) = R^*(x_1, x_2, x_3) = \sqrt[4]{\prod_{j=1}^{4} r_j(x)} \\ \Omega_x = \{-1{,}414 \le x_1, x_2, x_3 \le 1{,}414\} \end{cases} \qquad (14)$$

where,

$$r_j(x) = \left(\frac{C_j - y_j(x)}{C_j - y_{jmin}}\right)$$

When,

$$y_j(x) < C_j \qquad (15)$$

$$r_j(x) = 0$$

When,

$$y_j(x) \ge C_j \qquad (16)$$

$$j = 1 \div 4 \qquad (17)$$

From (15), it was obvious that $0 \le r_j(x) \le 1$, when $y_j(x) \to y_{jmin}$ then $r_j(x) \to r_{jmax}(x) = 1$. For this reason, the multi-objective optimization problem (13) can be written as follow: Finding optimal Pareto root $x^R = (x_1^R, x_2^R, x_3^R) \in \Omega_x = \{-1.414 \le x_1, x_2, x_3 \le 1.414\}$ in order that:

$$\begin{cases} R^*_{max}(x) = R^*(x_1^R, x_2^R, x_3^R) = \max\left\{\sqrt[4]{\prod_{j=1}^{4} r_j(x)}\right\} \\ \Omega_x = \{-1{,}414 \le x_1, x_2, x_3 \le 1{,}414\} \end{cases} \qquad (18)$$

Solving the optimization problem (18) by the meshing method programmed in Matlab R2008a software, the result determined as follow:

$$R^*(x)_{max} = \mathrm{Max}\{R^*(x_1, x_2, x_3)\}$$
$$= R^*(x_1^R, x_2^R, x_3^R) = 0.7586$$

where, $x_1^R = -0.129$; $x_2^R = -0.255$; $x_3^R = -0.387$.
Then, transforming into real variables (Dzung, 2012b):

$$Z_1^{opt} = 24.35°C$$
$$Z_2^{opt} = 0.368 \text{ mmHg}$$
$$Z_3^{opt} = 19.225 \text{ h}$$

Substituting x_1^R, x_2^R, x_3^R into these Eq. (8), (9), (10) and (11), the results were obtained as (Dzung, 2012a, b):

$$y_{1P}^R = 3.510;\ y_{2P}^R = 1.968;\ y_{3P}^R = 1.839;\ y_{4P}^R = 1.799$$

where, $x^R = (x_1^R, x_2^R, x_3^R)$ called the optimal Pareto root; $y_P^R = (y_{1P}^R, y_{2P}^R, y_{3P}^R, y_{4P}^R)$ called the optimal Pareto effect.

Through the calculation from the experimental models (8), (9), (10) and (11), the parameters of the freeze drying process which satisfied the maximum R*-Optimal combination criterion were determined as: temperature of freeze drying chamber was Z_1^{opt} = 24.35°C, pressure of freeze drying chamber was Z_2^{opt} = 0.368 mmHg, time of freeze drying was Z_3^{opt} = 19.225 h. The residual water content of the product was y_{1P}^R = 3.510%≤4.5%; the loss of total protein, carbohydrate and lipid were y_{2P}^R = 1.968; y_{3P}^R = 1.839; y_{4P}^R = 1.799. Compared with the experimental results from the Table 3, these results above were suitable and satisfying with the objectives of the problem.

The simulation of the mathematical models of the objective functions (8), (9), (10) and (11) in 2D (x2 = -2, -1, 0, 1, 2) and 3D were performed by the method programmed in Matlab R2008a software. The results obtained were expressed in Fig. 5 to 12 (Dzung, 2012a, b and 2013).

From graph in Fig. 5 to 12, it proved that the simulation of the mathematical models are suitable with

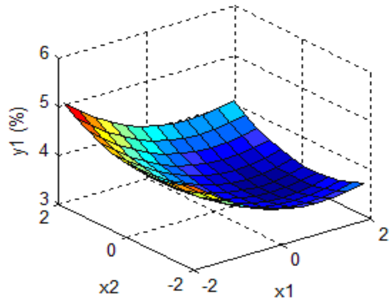

Fig. 5: The residual water content of Royal jelly after freeze drying was expressed in 3D

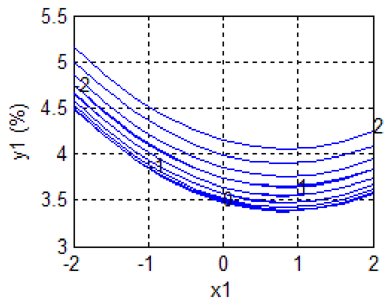

Fig. 6: The residual water content of Royal jelly after freeze drying was expressed in

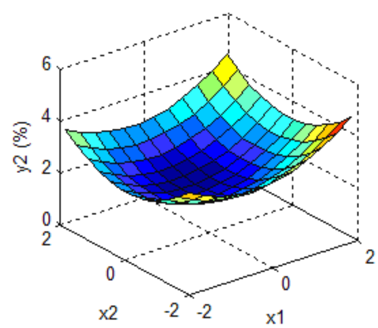

Fig. 7: The loss of total protein in Royal jelly after freeze drying was expressed in 3D

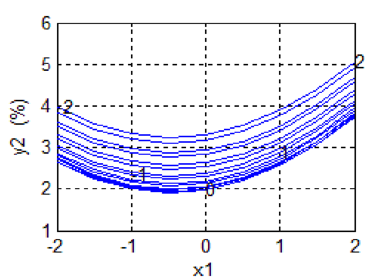

Fig. 8: The loss of total protein in Royal jelly after freeze drying was expressed in 2D

calculated results from experiment. The optimal point of problem is completely suitable with the root of the multi-objective optimization problem.

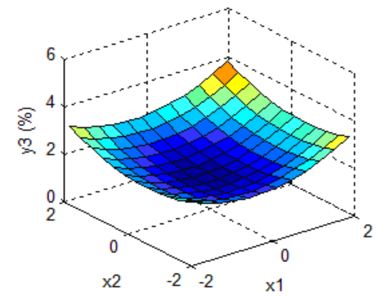

Fig. 9: The loss of carbohydrate in Royal jelly after freeze drying was expressed in 3D

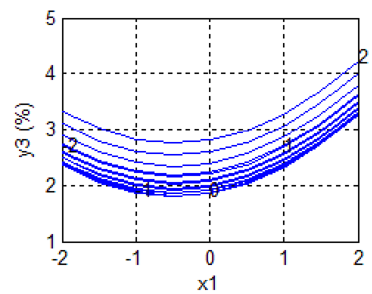

Fig. 10: The loss of carbohydrate in Royal jelly after freeze drying was expressed in 2D

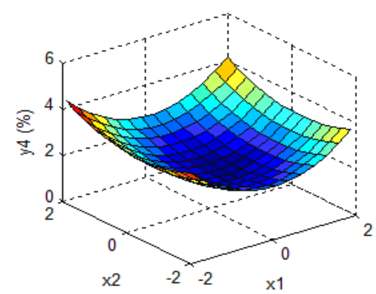

Fig. 11: The loss of lipid in Royal jelly after freeze drying was expressed in 3D

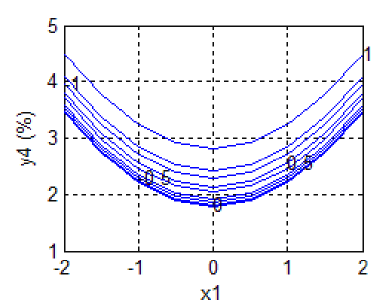

Fig. 12: The loss of lipid in Royal jelly after freeze drying was expressed in 2D

Testing the results of multi-objective optimization problem by experiment: The freeze drying process of Royal jelly was carried out at the optimal root:

temperature of freeze drying chamber of Z_1^{opt} = 24.35°C, pressure of freeze drying chamber of Z_2^{opt} = 0.368 mmHg and time of freeze drying Z_3^{opt} = 19.225 h. The experimental results were determined the residual water content of the final product of Royal jelly and the loss of total protein, carbohydrate and lipid in Royal jelly after the freeze drying as follow: $y_{Ex} = (y_{1Ex} = 3.59\%; y_{2Ex} = 2.042\%; y_{3Ex} = 1.934\%; y_{4Ex} = 1.826\%)$. It was very noticeable that the experimental results bring into comparison with the optimal Pareto effect $y_p^R = (y_{1P}^R = 3.510; y_{2P}^R = 1.968; y_{3P}^R = 1.839; y_{4P}^R = 1.799)$ is completely approximate.

The causes lost total protein, carbohydrate and lipid in Royal jelly after the freeze drying not for temperature of environment freeze drying, pressure of environment freeze drying or time of freeze drying process but for sublimation of ice inside freezing Royal jelly. When ice inside freezing Royal jelly sublimate, ice exchange to moisture, after that moisture is attracted and removed by vacuum pump go out sublimation chamber. Besides, some substance such as protein, lipid, glucid, ash, free fat acids and so on will be attracted in this process. With the loss of total protein, carbohydrate and lipid in Royal jelly after the freeze drying lower than 3% and the residual water content of the final product of Royal jelly under 4.5% was suitable for requirement of the technological freeze drying. Consequently, the optimal Pareto root of multi-objective optimization problem of the freeze drying process of Royal jelly was possibly applied to determine the technological mode of the freeze drying process of Royal jelly in the industrial production (Wytrychowski *et al*., 2013).

CONCLUSION

The above results could be concluded that the mathematical models of Eq. (8), (9) (10) and (11) were built by the experiment and completely suitable for experimental data. They had well described relationships between the loss of total protein, carbohydrate and lipid in Royal jelly, the residual water content of the final product of Royal jelly and technological factors of the freeze drying process of Royal jelly. Therefore, these mathematical models were used to set up the technological mode of the freeze drying process of Royal jelly (Holman, 1986; Gebhart, 1992).

The loss of total protein, carbohydrate and lipid in Royal jelly after the freeze drying are lower than 3%. While the residual water content of the final product of Royal jelly after the freeze drying lower than 4.5%. It proved that final product of freeze drying process of Royal jelly had very good quality. As a result, this product makes material in order to produce pharmaceutical product (Wytrychowski *et al*., 2013).

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