CHAPTER 7

Shelf-life evaluation of isoflavone aglycones powder

7.1 Introduction

The stability of isoflavone aglycones was an essential factor of powder quality. Insufficient stability of product would result in a fast reduction of isoflavone aglycones content. Besides, the shelf-life of the product need to be determined to ensure effective quaility upon usage.

Isoflavone aglycones powder was produced by two processes. One is addition of binding agent or carrier and the other is drying. To produce a powder, carrier or binding agent is needed to increase solid proportion. Based on the theory, the more binding agent is added, the more powder can be produced (Fernández-Pérez *et al.*, 2004). There are many commercially available binding agents such as sodium alginate, gelatin, gum arabic, cellulose, maltodextrin, fructooligosaccharide and inulin (Cano-Chauca *et al.*, 2005; Jaya and Das, 2004). Krishnan *et al.* (2005) explained that some binding agents like gelatin and sodium alginate were more viscous than maltodextrin and better in encapsulating substances. The powder yield and quality depend on drying methods and drying conditions (Wang and Chen, 2006). Spray drying is a common method for commercial powder production. However, this method requires a control of several variables; for example, inlet and outlet temperatures. More importantly, the powder quality may be negatively affected from the high-temperature used during drying (Bhandari *et al.*, 1992). On the contrary, Freeze drying is generally considered the most appropriate method to maintain high powder quality. The freeze dried product will have the least quality change and long stability at room temperature.

In the preliminary experiment, four binding agents including sodium alginate, maltodextrin, inulin and fructooligosaccharide were used to produce isoflavone aglycones powders by freeze drying. The powders were packed in aluminium foil bags. The powders were stored at 35 °C for 120 days. It was found that, the powder encapsulated with sodium alginate showed the best stability of apperance and colour while powders encapsulated with maltodextrin, fructooligosaccharide and inulin were coagulated and brown in colour (Figure 7.1). So, the powder studied this chapter would use sodium alginate as binding agent for isoflavone aglycone powder production.

35 °C, 0 day

35 °C, 120 days

a **b** **c** **d**

Figure 7.1 Isoflavone aglycones powder with various binding agent: (a) sodium alginate, (b) maltodextrin, (c) inulin and (d) fructooligosaccharide

From the previous experiment, isoflavone aglycones in fermented soygerm were extracted by solid-liquid extraction method with 80% ethanol. The crude extract was purified by gradient elution with ethanol in Amberlite XAD-4 column chromatography. In this chapter, the purified solution was added with sodium alginate (binding agent) to produce isoflavone aglycones powder. Finally, the powder was evaluated by measuring their shelf-life.

7.2 Methods

7.2.1 Fermented soygerm and inoculum preparation

Soygerm from soybean variety SJ2 was used to prepare fermented soygerm. Soygerm was mixed with water (1:2 w/w) and autoclaved at 121°C for 15 min. Added with 15% (v/w) of *Bacillus coagulans* PR03 and fermented at 30-35°C for 96 h with 6 L/min air feeding. Inoculum of *B. coagulans* PR03 was prepared in nutrient broth (NB) by transferring cells of *B. coagulans* PR03 to 100 ml NB and incubating at 120 rpm for 18 h before used.

7.2.2 Purified isoflavone aglycones preparation

Five kilograms of fermented soygerm was mixed with 25 L of 80% ethanol and extracted with extraction machine. After that, the mixed solution was filtered with filter cloth and filter paper (Whatman® No. 1) until a clear filtrate was obtained. The ethanol was evaporated from extracted solution by rotary evaporator. The crude extract was purified with Amberlite XAD-4 resins using column chromatography. The process of purification followed the process described in section 6.2.4. Fractions at 40% and 60% ethanol were collected and ethanol was evaporated from purified solution by rotary evaporator. The purified isoflavone aglycones extract was used for isoflavone aglycones powder production.

7.2.3 Isoflavone aglycones powder preparation

Three hundred grams of purified isoflavone aglycones extract and 900 g of 1% sodium alginate solution were mixed with homogenizer (Polytron: PT 10/35, Switzerland) for 5 min. Then, the mixed solution was freeze dried at -40 $^{\circ}$C and <133×10^{-3} mmHg with freeze dryer (Labconco: Freezone 12, USA). After that, freeze dried cake was grinded into a powder and packed in aluminium foil bags (0.30 g per bag).

7.2.4 Shelf-life evaluation of isoflavone aglycones powder

Isoflavone aglycones powder in aluminuim foil bags (from section 7.2.3) were stored in controlled temperature cabinet at 4, 25, 35, 45 and 55 ^{o}C for 120 days. The powder of each condition was randomly sampled every 15 days and analyzed for isoflavone aglycones content (see Appendix A). The total isoflavone aglycones content was used to evaluate shelf-life of the powder. The change of isoflavone aglycones during storage was explained by the order of reaction (Ellis, 1994; Pongsawatmanit, 2009). For food system, the reaction order often found in zero order, first order and second order reaction (Pongsawatmanit, 2009). The mathematic equations of each reaction order were shown below:

Zero order: $C_A/C_0 = -kt$ ----------------- 7.1

First order: $\ln(C_A/C_0) = -kt$ ----------------- 7.2

Second order: $1/(C_A/C_0) = kt$ ----------------- 7.3

When C_A = total isoflavone aglycones content at any time

C_0 = initial total isoflavone aglycones content

k = reaction rate constant

t = time

The equation 7.1, 7.2 and 7.3 follow linear regression model:

$Y = a.X$

When Y = concentration => C_A/C_0, $\ln(C_A/C_0)$ and $1/(C_A/C_0)$

a = slope of regression => k

X = time => t

The method of shelf-life evaluation was divided into 5 steps.

Step 1: Plot graphs between total isoflavone aglycones content and time based on the order of reaction. Zero order graph was plotted between C_A/C_0 and time. First order graph was plotted between $\ln(C_A/C_0)$ and time. And, second order graph was plotted between $1/(C_A/C_0)$ and time.

Step 2: Find the suitable order of reaction using coefficient of determination (R^2). The highest value was selected. The slope of selected regression model would be reaction rate constant at that temperature (k_T).

Step 3: Plot graph between Kelvin temperatures^{-1} (1/T) and reaction rate constant ($\ln k_T$). Find the suitable regression model using coefficient of determination (R^2).

Step 4: Find reaction rate constant (k) at a temperature by substituting the Kelvin temperature value to the variable (T) in regression model from step 3.

Step 5: Calculate shelf-life of the product (t) by using the mathematical model of selected order of reaction in step 2. The calculation was performed by substituting the values of total isoflavone aglycones content at reject point (C_A), initial total isoflavone aglycones content (C_0) and reaction rate constant into the equation.

7.3 Results and discussion

7.3.1 Isoflavone aglycones powder preparation

The isoflavone aglycones powder production was conducted as shown in Figure 7.2. Three hundred grams of purified isoflavone aglycones extract and 900 g of 1% sodium alginate solution were able to produce 36 g of isoflavone aglycones powder. The color of powder was light yellow. The yield of powder was 3% and consisted of 22,778.50 mg total isoflavone aglycones per 100 g powder. The isoflavone aglycones in 100 g or the powder were composed of 13,446.51mg daidzein, 3,310.06 mg genistein and 6,001.92 mg glycitein.

Three hundred grams of purified isoflavone aglycones extract

↓

Mixed with 1% sodium alginate solution 900 g by high speed homogenizer for 5 min

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Freeze dried at -40°C and <133x10^{-3} mmHg with freeze dryer

↓

Grinded freeze dried cake into powder

Figure 7.2 Isoflavone aglycones powder production.

7.3.2 Shelf-life evaluation of isoflavone aglycones powder

The isoflavone aglycones contents in powder during storage at 4, 25, 35, 45 and 55 ^{o}C for 120 days are shown in Table 7.1. It was found that longer storage time and higher temperature decreased isoflavone aglycones content. For instance, total isoflavone aglycones content was reduced from 22,778.50 mg/ 100 g powder to 20,415.73, 20,469.86, 20,158.35, 19,940.36 and 19,026.75 mg/ 100 g powder when stored for 120 days at 4, 25, 35, 45 and 55 °C, respectively.

Table 7.1 Total isoflavone aglycone contents (mg/ 100 g powder) during storage in accelerated temperature at 4, 25, 35, 45 and 55 °C for 120 days

Storage time (days)	Storage temperature (^{o}C)				
	4	25	35	45	55
0	22,778.50±13.91	22,778.50±113.91	22,778.50±113.91	22,778.50±113.91	22,778.50±113.91
15	22,108.29±79.07	21,678.07±172.94	21,605.89±381.04	22,484.77±350.60	21,528.62±218.88
30	21,884.33±791.17	21,297.38±31.08	21,542.13±82.11	22,214.82±260.30	21,116.18±320.73
45	21,405.65±189.41	21,024.93±838.71	21,152.06±485.36	22,017.03±607.31	21,079.73±302.28
60	21,165.44±12.70	20,765.43±625.78	20,732.17±99.03	21,347.30±700.01	20,965.80±443.12
75	20,968.35±315.60	20,735.71±517.61	20,536.46±619.09	21,148.09±582.39	20,354.80±439.48
90	20,909.70±49.14	20,727.84±475.71	20,447.63±229.87	21,045.85±510.85	20,138.53±701.82
105	20,672.82±76.02	20,667.23±535.25	20,216.90±421.65	20,070.79±737.81	19,330.54±386.18
120	20,415.73±128.31	20,496.86±222.09	20,158.35±151.96	19,940.36±143.27	19,026.75±195.98

The shelf-life of isoflavone aglycones powder was evaluated by plotting graphs between total isoflavone aglycones content and time based on the order of reaction. Zero order graph was plotted between C_A/C_0 and time. First order graph was plotted between $\ln(C_A/C_0)$ and time. And, second order graph was plotted between $1/(C_A/C_0)$ and time. The graphs with regression model and determination coefficient (R^2) of each graph order are shown in Figure 7.3, 7.4 and 7.5. The slope of selected regression model would be reaction rate constant at that temperature (k_T) and determination coefficient (R^2) of each graph order were summarized in Table 7.2.

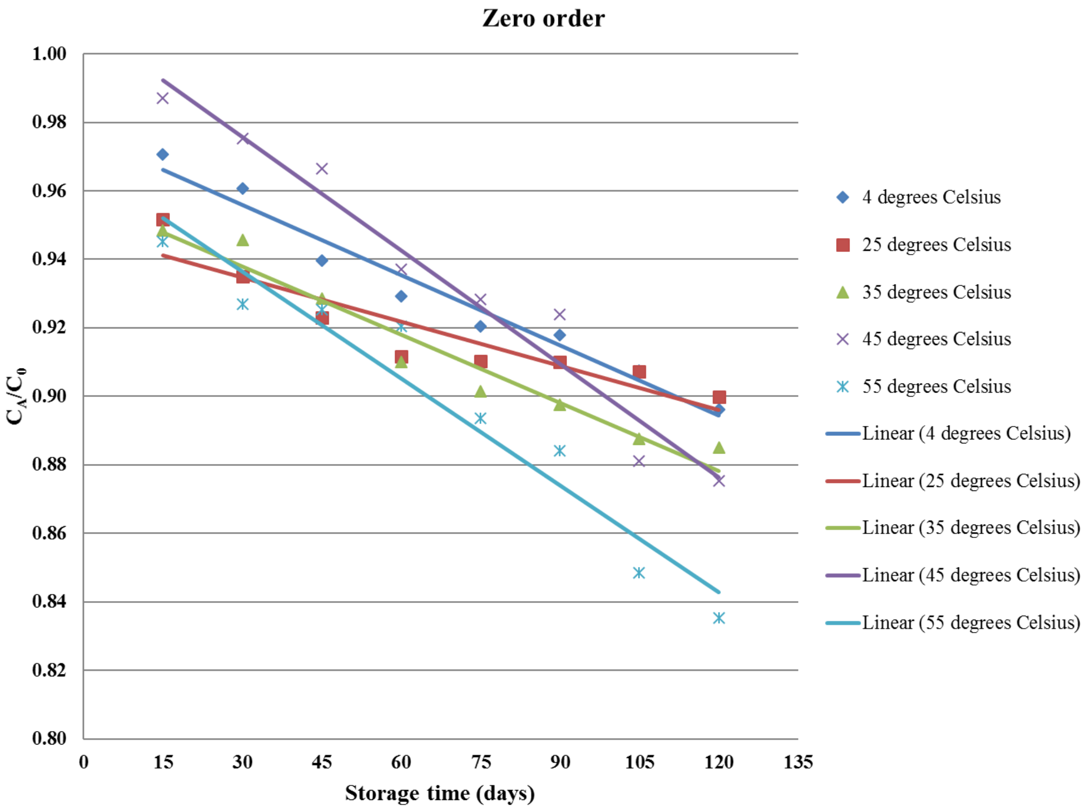

Figure 7.3 Zero order reaction plots of total isofalvone aglycones content during storage at different temperature.

The Linear regression models of zero order reaction at 4, 25, 35, 45 and 55 °C are shown below:

$$Y_{4\,^{o}C} = -6.821 \times 10^{-4}\,(X) + 0.9764 \quad R^2 = 0.9651$$

$$Y_{25\,^{o}C} = -4.302 \times 10^{-4}\,(X) + 0.9476 \quad R^2 = 0.8506$$

$$Y_{35\,^{o}C} = -6.641 \times 10^{-4}\,(X) + 0.9579 \quad R^2 = 0.9517$$

$$Y_{45\,^{o}C} = -1.103 \times 10^{-3}\,(X) + 1.0088 \quad R^2 = 0.9610$$

$$Y_{55\,^{o}C} = -1.041 \times 10^{-3}\,(X) + 0.9677 \quad R^2 = 0.9396$$

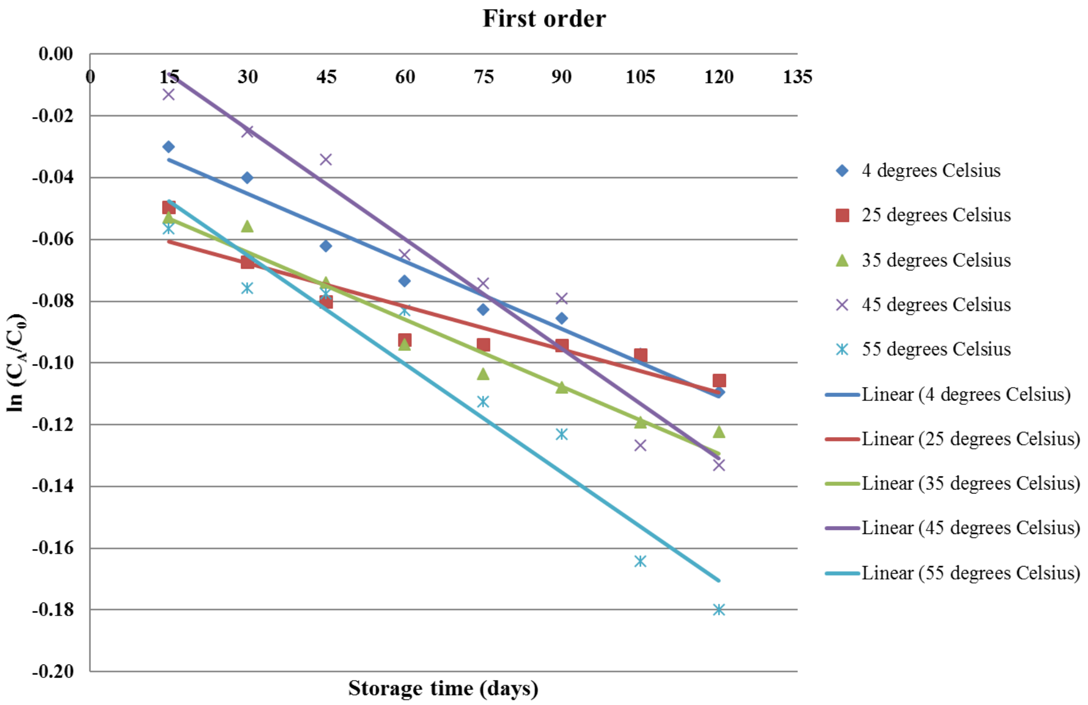

Figure 7.4 First order reaction plots of total isofalvone aglycones content during storage at different temperature.

The Linear regression models of first order reaction at 4, 25, 35, 45 and 55 °C are shown below:

$Y_{4}\,^{o}C = -7.137x10^{-4}\ (X) - 0.0232 \quad R^2 = 0.9681$

$Y_{25}\,^{o}C = -4.655x10^{-4}\ (X) - 0.0536 \quad R^2 = 0.8548$

$Y_{35}\,^{o}C = -7.254x10^{-4}\ (X) - 0.0423 \quad R^2 = 0.9544$

$Y_{45}\,^{o}C = -1.185x10^{-3}\ (X) + 0.0112 \quad R^2 = 0.9572$

$Y_{55}\,^{o}C = -1.169x10^{-3}\ (X) - 0.0301 \quad R^2 = 0.9341$

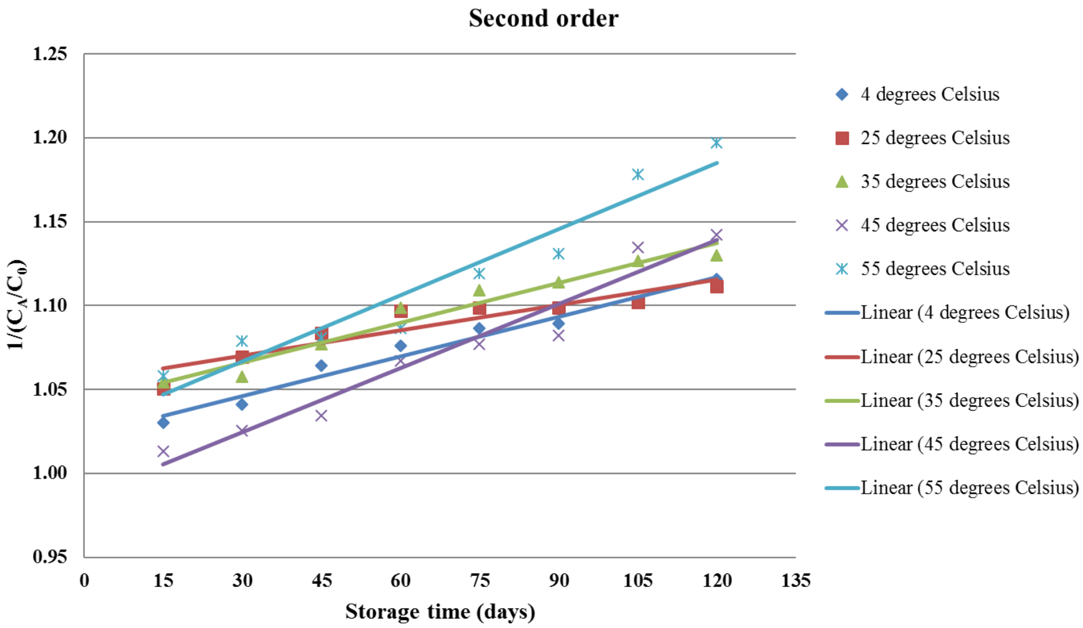

Figure 7.5 Second order reaction plots of total isofalvone aglycones content during storage at different temperature.

The Linear regression models of second order reaction at 4, 25, 35, 45 and 55°C are shown below:

$Y_{4\,^{o}C} = 7.847\text{x}10^{-4}\,(X) + 1.0226$ $R^2 = 0.9708$

$Y_{25\,^{o}C} = 5.040\text{x}10^{-4}\,(X) + 1.0549$ $R^2 = 0.8589$

$Y_{35\,^{o}C} = 7.923\text{x}10^{-4}\,(X) + 1.0424$ $R^2 = 0.9570$

$Y_{45\,^{o}C} = 1.274\text{x}10^{-3}\,(X) + 0.9861$ $R^2 = 0.9529$

$Y_{55\,^{o}C} = 1.314\text{x}10^{-3}\,(X) + 1.0275$ $R^2 = 0.9282$

Table 7.2 Reaction rate constants (k_T) and determination coefficients (R^2) of reaction orders at 4, 25, 35, 45 and 55 °C

Temperature (°C)	Zero order reaction		First order reaction		Second order reaction	
	k_T	**R^2**	**k_T**	**R^2**	**k_T**	**R^2**
4	6.821×10^{-4}	0.9651	7.137×10^{-4}	0.9681	7.847×10^{-4}	0.9708
25	4.302×10^{-4}	0.8506	4.655×10^{-4}	0.8548	5.040×10^{-4}	0.8589
35	6.641×10^{-4}	0.9517	7.254×10^{-4}	0.9544	7.923×10^{-4}	0.9570
45	1.103×10^{-3}	0.9610	1.185×10^{-3}	0.9572	1.274×10^{-3}	0.9529
55	1.041×10^{-3}	0.9396	1.169×10^{-3}	0.9341	1.314×10^{-3}	0.9282

Note: For comparison, the negative symbols of slopes (k_T) in zero order and first order equations, which showed the decreasing trend, were omitted in this Table.

From Table 7.2, the magnitudes of reaction rate constant values (k_T) of zero order and first order reaction were decreased with increasing temperature from 6.821×10^{-4} and 7.137×10^{-4} at 4 °C to 4.302×10^{-4} and 4.655×10^{-4} at 25 °C, respectively. After that, the values were increased along with increasing temperature to the highest values at 45 °C which were 1.103×10^{-3} and 1.185×10^{-3}, respectively. The magnitudes of reaction rate constant value (k_T) of second order reaction showed a similar trend which was decreased from 7.847×10^{-4} at 4 °C to 5.040×10^{-4} at 25 °C, but increased to the highest value at 55 °C with the value of 1.314×10^{-3}. According to the results, an increase in reaction rate constant value (k_T) accelerated isoflavone aglycones degradation, so powder stored at low temperature would show less isoflavone aglycones degradation than the one stored at high temperature. The results were relevant to reports from Kim *et al.* (2005) and Grün *et al.* (2001), which showed that isoflavone aglycones, especially daidzein, were degraded when stored at room temperature or higher. The determination coefficient values (R^2) of all reaction orders at various temperatures were similar. However, the first order reaction is commonly found in various chemical reactions such as vitamin loss, microbial growth, increasing volatile and pharmaceutical degradation (Giannakourou and Taoukis, 2003; Nuin *et al.*, 2008; Tsironi *et al.*, 2009). Therefore, the reaction rate constants (k_T) of first order reaction were used to evaluate shelf-life of isoflavone aglycones powder by plot graph between Kelvin temperatures^{-1} (1/T) and natural logarithm of reaction rate constant (ln k_T) as shown in Table 7.3 and Figure 7.6.

Table 7.3 Temperatures and reaction rate constants of first order reaction at 4, 25, 35, 45 and 55 °C

Storage temperature (T; Kelvin)		**Reaction rate constant**	
T	**1/T**	**k_T**	**ln k_T**
277	6.821×10^{-4}	0.965	7.137×10^{-4}
298	0.003356	4.655×10^{-4}	-7.6724
308	0.003247	7.254×10^{-4}	-7.22879
318	0.003145	1.185×10^{-3}	-6.73801
328	0.003049	1.169×10^{-3}	-6.75161

Note: Storage temperature at 4, 25, 35, 45 and 55 °C equal to 277, 298, 308, 318 and 328 Kelvin respectively.

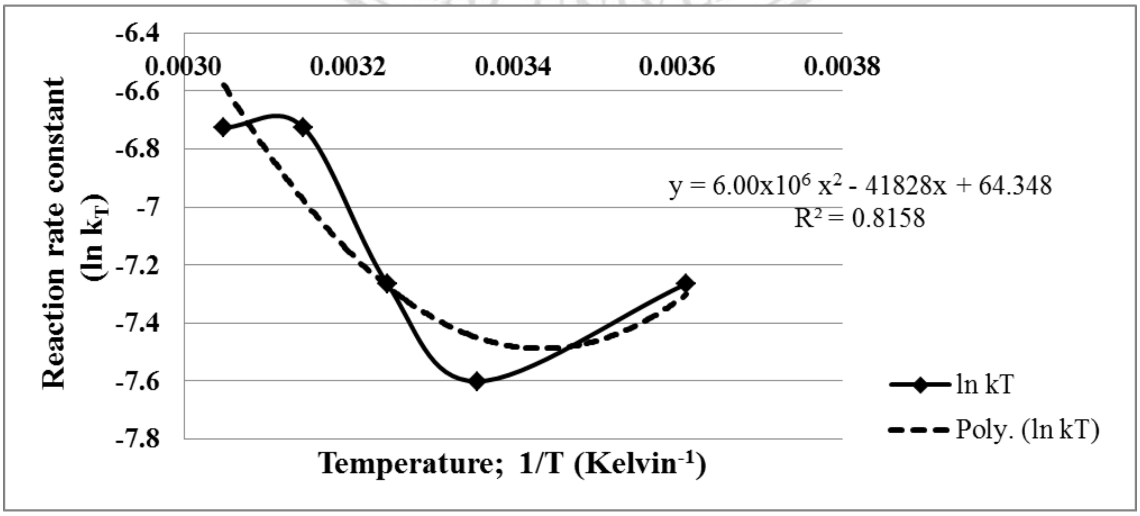

Figure 7.6 Relationship between reaction rate constant and temperature

From Figure 7.6, regression equation of reaction rate constant is:

$$y = 6.00x10^{6} (x)^{2} – 41828 (x) + 64.348 \quad R^2 = 0.8158$$

So $$\ln k = 6.00x10^{6} (1/T)^{2} – 41828 (1/T) + 64.348 \quad \text{----------} \quad 7.4$$

When k_T = reaction rate constant

T = Temperature (Kelvin)

The chosen order of reaction in this experiment was first order reaction (equation 7.2).

$$\ln(C_A/C_0) = - k_T t$$

Rearrange: $$t = - [\ln(C_A/C_0)] \times (1/ k_T) \quad \text{-----------------} \quad 7.5$$

When t = shelf-life of powder

C_A = total isoflavone aglycones content at reject point

C_0 = initial total isoflavone aglycones content

k_T = reaction rate constant

This experiment aimed to find shelf-life of isoflavone aglycones powder at 3 conditions: refrigerating temperature (4 ^{o}C), air conditioning temperature (25 ^{o}C) and room temperature (30, 35 and 40 ^{o}C). Therefore, shelf-lifes of the powder were found at temperature values of 277, 298, 303, 308 and 313 Kelvin.

The reaction rate constant (k) at each temperature was found by substituting the corresponding temperature in Kelvin into equation 7.4. From Table 7.1, the initial isoflavone aglycones content (C_0) was 22,778.50 mg/ 100 g powder while the lowest isoflavone aglycones content was reached after stored at 55 ^{o}C for 120 (at the value of 19,026.75 mg/ 100 g powder). Thus, the lowest isoflavone aglycones content was equal to 83.53% of the initial content. So, the chosen reject point (C_A) of isoflavone aglycones content in this experiment was at 85% of initial content or 19,361.30 mg/ 100 g powder. After that, shelf-life of the powder at various temperatures were found by substituting the reaction rate constants (k), initial isoflavone aglycones content (C_0) and isoflavone aglycones at reject point (C_A) in equation 7.5. As a result, the shelf-life of powder at 4, 25, 30, 35 and 40 °C were 2.09, 2.08, 1.87, 1.63 and 1.38 years, respectively (Table 7.4).

Table 7.4 Shelf- life of isoflavone aglycones powders at 3 conditions: refrigerating (4 °C), air conditioning (25 °C) and room temperatures (30, 35 and 40 °C)

Storage temperature (°C)	**k_T**	**Shelf-life; t**	
		days	**years**
4	2.121 x10^{-4}	766.24	2.09
25	2.139 x10^{-4}	759.81	2.08
30	2.375 x10^{-4}	684.24	1.87
35	2.723 x10^{-4}	597.01	1.63
40	3.210 x10^{-4}	506.28	1.38

7.4 Conclusion

The isoflavone aglycones powder production was conducted with 1:3 ratio of purified isoflavone aglycones extract and 1% sodium alginate solution. The color of powder was light yellow with the yield of 3%. The resulting powder contained 22,778.50 mg/ 100 g powder of isoflavone aglycones, which comprised of daidzein, genistein and glycitein at 13,446.51, 3,310.06 and 6,001.92 mg/ 100 g powder, respectively.

For the shelf-life evaluation with accelerate temperature, shelf-life of the isoflavone aglycones powders at refrigerating temperature (4 °C), air conditioning (25 °C) and room temperature (30, 35 and 40 °C) were 2.09, 2.08, 1.87, 1.63 and 1.38 years, respectively.