Journal of Vocational Studies on Applied Research

Efficiency of Betacyanin and Betaxanthin Extraction from Red Beets (*Beta vulgaris* L.) using Microwave-Assisted Extraction (MAE)

Satya Pramudika[1)] and Vita Paramita*[1)]

[1)]*Industrial Chemical Engineering, Faculty of Vocational School, Diponegoro University*
Jl. Prof. Soedarto, SH, Tembalang, Semarang

[*)] Corresponding author: vparamita@live.undip.ac.id

Abstract – *Red beet pigments, betacyanin and betaxanthin, are gaining significant interest due to their potential health benefits and applications as natural colorants in the food and pharmaceutical industries. This study explored the feasibility of Microwave-Assisted Extraction (MAE) as an alternative, efficient technique for extracting betacyanin and betaxanthin from red beets. Pretreated beets were subjected to MAE employing varying microwave power levels (200, 400, and 600 W) and solvents (water, ethanol, and ethanol + citric acid). Betacyanin content varied from 23.77 to 59.28 mg/100g. Higher microwave power significantly increased betacyanin yield ($p < 0.05$), while the type of solvent did not exert a significant influence. Betaxanthin content was also affected by both microwave power and solvent polarity. Higher power settings and the more polar solvent mixture (ethanol + citric acid) resulted in greater extraction, due to the higher dielectric constant. These findings demonstrate the effectiveness of MAE for extracting both pigments from red beets. While the study identifies conditions that influence betacyanin and betaxanthin content, further investigation is needed to determine the optimal extraction parameters for each compound.*

Keywords: *beetroot, microwave assisted extraction, betacyanin, betaxanthin*

Received: January 31, 2024 Revised: February 12, 2024 Accepted: March 05, 2024

Doi: http://dx.doi.org/10.14710/jvsar.v5i2.22011
[How to cite this article: Pramudika, S., and Paramita, V. (2023). Efficiency of Betacyanin and Betaxanthin Extraction from Red Beets (*Beta vulgaris* L.) using Microwave-Assisted Extraction (MAE). *Journal of Vocational Studies on Applied Research, 5*(2), 68-73. doi: http://dx.doi.org/10.14710/jvsar.v5i2.22011]

INTRODUCTION

Red beets, a versatile food ingredient, offer numerous benefits, including imparting natural color to food products. The vibrant pigment within red beets, betalain, belongs to the antioxidant family. Beetroot boasts a rich nutritional profile, encompassing protein, fat, calcium, phosphorus, iron, vitamins A, B, and C, water, and significant amounts of starch and fiber. Widyaningrum and Suhartiningsih (2014) reported that 100g of beetroot contains 35.81% starch and 2.14% fiber. Being a polar dye, Betacyanin readily dissolves in polar solvents. This nitrogenous compound, present in the red pigment, exhibits potent antioxidant activity.

Red beet (*Beta vulgaris*) stands out as a unique source of betalain, harboring two main betalain pigments: the red betanin and yellow vulgaxanthin I. Cultivated and consumed worldwide, red beets contribute significantly to global beet production, estimated at around 275 million metric tons in 2018 (Fu Y et al., 2020). Betacyanin constitutes 75-95% of beet pigment, while betaxanthin comprises the remaining 5-25% (Ninfali et al., 2013). Red beets also contain isobetanin, an epimer of betanin. The betalain concentration in red beets ranges between 200-2100 mg/kg fresh weight, with variations observed across cultivars, with newer varieties boasting higher betalain content (Ninfali et al., 2013).

Betacyanin's excellent water solubility makes it a desirable natural dye. This pigment finds presence in the flowers, fruits, and leaves of plants exhibiting a purplish-red hue. However, betacyanin exhibits sensitivity towards various environmental factors like temperature, pH, light, oxygen, and metal ions, impacting its stability. Extraction of betacyanin utilizes various solvents, including water, ethanol, methanol, and n-hexane. Notably, using water in high-heat concentration processes can damage the betacyanin compound due to its boiling point of 100°C (Rengku et al., 2017). Sari et al.'s research employed a maceration method with ethanol concentrations of 80%, 50%, 20%, and 0%, achieving the best result with 50% ethanol solvent, yielding 0.089 mg/100 g of betacyanin. Novatama et al.'s maceration method using 70% ethanol solvent yielded 37.64 mg/100 g of betacyanin. However, both studies suffer from long extraction times, requiring 48 hours.

Non-conventional extraction methods like Microwave Assisted Extraction (MAE) offer a promising alternative compared to traditional methods. This method delivers enhanced quality and quantity of the desired product due to its use of less solvent and shorter exposure times at high temperatures (Yuan et al., 2018). Reduced operating time and solvent consumption, along with lower energy consumption due to direct radiation to the matrix, constitute the key advantages of microwave extraction (Seoane et al., 2017). Cardoso-Ugarte et al.'s (2014) research achieved the same betalain concentration ten times faster using microwave irradiation compared to conventional methods, with betalain yields doubling. However, dielectric heating during microwave processing can accelerate chemical reactions of target compounds, including epimerization, oxidation, and polarization, which necessitates consideration (Bastos et al., 2017).

During MAE, sample cells experience thermal increases in temperature and pressure due to microwave irradiation, leading to cell wall rupture and the release of intracellular compounds (Pap et al., 2012).

This research aims to investigate the effectiveness of MAE in extracting betacyanin and betaxanthin from red beets and to determine the optimal extraction conditions.

RESEARCH METHOD

Materials and Tools

The materials used were Red Beets ethanol (96% purity), Citric Acid, and water. using tools microwave-assisted extraction, oven, analytical balance, beaker glass, and UV-Vis Spectrophotometer.

Red Beets Pretreatment

Beets were obtained from a local supermarket in Semarang, Indonesia. After thorough washing, their skin was peeled with a knife. The flesh was then diced into 1 cm cubes and distributed equally into 9 airtight containers, each containing 20 grams. The beets were stored for 72 hours at ±-10°C.

Microwave-Assisted Extraction (MAE)

Betacyanin and betaxanthin extraction from red beet was performed using microwave-assisted extraction (MAE). A mixture of 1:20 solids to solvent ratio were selected for MAE of betalain pigments Solid-liquid extraction began by grinding 20 grams of beet pulp in a blender with 400 mL of each solvent: distilled water, ethanol, and ethanol + citric acid, each used in a separate extraction. The MAE process was conducted for 10 minutes per experiment at varying microwave power levels: 200, 400, and 600 W. The MAE system was equipped with a condenser to prevent solvent loss. Immediately after extraction, the samples were stored in sealed bottles and kept in a cooler at 18°C to minimize pigment degradation due to temperature. Analysis was conducted the following day.

Quantification of betacyanin and betaxanthin

The total betacyanin and betaxanthin contents were quantified using a spectrophotometric method at specific absorbance wavelengths: 537 nm for betacyanin and 480 nm for betaxanthin. The content was expressed in mg per 100 g of sample, as calculated using formula (1).

$$\text{BC/BxC (mg/100g)} = \frac{A\ .\ DF\ .\ MW\ .\ 1000}{\varepsilon\ .\ L} \qquad (1)$$

Where A is the maximum absorbance value at 537 nm for betacyanin and 480 nm for betaxanthin; DF is the dilution factor; MW is the molecular weight: 550 g/mol for betacyanin and 308 g/mol for betaxanthin; L is the length of the tank path (1 cm); ε is the absorbance coefficient: 60,000 L/mol cm in water for betacyanin and 48,000 L/mol cm in water for betaxanthin.

Statistical analysis

Two-way ANOVA was employed to analyze the combined effects of solvent and microwave power on betacyanin yield. During data analysis of the extraction process, Two-way ANOVA was used to assess differences in the average extraction results based on two independent variables: solvent and microwave power. The analysis examined the mean betacyanin yield obtained in each extraction and calculated the p-values for both factors and their interaction. Three replicates were performed for each condition, and a 5% tolerance limit was applied. The Two-way ANOVA analysis was conducted using Microsoft Excel 2021.

RESULTS AND DISCUSSION

The Influence of Extraction Parameters on Betacyanin Content

This study explored the impact of varying microwave power and solvent type on betaxanthin extraction from beets. The experiment revealed a range of betacyanin content, from 23.77 to 59.28 mg/100g. A two-way ANOVA (Table 1) was used to analyze the significance of these factors in influencing the extraction process.

Treatment with microwave power levels of 200, 400, and 600 watts yielded average betacyanin contents of 28.98, 46.2, and 40.07 mg/100g, respectively. Based on the F-value (2.92774), which exceeds the F-critical (2.38877), the alternative hypothesis (Ha) stating a

Table 1. Two-way Anova of Betacyanin and Betaxanthin Content with Replication

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Betacyanin Content						
Sample	1370.0412	2	685.02059	34392.562	5.7409E-33	3.5545571
Columns	490.05747	2	245.02873	12302.062	5.9638E-29	3.5545571
Interaction	1665.3708	4	416.3427	20903.156	9.9102E-33	2.9277442
Within	0.3585185	18	0.0199177			
Total	3525.828	26				
Betaxanthin Content						
Sample	153.7526	2	76.87632	15753.87	6.45E-30	3.554557
Columns	288.2421	2	144.1211	29534	2.26E-32	3.554557
Interaction	199.4584	4	49.8646	10218.5	6.2E-30	2.927744
Within	0.087837	18	0.00488			
Total	641.541	26				

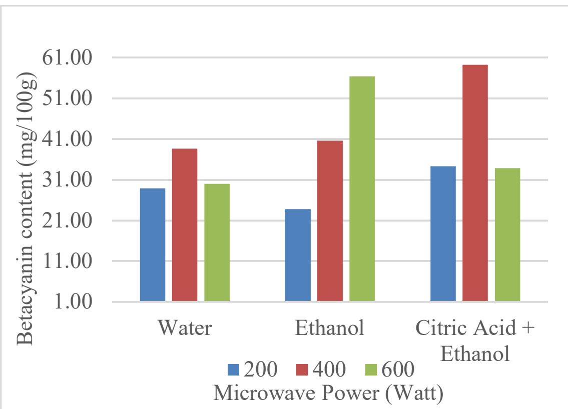

Figure 1. Betacyanin content

significant difference between average betacyanin values at different power levels was accepted. This confirms that microwave power significantly affects the extraction efficiency. This is also in accordance with research by Aruna Singh et al (2017) which explains that at a power of 384.5 W it produces betalain which is higher than at a power of 224.61 W. In microwave extraction, ethanol yielded higher values compared to distilled water. Notably, the addition of citric acid to the ethanol solvent further increased betacyanin levels. This enhanced extraction can be attributed to the acidic environment created by citric acid. Betalain compounds, including betacyanin, exhibit optimal stability within a pH range of 3-7 (Enriquez et al., 2020).

The impact of microwave power can be explained by the principle of increased energy input at higher power settings. This leads to faster and more uniform heating, facilitating efficient extraction and resulting in higher average betacyanin values. The highest average value was observed at 600 watts, followed by 400 and 200 watts, further supporting this explanation.

While solvent type did not significantly affect the average betacyanin yield, as evidenced by the F-value (0.588527) being lower than the F-critical value (6.944272). This supports the null hypothesis (H_0) stating no significant difference in average values between the aquadest, ethanol, and citric acid solvents.

This can be explained by the diverse solubilities of different compounds in various solvents. However, in the context of this study, the differences in solvent-specific extraction abilities were not substantial enough to significantly impact the average betacyanin content.

Therefore, we conclude that microwave power exerted a significant influence on the average betacyanin yield compared to solvent variation. Higher microwave power settings led to correspondingly higher average betacyanin content.

The Influence of Extraction Parameters on Betaxanthin Content

This study explored the impact of varying microwave power and solvent type on betaxanthin extraction from beets. The microwave power used significantly impacted the extraction yield. Higher power levels led to increased extraction, presumably due to the generated higher temperature facilitating a faster and more efficient extraction process.

Solvent variations also played a role in extraction yield. More polar solvents, like ethanol in combination with citric acid, proved more effective in extracting the polar betaxanthin molecules. This enhanced efficacy likely arises from improved interactions between the solvent and the target compound.

Based on Figure 2, it can be concluded that both microwave power and solvent polarity influence betaxanthin extraction yield. Higher power settings and more polar solvents generally lead to increased extraction efficiency.

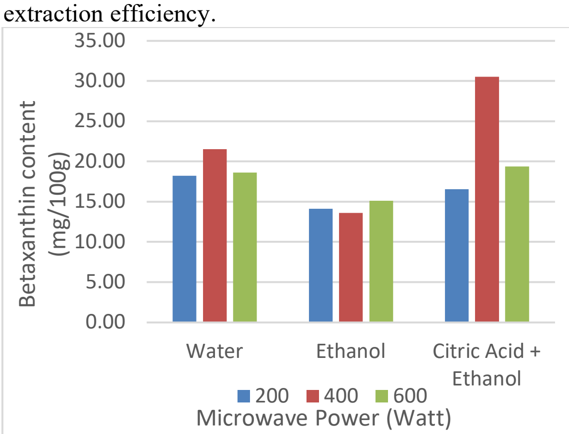

Figure 2. Betaxanthin content

CONCLUSION

This study confirms Microwave-Assisted Extraction (MAE) as a viable method for extracting betacyanin and betaxanthin from red beets. Citric acid addition significantly enhances betacyanin yield, while microwave power exerts a significant influence on betacyanin content. The combination of citric acid + ethanol at 600 W power delivers the highest betacyanin yield (59.28 mg/100g), while dropping to 40.64 mg/100g at 400 W. Higher microwave power settings consistently lead to increased average betacyanin and betaxanthin content compared to lower settings. Solvent type, however, does not significantly affect betacyanin and betaxanthin content. All three solvents used in this study exhibit comparable performance in betacyanin and betaxanthin extraction. Both microwave power and solvent polarity play key roles in betacyanin and betaxanthin content. Higher power settings and more polar solvents, like ethanol + citric acid, significantly improve betacyanin and betaxanthin extraction efficiency.

REFERENCES

Bastos, E.L.; Gonçalves, L.C.P. (2017). Microwave-Assisted extraction of betalains. In Water Extraction of Bioactive Compounds: From Plants to Drug Development; González, H.D., Muñoz, M.J.G., Eds.; Elsevier: Amsterdam, The Netherlands, pp. 245–267

Cardoso-Ugarte, G.A.; Sosa-Morales, M.E.; Ballard, T.; Liceaga, A.; San Martín-González, M.F. (2014). Microwave-Assisted extraction of betalains from red beet (Beta vulgaris). LWT Food Sci. Technol. 59, 276–282.

Castro-Enríquez, D.D.; Montaño-Leyva, B.; Del Toro-Sánchez, C.L.; Juaréz-Onofre, J.E.; Carvajal-Millan, E.; Burruel-Ibarra, S.E.; Tapia-Hernández, J.A.; Barreras-Urbina, C.G.; Rodríguez-Félix, F. Stabilization of Betalains by Encapsulation—A Review. J. Food Sci. Technol. 2020, 57, 1587–1600.

Ceclu, L., and Nistor, O. V. (2020). Red Beetroot: Composition and Health Effects - A Review. J. Nutri Med Diet Care. 6:043

Coy-Barrera, E. (2020). Analysis of betalains (betacyanins and betaxanthins). Chapter 17 : Recent Advances in Natural Products Analysis

Fu, Y.; Shi, J.; Xie, S.Y.; Zhang, T.Y.; Soladoye, O.P.; Aluko, R.E. (2020). Red Beetroot Betalains: Perspectives on Extraction, Processing, and Potential Health Benefits. J. Agric. Food Chem. 68, 11595–11611.

Gharekhani, M., M. Ghorbani, and N. Rasoulnejad. (2012). Microwave-Assisted Extraction Of Phenolic And Flavonoid Compounds From Eucalyptus Camaldulensis Dehn Leaves As Compared With Ultrasound-Assisted Extraction. Latin American Applied Research 42(July):305–310.

Lazăr (Mistrianu), Silvia, Oana Emilia Constantin, Nicoleta Stănciuc, Iuliana Aprodu, Constantin Croitoru, and Gabriela Râpeanu. 2021. "Optimization of Betalain Pigments Extraction Using Beetroot by-Products as a Valuable Source" Inventions 6, no. 3: 50.

Lembong, E., & Utama, G. L. (2021). Potensi pewarna dari bit merah (Beta vulgaris L.) sebagai Antioksidan. Jurnal Agercolere, 3(1), 7–13.

Ninfali, P.; Angelino, D. (2013). Nutritional and functional potential of Beta vulgaris cicla and rubra. Fitoterapia, 89, 188–199.

Nurbaya, S. R., Putri, W. D. R., & Murtini, E. S. (2018). Pengaruh Campuran Pelarut Aquades-Etanol Terhadap Karakteristik Ekstrak Betasianin Dari Kulit Buah Naga Merah (*Hylocereus polyrhizus*). Jurnal Teknologi Pertanian, 19(3), 153–160.

Petrucci, Ralph H. 1987. Kimia Dasar Prinsip dan Terapan Modern Jilid 1. Jakarta: Erlangga

Righi Pessoa da Silva, H., da Silva, C., & Bolanho, B. C. (2018). Ultrasonic-assisted extraction of betalains from red beet *(Beta vulgaris* L.). Journal of Food Process Engineering, 41(6).

Polturak, G., and Aharoni, A. (2018). ‘‘La Vie en Rose’’: Biosynthesis, Sources, and Applications of Betalain Pigments. Molecular Plant 11, 7–22.

Purnami, G. A. I., Puspawati, G. ayu K. D., & Pratiwi, I. D. P. K. (2022). Pengaruh Jenis Pelarut dan Waktu Ekstraksi pada Metode Microwave Assited Extraction Terhadap Karakteristik Pewarna Ekstrak Kulit Buah Naga Kuning (Selenicereus megalanthus). Jurnal Ilmu dan Teknologi Pangan, 11(2), 309–321.

Sari, N. M. I., Hudha, A. M., & Prihanta, W. (2016). Uji Kadar Betasianin Pada Buah Bit (Beta Vulgaris L.) Dengan Pelarut Etanol Dan Pengembangannya Sebagai Sumber Belajar Biologi. Jurnal Pendidikan Biologi Indonesia, 2, 72–77.

Seoane, P.R.; Flórez-Fernández, N.; Piñeiro, E.C.; González, H.D. (2017). Microwave-assisted water extraction. In Water Extraction of Bioactive Compounds: From Plants to Drug Development; González, H.D., Muñoz, M.J.G., Eds.; Elsevier: Amsterdam, The Netherlands, pp. 163–198.

Setiawan, M. A. W., Nugroho, E. K., & Lestario, L. N. (2015). Ekstraksi Betasianin Dari Kulit Umbi Bit (*Beta Vulgaris*) Sebagai Pewarna Alami. AGRIC, 27(1), 38–43.

Singh, A., Ganesapillai, M., & Gnanasundaram, N. (2017). Optimizaton of extraction of betalain pigments from beta vulgaris peels by microwave pretreatment. IOP Conference Series: Materials Science and Engineering, 263(3).

S.J. Calva-Estrada, M. Jim´enez-Fern´ andez , E. Lugo-Cervantes. (2022). Betalains and their applications in food: The current state of processing, stability and future opportunities in the industry. Food Chemistry: Molecular Sciences 4 100089

Slavov, A.; Karagyozov, V.; Denev, P.; Kratchanova, M.; Kratchanov, C. (2013). Antioxidant activity of red beet juices obtained after microwave and thermal pretreatments. Czech J. Food Sci. 31, 139–147.

Spórna-Kucab, A., Tekieli, A., Grzegorczyk, A., Swiatek, L., Boguszewska, A., and Skalicka-Wo´zniak. K. (2023). Betaxanthin Profiling in Relation to the Biological Activities of Red and Yellow Beta vulgaris L. Extracts, 13, 408

Veggi, P. C., Martinez, J., & Meireles, M. A. A. (2013). Fundamentals of microwave extraction. Dalam Food Engineering Series (hlm. 15–52).

Yuan, Y.; Zhang, J.; Fan, J.; Clark, J.; Shen, P.; Li, Y.; Zhang, C. (2018). Microwave assisted etraction 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 Res. Int. 113, 288–297.

Zin, M. M., Anucha, C. B., & Bánvölgyi, S. (2020). Recovery of phytochemicals via electromagnetic irradiation (Microwave-Assisted-Extraction): Betalain and phenolic compounds in perspective. Foods (Vol. 9, No 7). MDPI AG.