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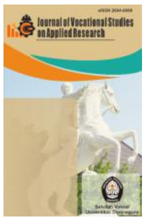

Research Article

Optimization Extraction of Anthocyanin from Roselle as a Natural Colorant using Natural Deep Eutectic Solvent with Microwave Assisted Extraction Method

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Abstract

Roselle (*Hibiscus sabdariffa* L.) contains high levels of anthocyanins with significant antioxidant properties. However, conventional extraction methods often result in low efficiency and degradation of bioactive compounds. This study aims to optimize the extraction of anthocyanins from roselle using Microwave Assisted Extraction (MAE) with Natural Deep Eutectic Solvent (NADES) combination of sodium acetate and glycerin then examine the effect of optimum anthocyanin content. The variables applied were microwave power (200, 400, 600 Watt) and extraction time (4, 6, 8 min), optimized using Response Surface Methodology (RSM) with 12 experimental runs. Analysis included antioxidant content (anthocyanins, vitamin C, tannins, and total phenolics) and stability tests (density and viscosity). The RSM results have shown that the optimization value of anthocyanin content is 0.3217%, which will be achieved at an extraction time of 6.02 min, and microwave assisted extraction power at 323.77 Watt. The result of the optimum anthocyanin variable shows anthocyanin content is 0.3072% confirming the model's accuracy. MAE combined with NADES provides an efficient and environmentally friendly method for extracting natural colorants with high antioxidant activity from roselle.

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1. Introduction

Roselle (*Hibiscus sabdariffa* L.) has a high antioxidant activity of 54.1% and contains 1.48 g of anthocyanin per 100 g of dry petals. Anthocyanin is a type of color pigment, classified as a flavonoid compound, that acts as an antioxidant to inhibit the oxidation of free radicals. In addition, rosella flower petals are rich in vitamin C (260-280 mg/100 g), vitamin D, B1, B2, calcium, polysaccharides, omega-3, iron, beta-carotene, amino acids, riboflavin and niacin. In food processing, colorants are often added, which can be either natural or synthetic (Sari et al., 2020). Synthetic colorants are harmful to health as they are toxic and carcinogenic, containing heavy metals that are difficult to break down in the digestive system and can accumulate in the body (Lellis et al., 2020). Therefore, safe natural colorants should be used, such as rosella flower extract, which contains anthocyanin pigments that can provide color to food products. The anthocyanin pigments from rosella flower petals can be extracted using extraction techniques. Choosing the right extraction method is also crucial to prevent the degradation of antioxidant components.

Microwave Assisted Extraction (MAE) is a modern extraction method that is faster and more efficient compared to conventional methods. MAE uses microwave heating that directly targets the molecules, resulting in better product quality and quantity with reduced solvent usage and shorter extraction time at high temperatures (Yuan et al., 2018). The heating mechanism of microwaves involves dipole rotation or ion conduction, generating heat through interactions with polar molecules or ions. The advantages of MAE include minimizing the use of organic solvents, speeding up the extraction process, and being environmentally friendly.

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Traditional extraction methods often employ organic solvents such as methanol, ethanol, and acetone, which may pose environmental and safety concerns. Conventional water extraction, while safe, typically yields lower extraction efficiency and longer processing times. In contrast, modern extraction techniques have emerged to address these limitations while maintaining or improving extraction efficiency.

Natural Deep Eutectic Solvent (NADES) is a type of solvent derived from natural substances that consist of compounds functioning as both hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD). The most common NADES are based on the hydrogen bond acceptor compound choline chloride (ChCl), carboxylic acids, and other hydrogen bond donors such as sugars, citric acid, succinic acid, amino acids, and glycerol, which are commonly found in living organisms' cells (Bajkacz & Adamek, 2018). NADES has a high ability to extract both polar and non-polar compounds, as well as several other secondary metabolites. NADES also offers other advantages, such as being easy to produce, non-toxic, and biodegradable (Cannavacciuolo et al., 2023).

NADES with the combination of sodium acetate and glycerin involves an interaction between the hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), which causes the compound's charges to become delocalized, thereby reducing the lattice energy between the two compounds. As a result, the melting point decreases, allowing them to mix perfectly into a solution that can be used as an environmentally friendly solvent (Sun et al., 2023).

2. Materials and Method

2.1 *Materials*

Roselle (*Hibiscus sabdariffa* L.) powder that used in this research were obtained from Semarang, Central Java, Indonesia, NADES (glycerin and sodium acetate) were analytical grade, distilled water, pH 1 and pH 4.5 buffer solution, Folin-Ciocalteau, ammonium molybdate and sodium carbonate.

2.2 *Extraction and Antioxidant Activity*

The Preparation of NADES by mixing sodium acetate and glycerin with ratio 1:20 (w/v) using a magnetic stirrer (Heidolph, Schwabach, Germany) for 1 h at 60 °C. Then, involves mixing powdered roselle with NADES, which is then placed into the MAE for extraction using the specified MAE power and time. After that, anthocyanin, vitamin C, tannin, and phenolic content are tested using UV-Vis spectrophotometry, and the density and viscosity are also measured.

2.3 *Determination of Total Anthocyanin Content*

Anthocyanin content in roselle is determined using the pH differential method. pH 1 buffer solution is prepared using HCl, while a pH 4.5 buffer solution is prepared with NaOH. 1 mL sample is dissolved in 10 mL of each buffer solution at pH 1 and pH 4.5. The absorbance of the sample is then measured using a UV-Vis spectrophotometer at wavelengths of 510 nm and 700 nm (Zhang et al., 2020). The absorbance of the dissolved sample (A) is determined using Equation (1).

$$A = \left(A_{510} - A_{700}\right)_{pH\ 1.0} - \left(A_{510} - A_{700}\right)_{pH\ 4.5} \tag{1}$$

The anthocyanin content in the sample is calculated using Equation (2).

$$Anthocyanin\left(\frac{mg}{L}\right) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times L} \tag{2}$$

2.4 *Determination of Vitamin C Content*

The vitamin C content of the extract is measured by diluting 3 mL of concentrated extract into a 10 mL volumetric flask. The diluted extract is then pipetted with 1 mL into a 10 mL volumetric flask, followed by the addition of 4 mL of 5% H_2SO_4 and limited with 5% ammonium molybdate. The mixture is incubated for 30 min and measured at the maximum wavelength of 681 nm (Syahputra et al., 2023). The absorbance measurement of the sample is then used to determine the vitamin C content using a calibration curve with the linear regression equation y = 0.0398x – 0.0421 (Rosalinda et al., 2023). The vitamin C content is then calculated using Equation (3).

$$Vitamin\ C\ content\left(\frac{mg}{100g}\right) = \frac{C \times V \times Fp}{W} \tag{3}$$

2.5 *Determination of Tannin Content*

The roselle extract solution obtained is then pipetted with 5-10 drops and added to 1 mL of Folin-Ciocalteu reagent, then shaken and left for 5 min. To this solution, 2 mL of 15% Na_2CO_3 solution is added, shaken homogenously, and left for another 5 min. Then, distilled water is added to make the volume up to 10 mL, and it is left to stabilize for the time range obtained. The absorbance of the extract solution is observed at the maximum wavelength of 765 nm. The absorbance measurement of the sample is then used to determine the tannin content using a standard gallic acid curve with the linear regression equation y = 0.3866x (Chatepa et al., 2023). The tannin content is then calculated using Equation (4).

$$Total\ Tannin\ content\left(\frac{mgTAE}{g}\right) = \frac{C \times V \times FP}{g} \tag{4}$$

2.6 Determination of Total Phenolic Content

Pipette 0.5 mL of the red roselle flower extract (*Hibiscus sabdariffa* L.) and add 0.4 mL of Folin-Ciocalteu reagent (a mixture of phosphomolybdate and phosphotungstate). Shake the mixture and allow it to sit for 4-8 min. Add 4.0 mL of a 7% Na_2CO_3 solution and shake until the solution is homogeneous. Add distilled water to bring the total volume to 10 mL and let the mixture stand for 2 h at room temperature. Measure the absorbance at the maximum absorption wavelength of 750 nm, which will result in the formation of a blue complex. The absorbance measurement of the sample is then used to determine the total phenolic content using a standard curve with the linear regression equation $y = 0.2506x - 0.0094$ (Hapsari et al., 2021). The total phenolic content is then calculated using Equation (5).

$$\text{Total phenolic content}\left(\frac{mgGAE}{g}\right)=\frac{C\times V\times FP}{g} \quad (5)$$

3.1 Research Design

The research was designed using Response Surface Methodology (RSM) with 12 runs and independent variables of the ratio of MAE power, and extraction time, as shown in (Table 1). RSM is a mathematical and statistical method that is useful for modeling and analyzing complex problems. Where the response will be influenced by many variables and the results will optimize the response with graphical visualization that will show the interaction pattern between each factor on a response.

Table 1. Summary for variable

Factor	Factor Name	Low Value	Center Value	High Value
1	MAE Power (Watt)	200	400	600
2	Time (min)	4	6	8

3. Results and Discussion

3.2 Antioxidant Analysis

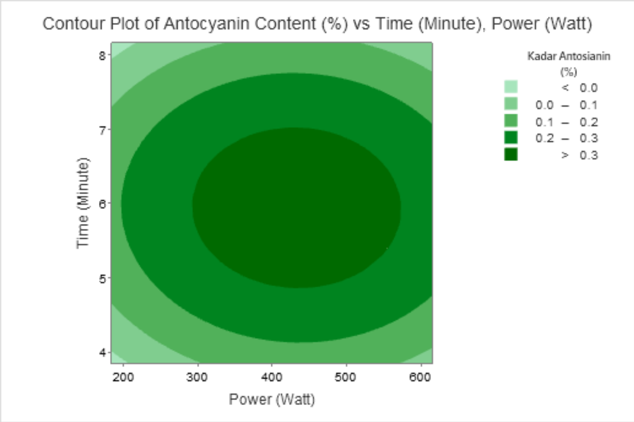
Roselle antioxidant analysis (Table 2) showed the highest anthocyanin value during the run with a microwave power of 400 Watt and an extraction time of 6 min, with an average value of 0.379%. The highest vitamin C content (2.139%) was obtained during the run with a microwave power of 200 Watt and an extraction time of 4 min. The highest tannin content (0.121%) was obtained during the run with a microwave power of 400 Watt and an extraction time of 8.15 min. And, the highest total phenolic content (0.215%) during the run with a microwave power of 184.38 Watt and an extraction time of 5 min. The use of NADES can improve extraction efficiency and antioxidant content compared to conventional solvents because NADES has a better ability to dissolve bioactive compounds (Imtiaz et al., 2024).

NADES with a mixture of sodium acetate and glycerol in rosella extraction has a high ability to extract polar and non-polar compounds and several other secondary metabolites. Microwave power also affected the antioxidant content of roselle extraction. Higher microwave power can increase the heating rate, which can enhance the extraction rate of active compounds from rosella flowers into the solvent. This rapid heating can also break down the plant cell walls, making the bioactive compounds easier to extract (Nguyen, 2020). However, if the microwave power is too high, it may lead to the degradation of heat-sensitive compounds, such as certain flavonoids and vitamin C.

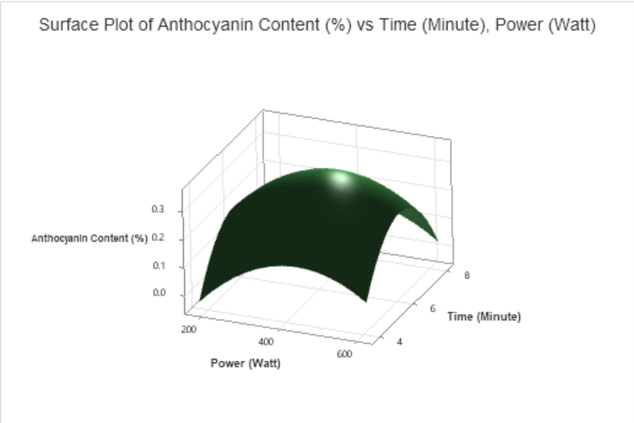
Density results tend to stabilize along with the amount of microwave power and the length of extraction time. While, viscosity results tend to increase along with an increase in microwave power and extraction time. This is because microwave power and longer extraction times also tend to increase the viscosity of the extract, because more bioactive compounds are extracted. These compounds will

Table 2. Antioxidant content of Roselle

Run	MAE Power (Watt)	Time (min)	Anthocyanin Content (%)	Ascorbic Acid Content (%)	Tannin Content (%)	Total Phenolic Content (%)	Viscosity (cP)	Density (g/mL)
1	200	4	0.093	2.139	0.073	0.125	10.308	1.302
2	200	8	0.106	1.296	0.073	0.144	16.690	1.314
3	600	4	0.086	1.396	0.110	0.129	18.207	1.306
4	600	8	0.076	1.788	0.133	0.170	17.167	1.309
5	184.382	6	0.033	1.155	0.073	0.215	19.886	1.310
6	615.618	6	0.280	1.306	0.118	0.178	28.833	1.317
7	400	3.84	0.103	1.075	0.119	0.152	30.980	1.310
8	400	8.15	0.033	1.055	0.121	0.136	18.968	1.312
9 C	400	6	0.667	1.145	0.112	0.178	18.033	1.313
10 C	400	6	0.116	1.055	0.103	0.131	16.160	1.315
11 C	400	6	0.601	1.185	0.116	0.181	17.007	1.314
12 C	400	6	0.133	1.015	0.098	0.133	16.929	1.315



(a)



(b)

Figure 1. (a) Contour plot effect of microwave power and extraction time of on anthocyanin levels content; (b) Surface plot effect of microwave power and extraction time on anthocyanin levels content.

increase the viscosity of the liquid (Weremfo et al., 2020).

3.3 *Statistical Analysis using Response Surface Methodology (RSM)*

In this research, RSM analysis aims to determine the effect of the independent variable of microwave power and extraction time on the resulting anthocyanin content and to seek optimization to produce the best response. The effect of the variables on the response was investigated using a second-order polynomial regression equation based on the effect estimation data. The resulting equation represents the effect of the independent variables used, namely the microwave power (X_1), and the extraction time (X_2) on the anthocyanin content value of roselle. The second-order polynomial regression equation is obtained as follows (Equation (6)):

$$\begin{aligned} \textit{Anthocyanin content}\,(\%) = & -1.86 + 0.00250X_1 \\ & + 0.564X_2 - 0.000003X_1^2 - 0.0470X_2^2 \\ & - 0.000014X_1X_2 \end{aligned} \quad (6)$$

Figure 1 shows the contour plot optimum values indicated by the increasingly dark green color. The darker the green, the better the response, and this occurs at the center value of the variables, which are 400 Watt of power and 6 min of time. On the other hand, the lighter the color on the graph, the smaller the effect of the variable on the anthocyanin content. In the surface plot, the optimum value can be seen at the top end of the curve, which corresponds to 400 Watt of power and 6 min of time. According to Purbowati & Maksum (2019), who conducted research on the anthocyanin content in rosella flower petals using the MAE method, the optimum results were achieved at 325 Watt of power and 5 min, with a yield of 75.755 mg/100g.

The Pareto diagram in Figure 2 shows that the variable the higher the extraction time; the more anthocyanin content results, the higher the resulting value. The Pareto diagram shows that the square of the time variable (square)(BB) has a significant impact on the anthocyanin content, although it does not reach the standard level of significance. Furthermore, the square of the power variable (square)(AA) also has a considerable impact, even though it does not achieve the standard level of significance on the anthocyanin content of the extract.

Analysis of Variance (ANOVA) model of anthocyanin extraction polynomial equation is

Table 3. Analysis of variant (ANOVA) of anthocyanin extraction polynomial equation model.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	0.198233	0.039647	0.76	0.608
Linear	2	0.009143	0.004571	0.09	0.917
Power (Watt)	1	0.008313	0.008313	0.16	0.703
Time (min)	1	0.000830	0.000830	0.02	0.904
Square	2	0.188957	0.094479	1.82	0.242
Power (Watt)*Power (Watt)	1	0.040412	0.040412	0.78	0.412
Time (min)*Time (min)	1	0.114251	0.114251	2.20	0.189
2-Way Interaction	1	0.000132	0.000132	0.00	0.961
Power (Watt)*Time (min)	1	0.000132	0.000132	0.00	0.961
Error	6	0.312002	0.052000		
Lack-of-Fit	3	0.050090	0.016697	0.19	0.896
Pure Error	3	0.261913	0.087304		
Total	11	0.510235			

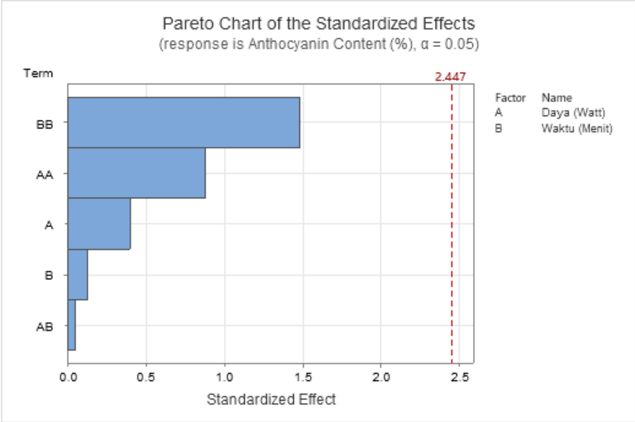


Figure 2. Pareto diagram of the standardized effects of anthocyanin levels content

presented in Table 3. The F value, or Fisher's variance ratio, is a reliable statistical indicator of how effectively a factor accounts for the variation in the average data, confirming whether the expected effect of the factor is real. In this case, the greater the F value, the more uniformity is shown from the F value of the Fisher test.

Analysis of the predicted value of anthocyanin levels content in Table 4 shows the most optimum yield parameters of anthocyanin content were obtained with the independent variables of microwave power and extraction time determined from the critical value. The critical value for optimizing the anthocyanin content was reached when the microwave power was 323.77 Watt, and the extraction time was 6.02 min. The predicted value of anthocyanin levels obtained was 0.3217. The higher the microwave power and extraction time the higher the anthocyanin content and the resulting value. The results of the critical value showed that the anthocyanin content is obtained at 0.3072%. The slight discrepancy between the predicted (0.3217 mg/L) and actual (0.3072 mg/L) anthocyanin content is within acceptable limits for RSM studies. This minor discrepancy is due to normal experimental variations and model limitations, indicating good model reliability. Similar accuracy has been observed in other MAE optimization studies.

4. Conclusion

This study optimized the extraction of anthocyanins from roselle (*Hibiscus sabdariffa* L.) using Microwave Assisted Extraction (MAE) with a Natural Deep Eutectic Solvent (NADES) combination of sodium acetate and glycerin. The optimization process, carried out through Response Surface Methodology (RSM), determined the optimal conditions for maximum anthocyanin content at a microwave power of 323.77 Watt and an extraction time of 6.02 min, resulting in an anthocyanin content of 0.3217%. The experimental results confirmed that both microwave power and extraction time significantly affect the antioxidant content of roselle, particularly anthocyanins, vitamin C, tannins, and total phenolics. These findings highlight the efficiency of MAE with NADES in extracting antioxidants from roselle, offering a promising and environmentally friendly method for producing natural colorants with high antioxidant activity.

Table 4. Analysis of variant model of anthocyanin extraction polynomial equation model.

Factor	Critical Value	Testing Result
Microwave Power (Watt)	323.77	323.77
Extraction Time (min)	6.02	6.02
Parameter		
Estimated Value of Anthocyanin Concentrations (%)	0.3217	0.3072

References

Bajkacz, S., Adamek, J. (2018). Development of a Method Based on Natural Deep Eutectic Solvents for Extraction of Flavonoids from Food Samples. *Food Analytical Methods*, 11(5), 1330–1344. DOI: 10.1007/s12161-017-1118-5

Cannavacciuolo, C., Pagliari, S., Frigerio, J., Giustra, C.M., Labra, M., Campone, L. (2023). Natural Deep Eutectic Solvents (NADESs) Combined with Sustainable Extraction Techniques: A Review of the Green Chemistry Approach in Food Analysis. *Foods*, 12(1), 56. DOI: 10.3390/foods12010056

Chatepa, L.E.C., Masamba, K.G., Sanudi, T., Ngwira, A., Tanganyika, J., Chamera, F. (2023). Effects of aqueous and methanolic solvent systems on phytochemical and antioxidant extraction from two varieties of Roselle (Hibiscus sabdariffa L.) var. sabdariffa plant from Central Malawi. *Food and Humanity*, 1, 1172–1179. DOI: 10.1016/j.foohum.2023.09.006

Hapsari, B.W., Manikharda, M., Setyaningsih, W. (2021). Methodologies in the Analysis of Phenolic Compounds in Roselle (Hibiscus sabdariffa L.): Composition, Biological Activity, and Beneficial Effects on Human Health. *Horticulturae*, 7(2), 35. DOI: 10.3390/horticulturae7020035

Imtiaz, F., Saif, Z., Sajid, A., Nazir, A., Manzoor, Q., Saleem, A., Mehr-un-Nisa, M., Farooq, A., Al-Mijalli, S.H., & Iqbal, M. (2024). Role of glycerol-based deep eutectic solvents for extraction of phytochemicals from Cichorium intybus seeds: Optimization by response surface methodology. *Microchemical Journal*, 199, 110083. DOI: 10.1016/j.microc.2024.110083

Lellis, B., Fávaro-Polonio, C.Z., Pamphile, J.A., Polonio, J.C., (2020), Effects of textile dyes on health and the environment and bioremediation potential of living organisms. *Biotechnology Research and Innovation*, 3(2), 275–290. DOI: 10.1016/j.biori.2019.09.001

Nguyen, M.P. (2020). Mircowave-Assisted Extraction of Phytochemical Constituents in Roselle (Hibiscus sabdariffa L.). *Journal of Pharmaceutical Research International*, 32(2), 1–12. DOI: 10.9734/jpri/2020/v32i230397

Purbowati, I.S.M., Maksum, A. (2019). The antioxidant activity of Roselle (Hibiscus sabdariffa Linii) phenolic compounds in different variations microwave-Assisted extraction time and power. *IOP Conference Series: Earth and Environmental Science*, 406(1), 012005. DOI: 10.1088/1755-1315/406/1/012005

Rosalinda, S., Azizah, I.W., Nurjanah, S. (2023). Identifikasi Kadar Vitamin C Ekstrak Rosela (Hibiscus sabdariffa L.) Hasil Ekstraksi Berbantu Gelombang Mikro. *Teknotan: Jurnal Industri Teknologi Pertanian*, 17(3), 167. DOI: 10.24198/jt.vol17n3.2

Sari, T.S., Kusumawati, I., Isnaeni, I. (2020). Color Stability and

Antioxidant Activity of Red Roselle (Hibiscus Sabdariffa L.) Calyx Infuse. *Berkala Ilmiah Kimia Farmasi*, 10(2), 36–41. DOI: 10.20473/bikfar.v10i2.51706

Sun, R., Niu, Y., Li, M., Liu, Y., Wang, K., Gao, Z., Wang, Z., Yue, T., Yuan, Y. (2023). Emerging trends in pectin functional processing and its fortification for synbiotics: A review. *Trends in Food Science and Technology*, 134, 80–97. DOI: 10.1016/j.tifs.2023.03.004

Syahputra, H., Rivai, M., Mahrani, S., Nazhifah, Y. (2023). Analysis of Vitamin C (ascorbic acid) in Cucumis mel L. var reticulatus Naudin and Averrhoa bilimbi L. Fruit using UV-Vis Spectrophotometry. *Indonesian Journal of Pharmaceutical and Clinical Research*, 6(2), 13–18. DOI: 10.32734/idjpcr.v6i2.13557

Weremfo, A., Adulley, F., Adarkwah-Yiadom, M. (2020). Simultaneous Optimization of Microwave-Assisted Extraction of Phenolic Compounds and Antioxidant Activity of Avocado (Persea americana Mill.) Seeds Using Response Surface Methodology. *Journal of Analytical Methods in Chemistry*, 2020(1), 7541927. DOI: 10.1155/2020/7541927

Yuan, Y., Zhang, J., Fan, J., Clark, J., Shen, P., Li, Y., Zhang, C. (2018). Microwave assisted extraction of phenolic compounds from four economic brown macroalgae species and evaluation of their antioxidant activities and inhibitory effects on α-amylase, α-glucosidase, pancreatic lipase and tyrosinase. *Food Research International*, 113, 288–297. DOI: 10.1016/j.foodres.2018.07.021

Zhang, J., Wen, C., Zhang, H., Duan, Y., Ma, H. (2020). Optimization of red pigment anthocyanin recovery from Hibiscus sabdariffa by subcritical water extraction. *Processes*, 10(12), 2635. DOI: 10.3390/pr10122635