CHEMICAL ENGINEERINGTRANSACTIONS

VOL. 56, 2017

A publication of
AIDIC
The Italian Association
of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Jiří Jaromír Klemeš, Peng Yen Liew, Wai Shin Ho, Jeng Shiun Lim

ISBN978-88-95608-47-1; **ISSN** 2283-9216

DOI: 10.3303/CET1756146

Optimisation of Supercritical CO_2 Extraction of Red Colour from Roselle (Hibiscus Sabdariffa Linn.) Calyces

Zuhaili Idham*[,a], Hasmida Mohd Nasir[a], Mohd Azizi Che Yunus[a,b], Lee Nian Yian[a], Wong Lee Peng[a], Hashim Hassan[b], Siti Hamidah Mohd Setapar[a,b]

[a]Centre of Lipids Engineering and Applied Research, Ibnu Sina Institute for Scientific &Industrial Research, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
[b]Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
zuhaili@cheme.utm.my

Roselle (Hibiscus sabdariffa Linn.) is a local tropical plant widely cultivated in Malaysia. Roselle produces red edible calyces which contain intense red pigments of anthocyanins. Supercritical fluid extraction with carbon dioxide (CO_2) is a particularly suitable isolation method for natural materials and gives an alternative to replace the mass usage of non-polar organic solvents in conventional methods. The advantage of using CO_2 as solvent is that no organic solvent residual inside the extracted sample since CO_2 is in gas form at room temperature. The red colour extract by using CO_2 is easier to be separated by decompression and has high recovery percentage. The objective of this research was to optimise the supercritical carbon dioxide (SC-CO_2) extraction conditions for obtaining the maximum yield of red colour extract. SC-CO_2 extraction of red colour of roselle was performed with ethanol as modifier at the pressures of 8, 10 and 12 MPa, temperatures of 50, 60 and 70 °C while the percentage of modifier flow rates was at 5, 7.5 and 10 %. Full 3^3 factorial design was used to optimise operating conditions for the extraction yield of roselle calyces in SC-CO_2.The other parameters were kept constant, such as total flow rate of CO_2 and modifier (6 mL/min), ratio of modifier (75 % of ethanol), the average particle size used (350 μm) and extraction regime (70 min). The findings revealed that the extraction yield was significantly influenced by three main effects investigated in this study, with p-value smaller than 0.05. The optimum operating conditions obtained for SC-CO_2 extraction of red colour extract were 8.90 MPa, 70 °C, and 9.49 % with predicted percentage yield of 26.73 %.

1. Introduction

The adverse health effect caused by artificial colourant is raising global concern, due to the limited availability of local natural colourant. This issue was due to an effort to replace the artificial colourant because of consumer concern and legislative actions such as the ban of FD&C Red 40 and FD&C Red 2 by the Federal Drug Association (Cevallos-Casals and Cisneros-Zevallos, 2003). Many industries prefer the use of artificial colourant due to the high cost of imported natural colourant. The continuously raising of public concern in this problem has stimulated research interest in local plant, the roselle extract, as the new source of natural colourant and as the alternatives to conventional artificial colourant.

Roselle (Hibiscus sabdariffa) calyces have a characteristic of deep red colour, which is mainly due to the presence of four anthocyanins including dephinidin 3-sambubioside or hybiscin and cyanidin 3-sambubioside as the major pigments, and delphinidin 3-glucoside and cyanidin 3-glucoside as the minor ones (Wong et al., 2002). The most common use of Roselle calyces is for obtaining aromatic infusions of intense red colour that are traditionally consumed either cool or hot. Roselle extracts are used as natural pigments for foods and beverages as well as for preparing jams, jellies, and concentrates possessing red colour with a characteristic sour taste. The roselle extract is known to possess several health benefits such as wound healing (Builders et al., 2013), antihypertensive (Herrera-Arellano et al., 2004), anticholesterol (Chen et al., 2003) and other pharmacological effects.

Please cite this article as: Idham Z., Nasir H.M., Yunus M.A.C., Lee N.Y., Wong L.P., Hassan H., Setapar S.H.M., 2017, Optimization of supercritical co2 extraction of red colour from roselle (hibiscus sabdariffa linn.) calyces, Chemical Engineering Transactions, 56, 871-876 DOI:10.3303/CET1756146

The conventional method used for extraction of roselle anthocyanins is solvent extraction using water and ethanol. However, these traditional extractions with large volume of liquid solvents has some drawbacks, such as long extraction time and large consumption of solvents requiring concentration steps that can result in a loss or degradation of active component (Santos et al., 2013). Anthocyanin is sensitive, unstable and very susceptible to degradation. The major degradation factors are the temperature, the presence of oxygen and light (Junior et al., 2010). Supercritical fluid extraction (SFE) could be an environmentally beneficial alternative to the conventional organic solvent extraction of these compounds. The SFE processes are fast, selective and the products are free of residual solvents. SFE provides relatively clean extracts, free from certain degradation of labile or easily oxidised compounds which may emanate from lengthy exposure to high temperatures and oxygen, which can occur during the traditional extraction techniques (Yunus et al., 2013). The carbon dioxide is readily available, relatively cheap and accepted as a solvent in the food industry. Quantitative extraction of polar analytes, such as anthocyanins, typically requires the addition of an organic modifier (King and Srinivas, 2009).

The employment of optimisation process using response surface methodology is necessary in the development of SC-CO_2 extraction of roselle calyces as it is important to study the best extraction technique, especially the process to be applied in industries which will help to reduce the cost and energy. The aim of this study is to obtain the optimum extraction conditions of the temperature, pressure and modifier percentage of the red colour extract from roselle calyces using SC-CO_2.

2. Materials and Methods

2.1 Materials

Dry roselle calyces were obtained from local market in Perak. The dried calyces were ground and sieved with the average size of 355 µm and were used as the extraction sample. Absolute ethanol (99.86 % purity) was purchased from Hayman (England) and Carbon dioxide (99.9 %) was purchased from KRAS Instrument Sdn Bhd.

2.2 Supercritical carbon dioxide (SC-CO_2) extraction

The schematic diagram of the experimental set up for the SC-CO_2 is shown Figure 1. SC-CO_2 system consisted of CO_2 tank, $CO_2$and modifier pump (Supercritical 24, Lab Alliance), automated back pressure regulator (Jasco BP 2080 Plus Automated BPR), and oven (Memmert). 1.5 ± 0.0010 g of powdered roselle sample was inserted into the extraction vessel and placed in an oven to achieve the operating temperature. Pressure was set at the back pressure regulator and the flow rates of solvent (CO_2 and 75 % ethanol) were adjusted so that the total flow rate for both was 6 mL/min. The extraction conditions analysed in this study was listed in Table 1. The extraction was performed dynamically for 70 min and the extracted collected in the vial was dried in 40 °C oven to maintain the compounds structure. The extraction yield was measured gravimetrically using dried weight of the roselle extract and was expressed in weight percentage.

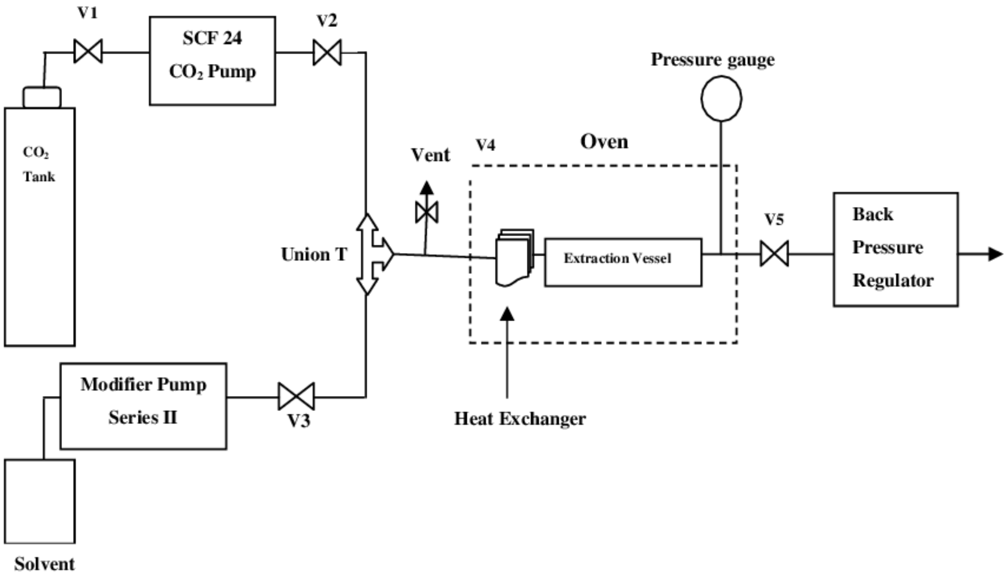

Figure 1: Schematic diagram of the set up for the supercritical fluid extraction

2.3 Experimental design

Optimisation of independent variables of roselle extraction in SC-CO_2 was performed using response surface methodology (RSM). Full 3^3 factorial design consisting 27 experiments were carried out and were run randomised (Table 1). The analysis of variance (ANOVA) was performed to evaluate the significance of the experimental variables studied in this work. Design Expert 7.0 software was used for optimisation purpose (Design-Expert, 2016).

Table 1: Coded and levels of the three independent variables for full factorial design of SFE

Variables	Coded factor	Level		
		Low (-1)	Medium (0)	High (+1)
Pressure (MPa)	X_1	8	10	12
Temperature (°C)	X_2	50	60	70
Modifier percentage (%)	X_3	5.0	7.5	10.0

3. Result and Discussion

3.1 Process optimisation and statistical analysis of experimental design

The full 3^3 factorial design to optimise the SC-CO_2 of total extraction yield is presented in Table 2. The table tabulated the percentage yield of roselle extracts obtained experimentally using SC-CO_2 and its predicted values acquired by Design Expert 7.0 software at different extraction conditions.

Table 2: Observed and predicted experimental yield for different extraction conditions of red colour extract from roselle obtained from Design Expert 7.0 software.

Run	Pressure (MPa)	Temperature (°C)	Modifier Percentage (%)	Coded Level			Yield	
							Observed (%)	Predicted (%)
1	8	50	5.0	-1	-1	-1	8.87	11.17
2	8	50	7.5	-1	-1	0	17.90	17.20
3	8	50	10.0	-1	-1	+1	17.75	17.25
4	8	60	5.0	-1	0	-1	11.99	12.45
5	8	60	7.5	-1	0	0	24.09	19.36
6	8	60	10.0	-1	0	+1	17.57	20.28
7	8	70	5.0	-1	+1	-1	16.80	16.44
8	8	70	7.5	-1	+1	0	26.88	24.23
9	8	70	10.0	-1	+1	+1	22.55	26.03
10	10	50	5.0	0	-1	-1	12.31	10.95
11	10	50	7.5	0	-1	0	17.11	16.94
12	10	50	10.0	0	-1	+1	17.46	16.92
13	10	60	5.0	0	0	-1	10.41	12.25
14	10	60	7.5	0	0	0	16.50	19.11
15	10	60	10.0	0	0	+1	21.53	19.97
16	10	70	5.0	0	+1	-1	14.45	16.25
17	10	70	7.5	0	+1	0	24.38	23.99
18	10	70	10.0	0	+1	+1	27.96	25.73
19	12	50	5.0	+1	-1	-1	5.93	4.97
20	12	50	7.5	+1	-1	0	9.46	10.90
21	12	50	10.0	+1	-1	+1	10.34	10.83
22	12	60	5.0	+1	0	-1	8.56	6.28
23	12	60	7.5	+1	0	0	12.04	13.08
24	12	60	10.0	+1	0	+1	13.97	13.89
25	12	70	5.0	+1	+1	-1	11.73	10.30
26	12	70	7.5	+1	+1	0	14.41	17.98
27	12	70	10.0	+1	+1	+1	21.44	19.66

The results of regression coefficient are summarised in Table 3. The regression analysis (Table 3) showed that the extraction yield was depending on linear term of the pressure, temperature and modifier amount ($p < 0.0001$) and quadratic term of pressure and modifier ($p < 0.05$). However, the interaction between each independent variable does not affecting the yield since $p \geq 0.05$. The negative signs present in the regression coefficient means that the extraction yield will reach the maximum limit at certain independent variables used in this study. The model was used for the construction of three dimensional response surface plots to predict the relationships between independent variables and the dependent variables.

Table 3: Regression coefficients for three independent variables

Factor	Coefficient	Sum of Squares	DF	Mean Square	F-value	p-value
Intercept	19.11	793.96	9	88.22	14.19	< 0.0001
X_1	-3.14	177.51	1	177.51	28.55	< 0.0001
X_2	3.53	223.92	1	223.92	36.01	< 0.0001
X_3	3.86	268.40	1	268.40	43.17	< 0.0001
X_1X_2	0.012	1.742×10^{-3}	1	1.742×10^{-3}	2.801×10^{-4}	0.9868
X_1X_3	-0.056	0.037	1	0.037	5.956×10^{-3}	0.9394
X_2X_3	0.88	9.23	1	9.23	1.48	0.2398
X_1^2	-2.88	49.93	1	49.93	8.03	0.0115
X_2^2	1.36	11.04	1	11.04	1.78	0.2003
X_3^2	-3.00	53.89	1	53.89	8.67	0.0091

The relationship between roselle extraction yield and variables can be presented in the form of Eq(1) where X_1 is the extraction pressure (MPa), X_2 indicates the extraction temperature (°C) and X_3 is the modifier amount (%).

$$Yield\ (\%) = 19.11 - 3.14X_1 + 3.53X_2 + 3.86X_3 + 0.012X_1X_2 - 0.056X_1X_3 + 0.88X_2X_3 - 2.88{X_1}^2 + 1.36{X_2}^2 - 3.00{X_3}^2 \tag{1}$$

Table 4 demonstrates the analysis of variance (ANOVA) for the roselle extraction in SC-CO_2 and ethanol system. The polynomial model generated in the previous equation was significant ($p < 0.05$) and can be used to estimate the extraction performance. Moreover, the correlation coefficient, R^2 of 0.88 shows the accuracy of the model to fit the experimental results. According to Joglekar and May (1987), the R^2 of the model should be 0.80 and above to get satisfactory optimisation data.

Table 4: Analysis of variance (ANOVA) for extraction yield of roselle in SC-CO_2-ethanol system

Source	Sum of squares	DF	Mean squares	F-value	p-value
Model	793.96	9	88.22	14.19	< 0.0001
Residual	105.71	17	6.22		
Total	899.67	26			

To sum up, the optimum operating conditions of roselle extract yield in SC-CO_2obtained using RSM of full 3^3 factorial design were pressure of 8.90 MPa, temperature (70 °C) and modifier percentage (9.49 %), which results in predicted maximum yield of 26.73 % with 0.944 desirability value.

3.2 Analysis of response surface

The best way to express the relationship between the variables and responses within the experimental region is by generating response surface plots (da Porto et al., 2012). Figure 2 illustrates the response surface to demonstrate the effect of pressure, temperature and modifier percentage on the extraction yield. At low to moderate pressure, extraction yield increases with the increase in extraction pressure due to the increase of fluid density, in which the distance between solute-solvent molecule will decrease and increasing the interaction between them (Lang and Wai, 2001). This behaviour boosts the solute solubility and efficiency of extraction process. The negative effect on Roselle extraction when raising the pressure might be due to the solute vapour pressure and analytes polarity. The pattern as shown in Figure 2(a) and 2(b) are explained by quadratic pressure term in the Eq(1).The finding in this study is in agreement to those observed by Maran et al. (2014) where the anthocyanin and phenolic compounds yield from jamun fruits increases with pressure at constant temperature until it reaches plateau and start to decrease with further rise of pressure.

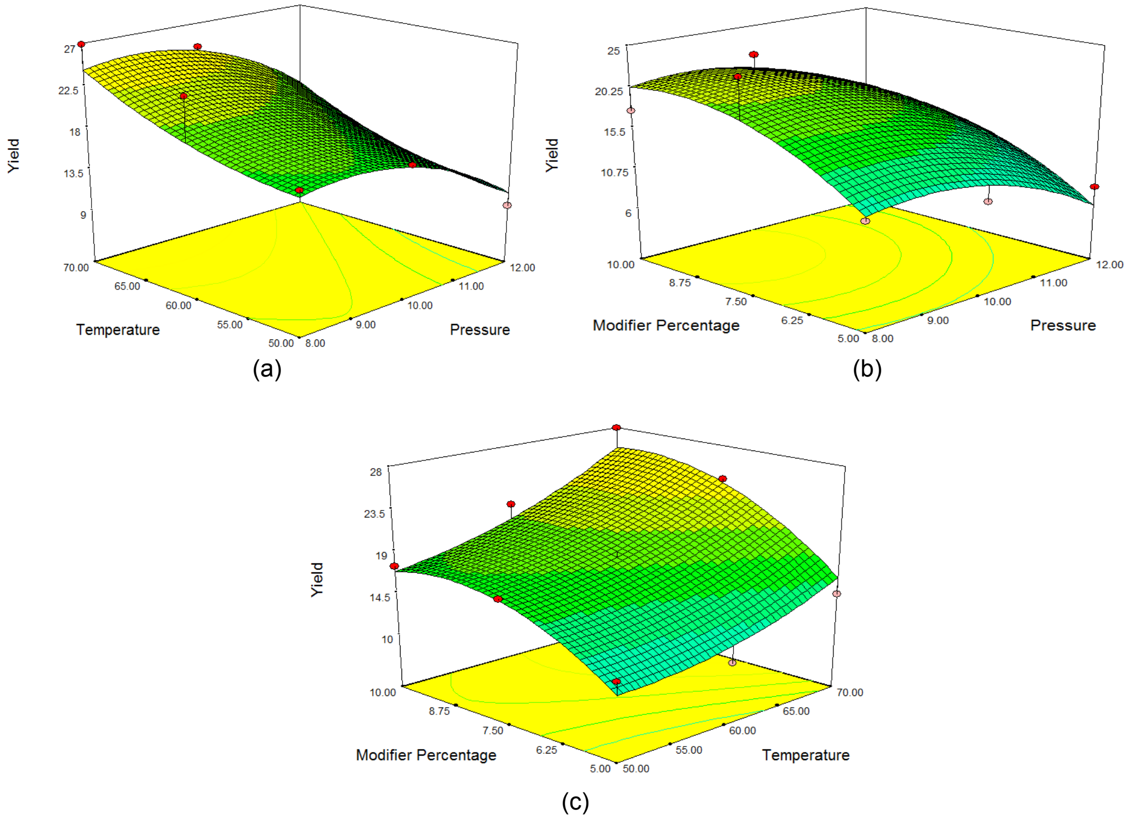

Figure 2: Response surface for the extraction yield as a function of (a) temperature and pressure at constant modifier percentage (7.5 %); (b) pressure and modifier percentage at constant temperature (T = 50 °C); (c) temperature and modifier percentage at constant pressure (P = 10 MPa).

Figure 2(a) and 2(c) show that the temperature is linearly affecting the extraction yield of roselle. The increasing temperature enhanced the solute solubility by rupturing cell wall of the sample which results in higher yields (Machmudah et al., 2006). Even though the solvent density theoretically decreases as temperature increase, but the changes is smaller compared to the rise of solute vapour pressure. The volatility of the solute plays a dominant factor in increasing the mass transfer rate during the extraction process. Silva et al. (2007) showed similar trend during the extraction of phenolic compounds from Inga edulis leaves. It has been reported that the extraction of anthocyanins from grapes is enhanced by increasing temperature (Vatai et al., 2009). Cardoso et al. (2003) reported that in SFE with CO_2 + ethanol, the use of low pressure (100 bar) and high temperature (65 °C) was desirable for the extraction of anthocyanins.

Given the non-polar property of CO_2, the addition of modifier helps the solvent to attract more polar solute molecules through intermolecular interaction and the hydrogen bonding formed between them. The increment of modifier percentage in increasing the total solute extracted are reflected from Figure 2(b) and(c) and positive effect in regression model has been discussed previously. Higher volume of modifier leads to better penetration of solvent to the solid matrix and lead to an increase in the availability of the analytes to be bind with the solvent. After the extraction yield reaching the maximum value, the different responses can be observed as indicated by the negative quadratic effect of the modifier amount. The data was comparable with the results presented by Cortés-Rojas et al. (2011) for extracting Biden spilosa L. using dynamic maceration.

4. Conclusions

SC-CO_2 is a promising technology in the extraction of red colour extract from roselle calyces by using the modifier such as ethanol. The optimisation of extraction process for red colour extract of roselle calyces with SC-CO_2 using a full 3^3 factorial design revealed that the extraction yield is dependent on the pressure, temperature and modifier percentage. The optimum operating condition for the extraction is 8.90 MPa, 70 °C and 9.49 %, giving predicted maximum yield of 26.73 %.

Acknowledgments

The authors gratefully acknowledge the Centre of Lipids Engineering and Applied Research (CLEAR), Universiti Teknologi Malaysia for the instruments provided and also to Ministry of Higher Education (MOHE) provided the grant of Research University Grant (Q.J130000.2609.11J55) for the financial support.

References

Builders P.F, Kabele-Toge B., Builders M., Chindo B.A., Anwunobi P.A., Isimi Y.C., 2013, Wound healing potential of formulated extract from Hibiscus sabdariffa calyx, Indian J. Pharm. Sci. 75 (1), 45-52.

Cardoso L.C., Serrano C.M., Quintero E.T., López C.P., Antezana R.M., de la Ossa E.J.M., 2013, High pressure extraction of antioxidants from Solanum stenotomun peel, Molecules 18, 3137-3151

Cevallos-casals B.A., Cisneros-zevallos L., 2003, Stoichiometric and kinetic studies of phenolic antioxidants from Andean purple corn and red-fleshed sweet potato, J. Agric. Food Chem. 51, 3313– 3319.

Chen C.C., Hsu J.D., Wang S.F., Chiang H.C., Yang M.Y., Kao E.S., Ho Y.C., Wang C, 2003, Hibiscus sabdariffa extract inhibits the development of atherosclerosis in cholesterol-fed rabbits, J. Agric. Food Chem. 51 (18), 5472-5477.

Cortés-Rojas D.F., Souza C.R.F., Oliveira W.P.,2011, Optimisation of the extraction of phenolic compounds and antioxidant activity from aerial parts of Biden spilosa L. using response surface methodology, Int. J. Food Sci. Tech. 46, 2420-2427.

Da Porto C., Voinovich D., Decorti D., Natolino A., 2012, Response surface optimization of hemp seed (Cannabis sativa L.) oil yield and oxidation stability by supercritical carbon dioxide extraction, J. Supercrit. Fluids 68, 45-51.

Design Expert 7.0, Stat-Ease Inc., Minneapolis, United States.

Herrera-Arellano A., Flores-Romero S., Chavez-Soto M.A., Tortoriello J.,2004, Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension; a controlled and randomized clinical trial, Phytomedicine 11, 375-382.

Joglekar A.M., May A.T., 1987, Product excellence through design of experiments, Cereal Foods World 32, 857–868.

Junior M.R.M., Leite A.V., Dragano N.R.V., 2010, Supercritical fluid extraction and stabilization of phenolic compounds from natural sources – review (supercritical extraction and stabilization of phenolic compounds), The Open Chemical Engineering Journal 4, 51-60.

King J.W., Srinivas K., 2009, Multiple unit processing using sub and supercritical fluids, J. Supercrit. Fluids 47, 598–610.

Lang Q. Y., Wai C.M., 2001, Supercritical fluid extraction in herbal and natural product studies – a practical review, Talanta 53, 771-782.

Machmudah S., Shotipruk A., Goto M., Sasaki M., Hirose T., 2006, Extraction of astaxanthin from Haematococcus pluvialis using supercritical CO_2 and ethanol as entrainer, Ind. Eng. Chem. Res. 45, 3652-3657.

Maran J.P., Priya B., Munikandan S., 2014, Modeling and optimization of supercritical fluid extraction of anthocyanin and phenolic compounds from Syzygium cumini fruit pulp, J. Food Sci. Tech. 51 (9), 1938-1946.

Santos W.J. D., Silva E.A., Taranto O.P., 2013, Supercritical fluid extraction from mango (Mangifera indica L.) leaves: experiments and modeling, Chemical Engineering Transactions 32, 2005-2010.

Silva E.M., Rogez H., Larondelle Y., 2007, Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology, Sep. Purif. Technol. 55, 381-387.

Vatai, T., Skerget, M., Knez, Z., 2009, Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide, J. Food Eng. 90, 246−254.

Wong P.K., Yusof S., Ghazali H.M., Man Y.B., 2002, Physico-chemical characteristics of roselle (Hibiscus sabdariffa L.), J. Nutr. Food Sci. 32, 68-73.

Yunus C.A.M., Arsad N.H., Zhari S., Idham Z., Mohd-Setapar S.H., Mustapha A.N., 2013, effect of supercritical carbon dioxide condition on oil yield and solubility of Pithecellobium jiringan (jack) prain seeds, Jurnal Teknologi (Science & Engineering) 60, 45-50.