Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. **00(0)**S1-S12 (2024) *Supplementary material*

SUPPLEMENTARY MATERIAL TO

Thermochemistry of pyrolyzed rutin and its esters prepared from facile biocatalytic route

NURUL NADIAH ABD RAZAK[1] AND MOHAMAD SUFFIAN MOHAMAD ANNUAR[2]*

[1]School of Bioscience, Faculty of Pharmacy and Biomedical Sciences, MAHSA University, Bandar Saujana Putra, Jenjarom, Selangor, and [2]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

Table SI. Factor and level set up for FFD screening experiment

Factor	Level		
	Lowest	Middle	Highest
Lauric acid (M)	0.05	0.15	0.25
Temperature (ºC)	35	45	55
Enzyme loading (g)	0.03	0.04	0.05

Mathematical basis and derivation for thermochemical analysis

Thermal degradation rate under a linear heating rate is expressed by equation (*S1*):

$$\beta\left(\frac{d\alpha}{dt}\right) = k(T)f(\alpha) \qquad (S1)$$

where β is heating rate (°C min^{-1}), k is the rate constant (min^{-1}), T is temperature (Kelvin), t is time (s) and $f(\alpha)$ is a function describing dependence of reaction rate on the extent of reaction, α. α is degree of conversion expressed by equation (*S2*):

$$\alpha = \frac{M_0 - M_t}{M_0 - M_f} \qquad (S2)$$

where M_0 refers to the initial weight, M_t refers to the sample weight at time t and M_f refers to the final weight.

The temperature (T) dependence of the rate constant is described by the Arrhenius equation. Thus, the rate of heterogenous solid state reaction can be described by:

*Corresponding authors. E-mail: suffian_annuar@um.edu.my

$$\frac{d\alpha}{dt} = Ae^{-\frac{E_a}{RT}}f(\alpha) \quad (S3)$$

where A pre-exponential Arrhenius factor, E_a activation energy, and R universal gas constant. Comparing eqs. (*S1*) and (*S3*) gives:

$$\frac{\delta\alpha}{\delta t} = \frac{A}{\beta}e^{-\frac{E_a}{RT}}f(\alpha) \quad (S4)$$

Integration of Eq. (*S4*) yields

$$\int_0^\alpha \frac{d\alpha}{f(\alpha)} = g(\alpha) = \frac{A}{\beta}\int_{T_0}^{T} e^{-\frac{E_a}{RT}}dT \quad (S5)$$

For the KAS method, the Coats−Redfern approximation of $p(x) \approx e^{-x}/x^2$ is applied for the temperature integration to obtain Eq. (*S6*):

$$g(\alpha) = \frac{A}{\beta}\cdot\frac{RT^2}{E_a}\cdot e^{-\frac{E_a}{RT}} \quad (S6)$$

By taking natural logarithm, Eq. (*S6*) is expressed as Eq. (*S7*):

$$\ln\left(\frac{\beta}{T^2}\right) = \ln\left(\frac{AR}{E_a g(\alpha)}\right) - \frac{E_a}{RT} \quad (S7)$$

The activation energy is obtained from a plot of ln (β/T^2) versus $1/T$ for a given value of conversion, α, where the slope is equal $-E_a/R$.

For the FWO method, Doyle's approximation, which is given as $\log(p(x)) = -2.315-0.4567x$ is applied for temperature integration as shown in Eq. (*S8*):

$$g(\alpha) = \frac{A}{\beta}0.00484e^{-\left(1.052\frac{E_a}{RT}\right)} \quad (S8)$$

By taking natural logarithm, Eq. (*S8*) is expressed as Eq. (*S9*):

$$\ln(\beta) = \ln\left(\frac{AE_a}{Rg(\alpha)}\right) - 5.331 - 1.052\frac{E_a}{RT} \quad (S9)$$

where a plot of ln (β) versus $1/T$ is fitted for E_a calculation from the slope. The enthalpy, ΔH is calculated using Eq. (*S10*):

$$\Delta H = E_a - RT \quad (S10)$$

The pre-exponential factor (A), the Gibbs free energy (ΔG), and the entropy (ΔS) are calculated using Eqs. (*S11*), (*S12*) and (*S13*), respectively:

$$A = \beta\cdot E_a\cdot e^{\left(\frac{E_a}{RT}\right)} \quad (S11)$$

$$\Delta G = E_a + RT\ln\left(\frac{K_B\cdot T}{h\cdot A}\right) \quad (S12)$$

$$\Delta S = \left(\frac{\Delta H-\Delta G}{T}\right) \quad (S13)$$

where K_B is Boltzmann constant and h is Planck constant.

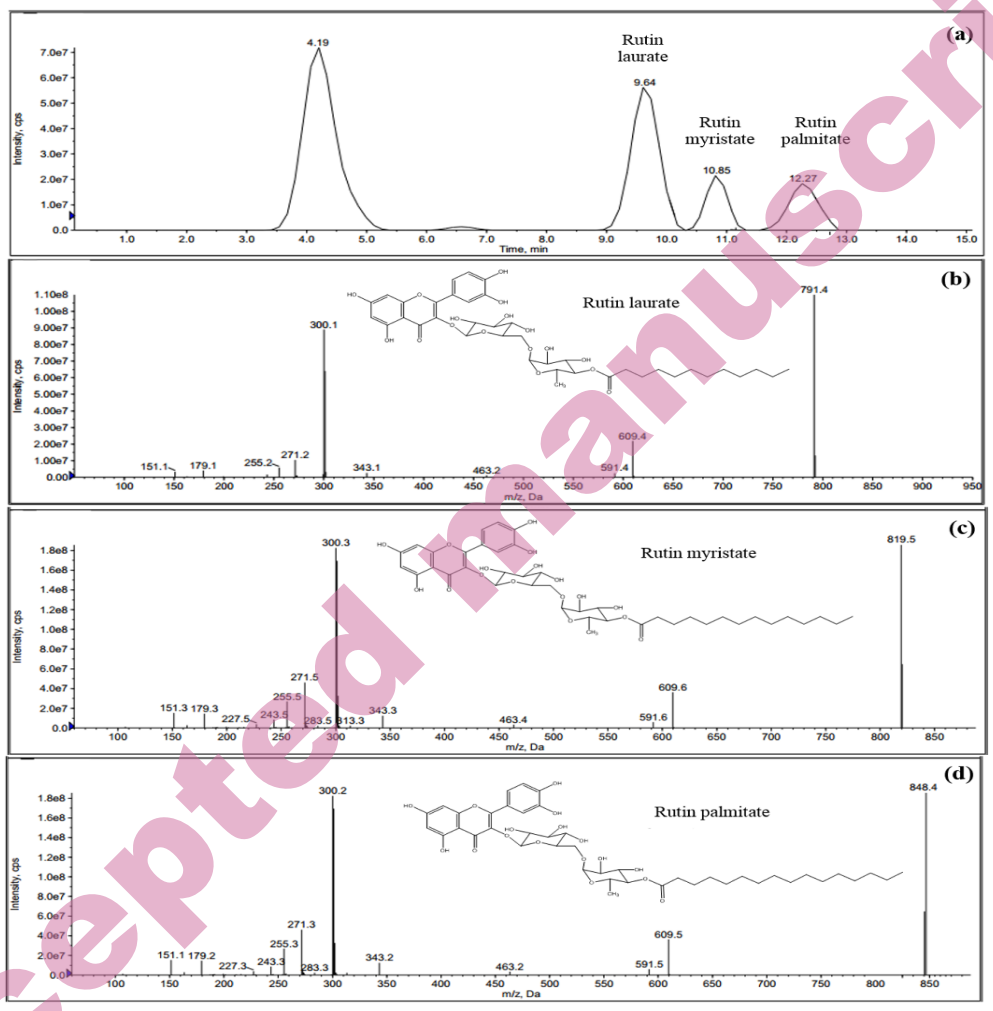

Figure *S1* LC/MS/MS chromatograms for rutin esters: (a) rutin and their ester peaks (b) fragmentation pattern of acylated rutin with lauric acid (c) fragmentation pattern of acylated rutin with myristic acid (d) fragmentation pattern of acylated rutin with palmitic acid.

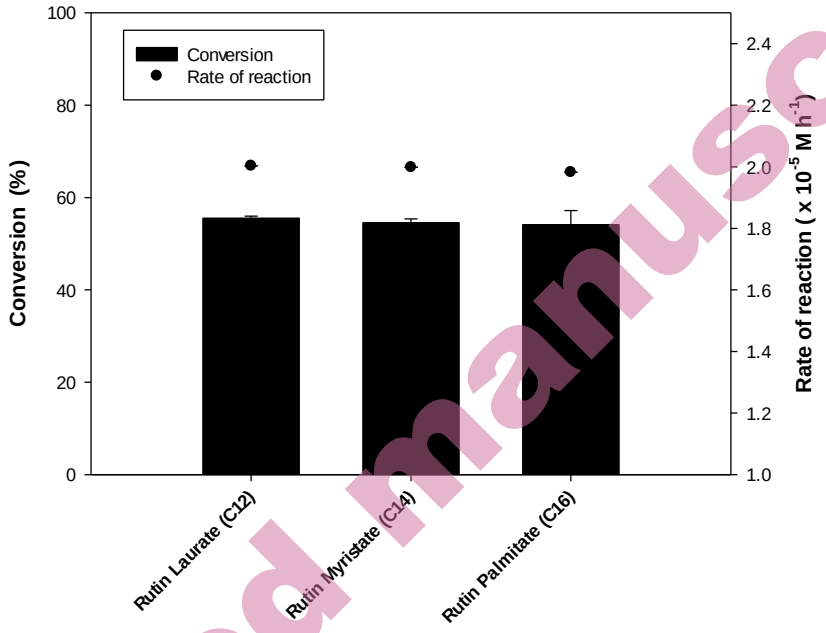

Figure *S2*. Conversion percentage and reaction rate of esterification of rutin with lauric acid, myristic acid and palmitic acid.

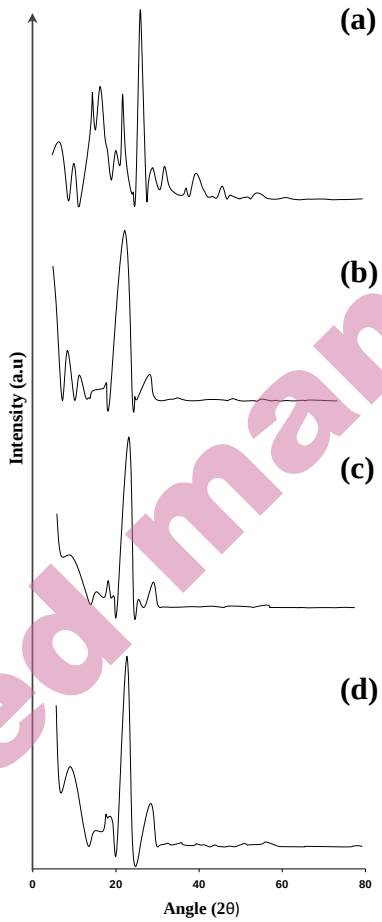

Figure *S3* X-Ray diffractograms of rutin and its corresponding esters (a) rutin (b) rutin laurate (c) rutin myristate (d) rutin palmitate

Table SII. Responses of FFD experimental runs as a function of different combinations of variables and their levels

Run	Lauric acid (M)	Enzyme loading (g)	Temperature (ºC)	Rutin laurate ($\times 10^{-6}$ M)
1	0.05	0.03	35	2.11
2	0.05	0.03	35	2.08
3	0.05	0.03	35	2.19
4	0.05	0.03	55	4.97
5	0.05	0.03	55	4.83
6	0.05	0.03	55	4.66
7	0.05	0.05	35	5.61
8	0.05	0.05	35	5.41
9	0.05	0.05	35	5.63
10	0.05	0.05	55	7.02
11	0.05	0.05	55	7.11
12	0.05	0.05	55	6.73
13	0.15	0.04	45	11.62
14	0.15	0.04	45	11.35
15	0.15	0.04	45	11.3
16	0.25	0.03	35	3.16
17	0.25	0.03	35	3.01
18	0.25	0.03	35	3.13
19	0.25	0.03	55	10.12
20	0.25	0.03	55	9.61
21	0.25	0.03	55	9.89
22	0.25	0.05	35	7.56
23	0.25	0.05	35	7.8
24	0.25	0.05	35	7.61
25	0.25	0.05	55	12.06
26	0.25	0.05	55	12.16
27	0.25	0.05	55	12.12

Analysis of Statistical Experimental Design Results

Analysis of variance (ANOVA) on full order model terms (main-, 2-way and 3-way effects) using Minitab® 15 software reveals good correlation with R^2- and R^2- *adjusted* value of 0.9986 and 0.9980 respectively (Table SIII). The effects of lauric acid molar concentration, enzyme and temperature, and their interaction effects (AB, AC, and BC) are found significant in all cases ($p < 0.05$). The single effect of full interaction (ABC) is also significant owing to all main effects are significant. The *F*-value for the studied factors shows temperature ($F = 3884.52$) variable exerts the strongest influence on the response followed by lauric acid molar concentration ($F = 2929.40$) and enzyme loading ($F = 2523.37$).

Table SIII. ANOVA for the effects of lauric acid molar concentration, temperature and enzyme loading on rutin laurate production

Factor	*DoF*	*Adj SS* ($\times 10^{-7}$)	*Adj MS* ($\times 10^{-7}$)	*F*	*p*
Main factor	3	21.2	7.1	3112.43	0.000
Lauric acid (A)		6.6	6.6	2929.40	0.000
Enzyme (B)		5.7	5.7	2523.37	0.000
Temperature (C)		8.8	8.8	3884.53	0.000
2-way interactions	3	2.5	0.8	360.75	0.000
Lauric acid*Enzyme (AB)		0.1	0.1	25.56	0.000
Lauric acid*Temperature (AC)		1.9	1.9	841.36	0.000
Enzyme*Temperature (BC)		0.5	0.5	215.33	0.000
3-way interactions	1	0.000	0.000	17.41	0.001
Lauric acid*Enzyme*Temperature (ABC)		0.000	0.000	17.41	0.001
Residual Error	18	0.000	0.000		
Total	26	6.6	6.6		

DoF: Degrees of freedom; *Adj SS*: Adjusted sum of squares; *Adj MS*: Adjusted mean of squares; *F*: *F*-statistic; *p*: *p*-statistic

The main effect plots were constructed based on the mean of rutin laurate molar concentration for each factor level. Slope of the plot determines the magnitude of response change with respect to variable change (Figure *S4*). The mean increases from low to high level when the effect of a factor is positive, and *vice versa* when the effect is negative. The effects of all factors are positive as shown by the increase in experimental means when each factor changes from low- to high level. Steeper line indicates a stronger effect of the variable on the experimental response. However, the differences in the relative steepness of the response slopes for lauric acid molar concentration, temperature and enzyme loading variables are not comparable *vis-à-vis* the variable change.

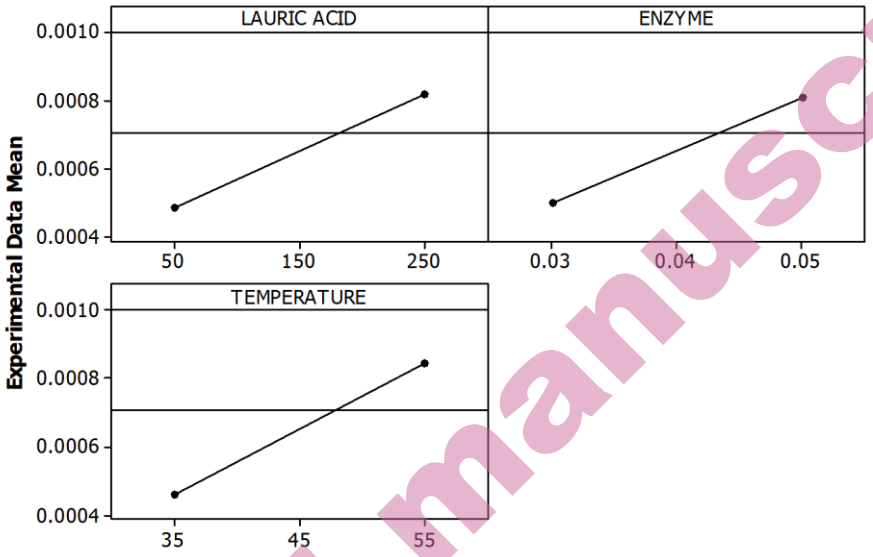

Figure *S4*. Main effect plots representing the effects of lauric acid molar concentration, temperature and enzyme loading on rutin laurate production

Normal probability plot is used to identify substantive departures from normality. One point on the plot is assigned to each effect. The points which are close to a line fitted to the middle group of points represent those estimated factors that do not demonstrate any significant effect on the response variables. The points far away from the line represent the real factor effects. The normal probability plot is shown in Figure *S5*. The effects of main factors (A, B, and C) and their interactions (AC) are considered to be real considering their significant distance away from the vertical line. Point ABC, BC and AB are attributed to random occurrences. Temperature shows the strongest effect since its point lie furthest from the line, also in agreement with the *F*-value in Table SIII. The factor effects decrease in the following order *viz*. C > A > B > AC > BC > AB > ABC.

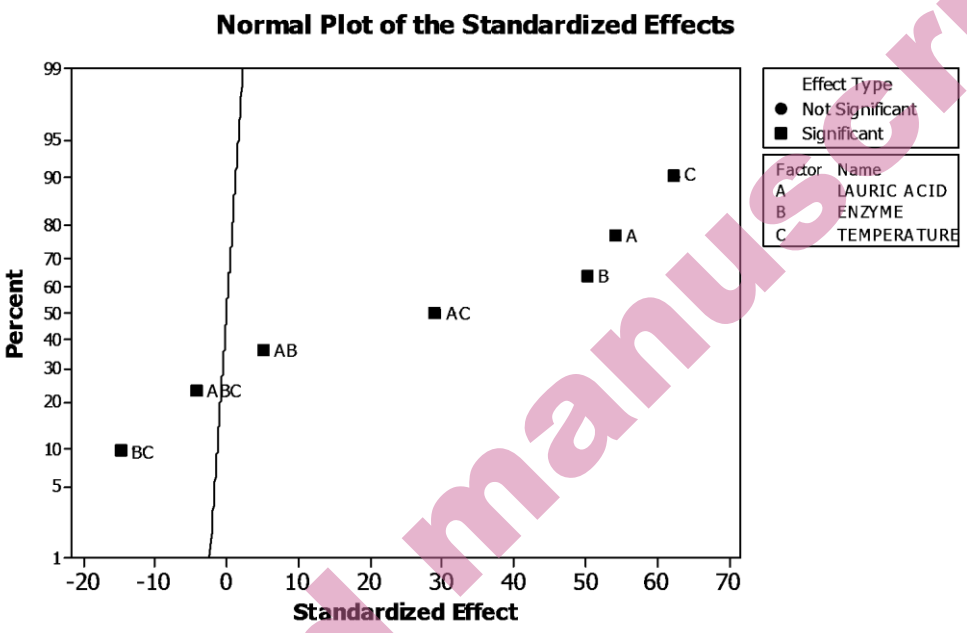

Figure *S5*. Normal plot of the standardized effects of lauric acid molar concentration, temperature and enzyme loading on rutin laurate production

To evaluate the fitted model, residual analysis was performed with standardized residuals (Figure *S6*). Residual values are derived from experimental values deducted by the model fitted values (Residuals = Experimental values – Model fitted values). The normal probability plot of residuals (Figure *S6a*) shows that the residuals are normally distributed as all standardized residual points exhibit approximately a linear pattern. By observing the residuals against fitted (predicted) values (Figure *S6b*), a random pattern of residuals on both sides of standardized zero line indicates low probability of systematic errors in the experiment (± 2). The normal probability assumption is also corroborated by the general bell-shape curve of the histogram constructed for standardized residuals (Figure *S6c*). It also indicates that the error associated with the experimental data are largely due to random occurrences. In addition, experimental order shows negligible effect on the data collected from the absence of clear pattern in standardized residuals versus observation order plot (Figure *S6d*). Generally, experimental points are reasonably scattered without obvious trend; hence supporting a valid assumption of normal distribution for the collected data.

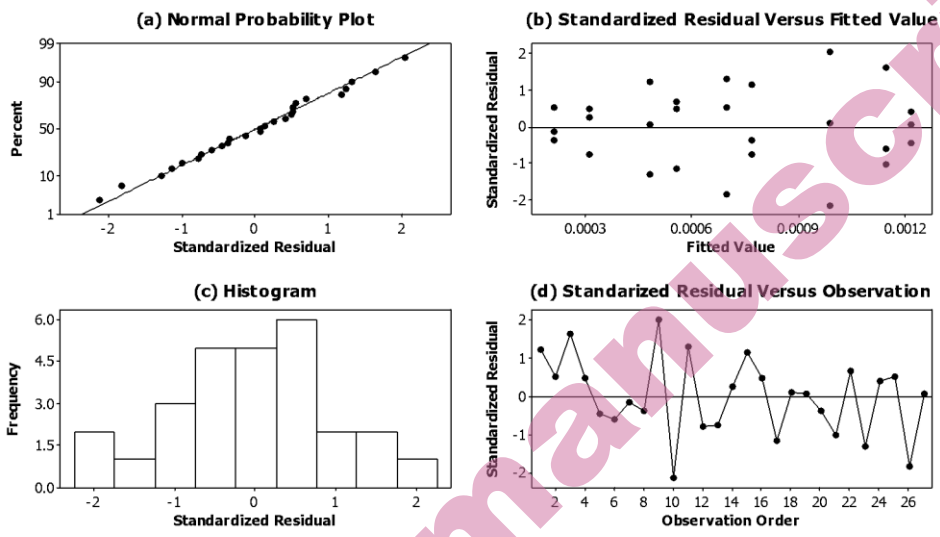

Figure *S6* Residual plots

Based on Anderson-Darling (*AD*) analysis for possible violation of normality assumption, the *AD* value (= 0.552) is found to be insignificant (p = 0.141). Using calculated data from the model, the normal probability plot of residuals shows that the observed values follow closely the theoretical distribution (not shown). It can be concluded that the pattern of residuals follows, in general, a normal distribution; thus, implying the collected data are less likely subjected to non-random errors.

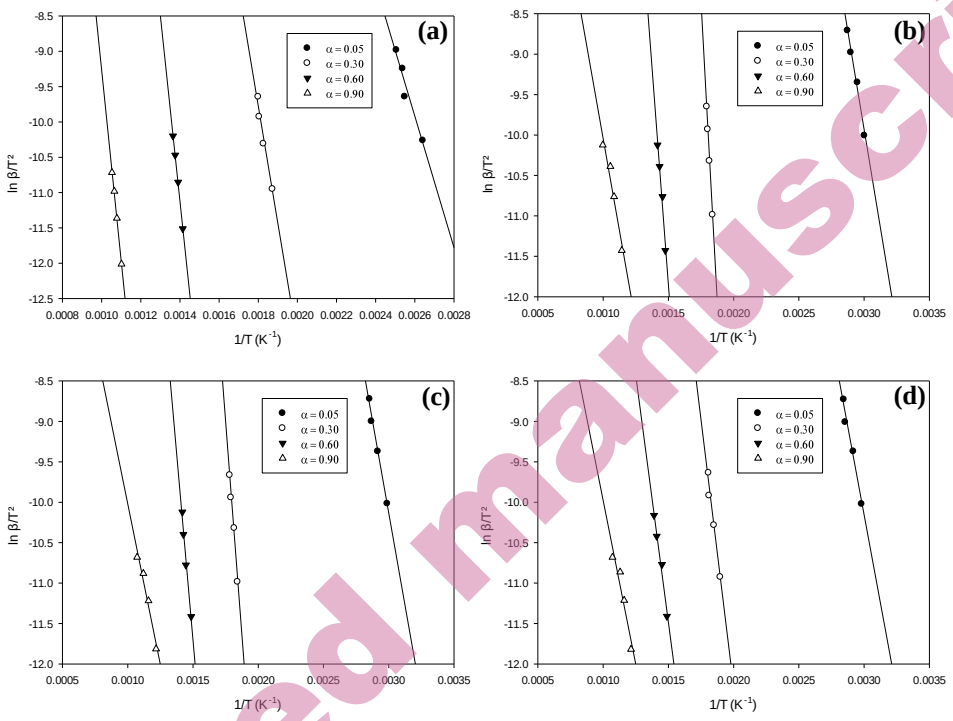

Figure *S7* Regression lines of rutin and its esters based on KAS method at heating rates of 5, 10, 15, 20 °C min^{-1} (a) rutin (b) rutin laurate (c) rutin myristate (d) rutin palmitate *(Standard deviation of the duplicate measurements was <7 %)*

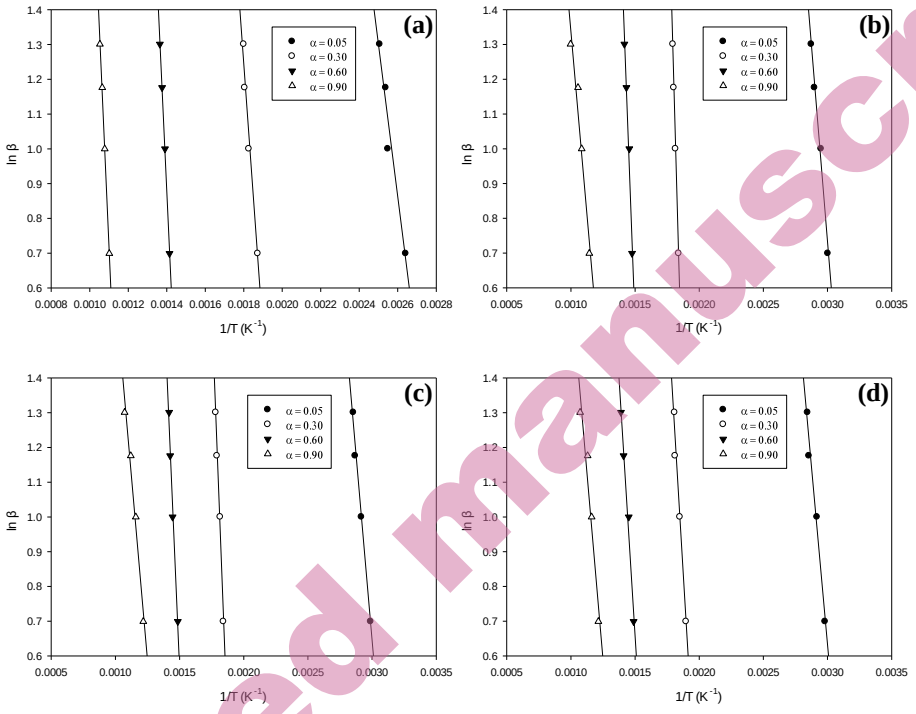

Figure *S8* Regression lines of rutin and its esters based on FWO method at heating rates of 5, 10, 15, 20 °C min^{-1} (a) rutin (b) rutin laurate (c) rutin myristate (d) rutin palmitate (*Standard deviation of the duplicate measurements was <7 %)*