Extraction and Characterization of Astaxanthin from Processed Rebon Shrimp Products as a Local Wisdom-Based Antioxidant Source

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Abstract

Astaxanthin, a carotenoid with pronounced antioxidant properties, represents a promising candidate for development as a functional food ingredient grounded in local wisdom. Traditional rebon shrimp derivatives including dried rebon, terasi, and cincalok are recognized sources of this pigment; however, systematic data on extraction efficiency, astaxanthin concentration, and antioxidant activity remain scarce. This study evaluated three extraction techniques and characterized antioxidant activity using 2,2-difenil-1-pikrilhidrazil (DPPH), azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), and 4,6-tris(2-pyridyl)-s-triazine (FRAP) assays. Samples were vacuum-dried, ground, and subjected to oil extraction, followed by qualitative thin-layer chromatography and quantitative UV-Vis spectrophotometry. Soxhlet extraction yielded the greatest oil output, whereas maceration preserved astaxanthin more effectively due to the absence of thermal degradation. Cincalok exhibited the highest astaxanthin content (10.943 mg/g), followed by terasi and dried rebon. DPPH analysis indicated that cincalok oil possessed the strongest antioxidant activity (IC_{50} 54.58 mg/L, Soxhlet), while dried rebon demonstrated the weakest. In contrast, ABTS and FRAP assays revealed lower responses for cincalok oil, suggesting that its primary antioxidant mechanism involves free-radical scavenging. These findings highlight cincalok as the most promising natural source of astaxanthin and local antioxidants, underscoring its potential for the development of coastal-based functional foods and health-promoting products.

Keywords: astaxanthin, rebon shrimp, extraction, antioxidant activity, cincalok

INTRODUCTION

Indonesia is recognized as one of the world's largest archipelagic countries, possessing extensive marine biodiversity and abundant fishery resources. Among these resources is rebon shrimp (*Acetes* spp.), a small pelagic shrimp widely distributed in coastal waters and commonly harvested by local fishing communities. Despite its abundance, rebon shrimp is generally considered a low-value commodity and is rarely consumed in fresh form due to its rapid perishability. Consequently, coastal communities traditionally process rebon into several preserved products, such as dried rebon, *cincalok* (fermented shrimp), and shrimp paste (*terasi*) (Junianto *et al*., 2023; Mutamimah *et al*., 2023; Ropikoh *et al*., 2022). These products are primarily utilized as condiments in traditional cuisine, while their potential as sources of functional bioactive compounds remains largely unexplored.

Previous studies have reported that rebon shrimp contains nutritionally valuable components, including high levels of omega-3 phospholipids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Balange *et al*., 2017). In addition, rebon shrimp has been reported to contain astaxanthin, a carotenoid pigment responsible for the characteristic reddish coloration of many marine crustaceans (Rahmalia *et al*., 2022). Astaxanthin is widely recognized for its strong antioxidant activity and has been associated with various health benefits, including protection against oxidative stress, cardiovascular diseases, cancer, and premature aging. Structurally, astaxanthin (3,3′-dihydroxy-β-carotene-4,4′-dione) belongs to the xanthophyll carotenoid group and is characterized by a conjugated polyene chain and terminal β-ionone rings containing hydroxyl and keto functional groups, which contribute to its high radical-scavenging capacity (Brotosudarmo *et*

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Diterima/Received : 12-02-2026, Disetujui/Accepted : 14-03-2026
www.ejournal2.undip.ac.id/index.php/jkt **DOI**: https://doi.org/10.14710/jkt.v29i2.31340

al., 2020; Debnath *et al*., 2025; Jaryal *et al*., 2025). Astaxanthin occurs in several chemical forms, including free astaxanthin, monoesters, and diesters with fatty acids such as palmitic, oleic, stearic, and linoleic acids, and it may also exist in association with proteins or lipoproteins. In addition, various stereoisomers and geometric isomers of astaxanthin have been identified (Berg *et al*., 2022; Hardo *et al*., 2022).

To date, most research on astaxanthin has focused on commercial sources such as the microalga *Haematococcus pluvialis* or krill (An *et al*., 2024; Nemani *et al*., 2024; Cui *et al*., 2025; Wang *et al*., 2025), which are widely used in nutraceutical and cosmetic industries. In contrast, the potential of traditional seafood products derived from local marine resources, such as processed rebon shrimp, has received limited scientific attention. Moreover, different processing methods, including drying, fermentation, and thermal treatment may influence the stability, isomerization, and bioactivity of astaxanthin, yet these effects remain poorly understood in the context of traditional rebon products.

Extraction efficiency also plays a crucial role in the recovery of carotenoids from biological matrices. Various extraction techniques, including maceration, soxhlet extraction, and ultrasonication, have been widely employed for carotenoid isolation, each with distinct advantages in terms of efficiency, solvent consumption, and preservation of bioactive compounds (Metibemu & Ogungbe, 2022; Kiruthika & Shivaswamy, 2024; Morón-Ortiz *et al*., 2024). However, comparative studies evaluating these methods for astaxanthin extraction from traditional rebon-based products are still scarce.

Therefore, this study aims to investigate the presence and characteristics of astaxanthin in several processed rebon shrimp products, including dried rebon, *cincalok*, and shrimp paste (*terasi*). In addition, different extraction techniques such as maceration, Soxhlet extraction, and ultrasonication are compared to determine their effectiveness in recovering astaxanthin. The extracted compounds are further characterized to identify astaxanthin isomers and to evaluate their antioxidant activity using DPPH, ABTS, and FRAP assays. The findings of this study are expected to provide baseline scientific data on the nutritional and functional properties of traditional rebon products and to highlight their potential as locally sourced natural antioxidants for applications in functional foods, nutraceuticals, and cosmetic formulations.

MATERIALS AND METHODS

Local processed rebon shrimp products, consisting of dried rebon shrimp, cincalok (fermented shrimp), and shrimp paste (terasi), will be collected from traditional producers in Teluk Majantu, West Kalimantan. Each sample will be subjected to vacuum drying, ground into a fine powder, and stored at low temperature until further extraction (Rahmalia *et al*., 2022).

The optimization of astaxanthin extraction was carried out using three different methods. The maceration method was modified from Nurbaeti *et al*. (2021). A total of 100 g of sample (dried rebon, cincalok, or shrimp paste) was extracted with 300 mL of n-hexane in a dark bottle and allowed to stand for 3 × 24 h. The filtrate was subsequently evaporated, and the astaxanthin yield was determined. The Soxhlet extraction method referred to Rahmalia *et al*. (2022). Briefly, 100 g of sample (dried rebon, cincalok, or shrimp paste) was wrapped in filter paper and extracted with 300 mL of n-hexane at 65 °C until the solvent became colorless. The filtrate was then evaporated, and the astaxanthin yield was analyzed. The ultrasonication method was modified from Prayitno *et al*. (2022). A total of 100 g of sample (dried rebon, cincalok, or shrimp paste) was mixed with 200 mL of n-hexane and extracted using an ultrasonic homogenizer at 10,000 rpm with extraction times ranging from 5–15 min and amplitudes of 20–40%. After centrifugation at 3000 rpm for 30 min, the filtrate was evaporated and the extraction yield was determined. All procedures were performed in triplicate to identify the method providing the highest yield. The extracts were initially subjected to thin-layer chromatography (TLC) for qualitative identification. The extract obtained from the most efficient

method was further analyzed using gas chromatography–mass spectrometry (GC–MS) to obtain a more detailed compound profile.

The antioxidant activity was evaluated using three different assays: DPPH, ABTS, and FRAP. A 1,000 ppm astaxanthin extract solution was prepared in ethanol and subsequently diluted to concentrations ranging from 1–11 ppm. DPPH assay, 2 mL of sample solution was mixed with 2 mL of 1 mM DPPH solution, incubated at 37 °C for 30 min, and the absorbance was measured at 516 nm. The percentage of inhibition was calculated and used to determine the IC_{50} value (Chintong *et al*., 2019). For the ABTS assay, stock solutions of ABTS and $K_2S_2O_8$ were incubated for 12 h, then mixed and diluted with ethanol to a final volume of 25 mL. A 500 µL aliquot of sample solution (1,000 µg/mL) was reacted with 1 mL of ABTS solution and ethanol was added to reach a total volume of 5 mL. The absorbance was measured at 745–755 nm, and antioxidant activity was calculated based on the change in absorbance (Chintong *et al*., 2019). FRAP assay, 1 mL of sample solution was mixed with 0.2 M phosphate buffer and 1% potassium ferricyanide, followed by incubation at 50 °C for 20 min. After the addition of 10% trichloroacetic acid (TCA) and centrifugation, the supernatant was reacted with 0.1% $FeCl_3$ and the absorbance was measured at 696 nm. Antioxidant activity was calculated based on the percentage reduction of Fe^{3+} and analyzed using linear regression to obtain the IC_{50} value (Al-Tarifi *et al*., 2020).

RESULT AND DISCUSSION

Astaxanthin is a red–orange carotenoid pigment with exceptionally strong antioxidant activity, exceeding that of vitamins C and E. The coastal region of West Kalimantan possesses abundant aquatic resources, including rebon shrimp, which are commonly processed into dried shrimp, shrimp paste (*terasi*), and fermented shrimp (*cincalok*). This study aimed to develop an appropriate method for astaxanthin extraction, characterize the types of astaxanthin present, and evaluate its antioxidant activity as baseline data for the nutritional value and functional benefits of processed rebon products. Sample preparation involved drying and size reduction. Drying was carried out using vacuum drying at 40–50 °C for 3–6 h to reduce moisture content and prevent microbial growth (Selviana *et al*., 2021). The dried samples were subsequently ground into a fine powder to increase surface area, thereby facilitating optimal extraction of bioactive compounds. The results of sample preparation are presented in Figure 1.

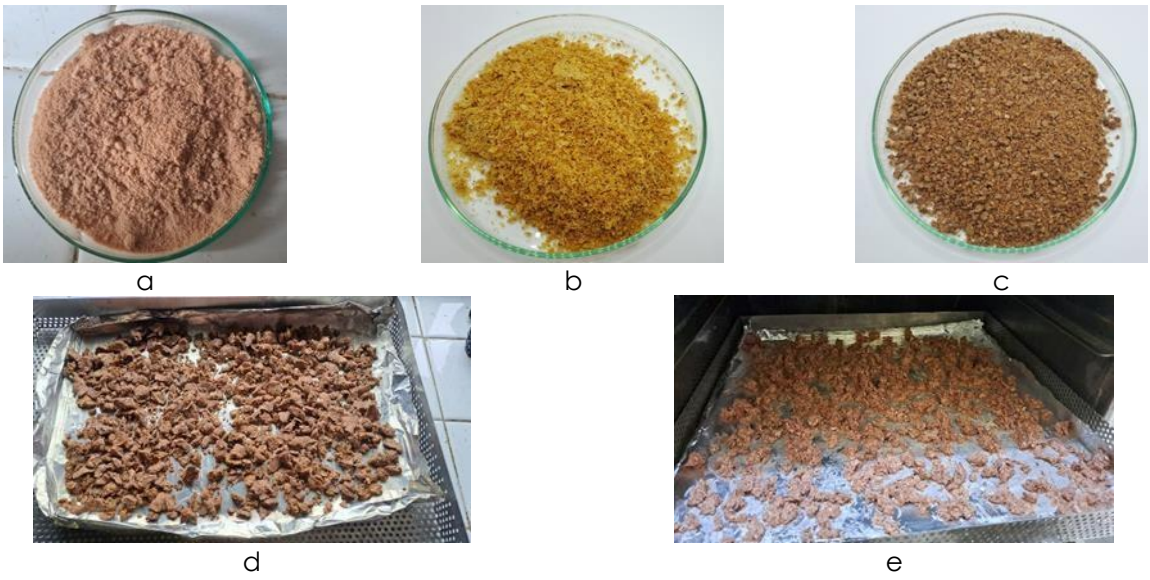


Figure 1. Proses Penghalusan: (a) Cincalok; (b) Rebon Kering; (c) Terasi; Proses Pengeringan: (d) Terasi; dan (e) Cincalok

The research was initiated by extracting the oil fraction from all three samples prior to astaxanthin analysis, as this compound generally does not occur in a free form but is predominantly associated with lipid matrices or protein–lipid complexes. Due to its lipophilic nature, astaxanthin strongly associates with the oil fraction in aquatic animal tissues, including rebon shrimp. Oil extraction enables the separation of astaxanthin from non-lipid components that are not relevant to the analysis, thereby facilitating its identification and quantification (Todorović *et al*., 2021). After obtaining the oil fraction, the astaxanthin content was analyzed more specifically to determine its concentration and binding form (free, monoester, or diester).

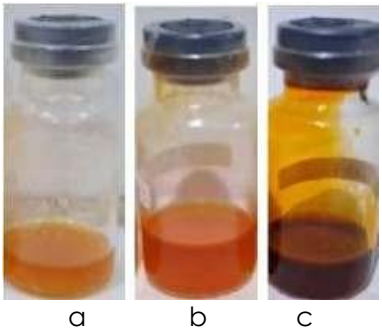
Maceration and Soxhlet extraction were employed as preliminary conventional methods before applying ultrasonication, to establish baseline information on the effectiveness of traditional techniques in extracting oil and astaxanthin from rebon. Maceration was selected due to its simplicity and the absence of high temperatures, which helps preserve the stability of heat-sensitive bioactive compounds (Fitri *et al*., 2025). Soxhlet extraction was used for comparison because, although it involves heating and repeated extraction cycles, it is widely recognized for achieving higher extraction efficiency and providing insight into the maximum extractable content from the sample (Sun *et al*., 2025).

Table 1 shows that the oil yield obtained through maceration was lower than that achieved by Soxhlet extraction, reflecting the differences in the working principles of the two methods. Maceration relies solely on soaking at room temperature, resulting in slow diffusion of oil into the solvent and less efficient lipid solubilization. In contrast, Soxhlet extraction employs continuous heating and repeated cycles of hot solvent percolation through the sample, significantly enhancing the solvent's ability to dissolve oil (Rosli & Idris, 2024).

The elevated temperature applied during Soxhlet extraction facilitates the disruption of lipid–matrix interactions within the biological material and accelerates diffusion, leading to greater oil recovery. Furthermore, the repeated solvent circulation allows gradual enrichment of the extract without the need for solvent replacement, thereby ensuring a more exhaustive extraction process (Ibrahim *et al*., 2025). Therefore, although maceration is considered safer for heat-sensitive compounds, Soxhlet extraction provides a higher oil yield under the conditions applied in this study.

Table 1 Oil Yield Obtained

Sample	Soxhlet Extraction Method (%)	Maceration Extraction Method (%)
Dried Rebon oil	3.762	2.622
Terasi oil	8.309	5.624
Cincalok oil	11.438	6.364



a b c

Figure 2. Oil obtained from dried rebon shrimp (a), shrimp paste (*terasi*) (b), and fermented shrimp (*cincalok*) (c)

Figure 2 illustrates the variation in oil color among the three samples: oil from dried rebon shrimp appears light orange, shrimp paste (*terasi*) oil is reddish-orange, and *cincalok* oil is dark red to nearly black. These differences indicate variations in astaxanthin content, as this carotenoid pigment produces an orange to red color spectrum depending on its concentration, binding form, and interactions with lipid components (Berg *et al*., 2022). The light orange color of rebon oil suggests a relatively low astaxanthin content or the predominance of the free, less stable form. The reddish-orange color of *terasi* oil indicates an increased pigment level, likely influenced by the fermentation process (El-Bialy & Abd El-Khalek, 2020). Meanwhile, the dark reddish coloration of *cincalok* oil suggests a higher astaxanthin concentration formed during prolonged fermentation (Li *et al*., 2024).

Confirm the presence of astaxanthin in each oil extract, qualitative analysis was performed using thin-layer chromatography (TLC), followed by quantitative analysis using UV–Vis spectrophotometry. The TLC results presented in Figure 3 show orange spots in all three samples with Rf values close to that of the astaxanthin standard (Rf ≈ 0.75). In rebon oil (Figure 3a), no distinct spot was clearly observed, indicating a very low astaxanthin content. Shrimp paste oil (Figure 3b) exhibited several orange spots with stronger intensity, suggesting a higher astaxanthin concentration and the possible presence of esterified forms resulting from fermentation. *Cincalok* oil (Figure 3c) showed the most intense and dominant orange spot at the same Rf position as the standard, confirming a higher astaxanthin concentration compared to the other two samples. The variation in spot intensity reflects differences in astaxanthin content and stability, which are influenced by the raw material type, processing method, and fermentation conditions (Dymek *et al*., 2024).

Quantitatively, the astaxanthin content in each sample was determined using UV–Vis spectrophotometry based on a calibration curve constructed from an astaxanthin standard. The calculated astaxanthin concentrations in each oil sample are presented in Table 2.

The quantitative analysis revealed that the astaxanthin content in the oil obtained by maceration was higher than that obtained by Soxhlet extraction. This finding can be attributed to the nature of astaxanthin as a carotenoid highly susceptible to heat, oxidation, and isomerization (Liu *et al*., 2024). During Soxhlet extraction, continuous heating may induce partial degradation or structural transformation of astaxanthin (e.g., trans to cis isomerization), resulting in reduced concentration despite a higher overall oil yield. In contrast, maceration is conducted at lower temperatures, thereby better preserving astaxanthin stability and allowing the pigment to be extracted in a more intact form. These results indicate that oil yield does not necessarily correlate with the retention of bioactive pigments. Thus, maceration is superior in maintaining astaxanthin quality and stability, whereas Soxhlet extraction is more efficient in maximizing oil recovery (X. Zhao *et al*., 2019). The choice of extraction method should therefore be guided by the primary objective, whether prioritizing total oil yield or specific bioactive compounds such as astaxanthin.

Overall, *cincalok* oil exhibited the highest astaxanthin content compared to dried rebon and shrimp paste oils. This difference is largely influenced by the processing method. Dried rebon does not undergo fermentation; consequently, astaxanthin remains in its natural state and at relatively lower levels due to the absence of biochemical mechanisms capable of releasing the pigment from protein–lipid matrices. In shrimp paste, which undergoes aerobic fermentation, microbial activity may hydrolyze cell walls and pigment-binding proteins, facilitating partial astaxanthin release. However, oxygen-rich conditions increase the risk of carotenoid oxidation, leading to pigment degradation. Conversely, the anaerobic fermentation process in *cincalok* enables enzymes and microorganisms to release astaxanthin from protein–lipid complexes without significant oxygen exposure, thereby enhancing pigment availability while minimizing oxidative damage (Rahmalia *et al*., 2022; *et al*., 2020). The combined effects of pigment release during fermentation and protection from oxidation explain the higher astaxanthin content observed in *cincalok* compared to the other two products.

The antioxidant activity of food materials is influenced by the type, concentration, and mechanism of action of their bioactive compounds. Processed rebon products such as dried rebon, shrimp paste, and *cincalok* contain carotenoid pigments, particularly astaxanthin, which function as natural antioxidants. Astaxanthin neutralizes free radicals through hydrogen atom donation, electron transfer, and metal ion reduction. Therefore, more than one analytical method is required to comprehensively evaluate its antioxidant activity. In this study, the antioxidant activity of oils from the three samples was assessed using DPPH, ABTS, and FRAP assays. The DPPH and ABTS methods measure free radical scavenging capacity, whereas FRAP evaluates the reducing power toward ferric ions ($Fe^{3+} \rightarrow Fe^{2+}$). The differing principles of these assays provide a more comprehensive understanding of the antioxidant potential and mechanisms of each sample. Accordingly, the IC_{50} values obtained not only reflect antioxidant strength but also indicate differences in the dominant mechanisms of action of active compounds, particularly astaxanthin, in dried rebon, shrimp paste, and *cincalok*. The following section discusses the IC_{50} results and their relationship to astaxanthin content and dominant antioxidant mechanisms in each assay.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is one of the most widely used techniques for evaluating antioxidant activity due to its simplicity, sensitivity, and effectiveness in measuring radical scavenging capacity. The DPPH radical is stable and exhibits a deep purple color, which decreases in intensity upon reaction with antioxidant compounds through hydrogen atom donation or electron transfer. The extent of absorbance reduction reflects the sample's ability to scavenge free radicals and is expressed as the IC_{50} value. This method was applied to compare the antioxidant activity of oils from dried rebon, shrimp paste, and *cincalok*, as well as to examine the contribution of astaxanthin as the primary active compound in the scavenging mechanism. The IC_{50} values for each sample are presented in Table 3.

Table 2 Astaxanthin Concentration in the Extracted Oil

Sample	Soxhlet Extraction Method (mg/g)	Maceration Extraction Method (mg/g)	Ultrasonication Extraction Method (mg/g)
Dried Rebon Shrimp oil	1.855	1.501	0.891
Terasi oil	5.726	2.555	1.543
Cincalok oil	10.943	8.078	5.444

a b c

Figure 3 Thin-layer chromatography (TLC) profiles of oil extracted from dried rebon shrimp (a), shrimp paste (*terasi*) (b), and fermented shrimp (*cincalok*) (c)

Table 3 Antioxidant Activity of Oils from Dried Rebon, Shrimp Paste (Terasi), and Cincalok

Oil Sample	Extraction Method	IC_{50}
Drying Rebon	Maceration	183.96
	Soxhlet	136.93
	Ultrasonication	192.93
Shirmp Paste (Terasi)	Maceration	137.44
	Soxhlet	124.31
	Ultrasonication	188.13
Cincalok	Maceration	75.23
	Soxhlet	54.58
	Ultrasonication	127.48

Table 3 shows that *cincalok* oil exhibited the highest antioxidant activity compared to shrimp paste (*terasi*) and dried rebon oils. This superiority is primarily attributed to the fermentation process, which enhances the release and stability of astaxanthin and phenolic compounds, thereby increasing the antioxidant capacity of fermented products relative to non-fermented ones (Adebo & Medina-Meza, 2020; Y. S. Zhao *et al*., 2021). Fermentation by lactic acid bacteria also creates an acidic environment that protects pigments from oxidation while generating bioactive peptides and organic acids that contribute synergistically to antioxidant activity (Cabanillas-Bojórquez *et al*., 2021; Mauludia *et al*., 2021). The combination of these factors explains the elevated antioxidant activity observed in *cincalok* oil compared to the other two samples.

Shrimp paste oil demonstrated moderate antioxidant activity. Although extended fermentation may enhance the formation of antioxidant compounds, processes such as salting, intensive drying, and exposure to aerobic conditions can induce partial degradation of astaxanthin, which is highly sensitive to heat, light, and oxygen. Consequently, its antioxidant activity is lower than that of *cincalok* oil but still higher than that of dried rebon oil. Dried rebon oil exhibited the lowest antioxidant activity because it does not undergo fermentation and is susceptible to oxidative degradation during drying. Moreover, the hardened tissue structure may hinder the release of bioactive compounds (Takeungwongtrakul & Benjakul, 2016).

Soxhlet extraction resulted in higher antioxidant activity than maceration and ultrasonication. Continuous solvent contact under controlled heating enhances the solubilization of lipophilic compounds such as astaxanthin, provided that the temperature does not exceed the degradation threshold. In this study, Soxhlet extraction proved to be more effective in extracting antioxidant compounds than the other methods due to continuous solvent circulation at a stable temperature, which improved astaxanthin solubility.

Ultrasonication did not enhance antioxidant activity. Cavitation effects and localized temperature increases may accelerate astaxanthin degradation; therefore, its application for carotenoid extraction must be carefully optimized. Overall, the antioxidant activity of rebon oil is influenced by fermentation processes, astaxanthin content and stability, and the extraction method employed. *Cincalok* oil demonstrated the highest potential, whereas dried rebon oil exhibited the lowest activity, consistent with previous findings emphasizing the importance of integrating processing methods with appropriate extraction techniques.

Based on the DPPH assay, *cincalok* oil showed the strongest antioxidant activity and was therefore further evaluated using ABTS and FRAP assays to elucidate its radical cation scavenging and metal-reducing capacities. However, as presented in Table 4, *cincalok* oil did not demonstrate significant activity according to the ABTS and FRAP mechanisms, indicating that its antioxidant effectiveness is predominantly associated with the free radical scavenging mechanism measured by the DPPH assay.

Table 4. Antioxidant Activity of Oils from Dried Rebon, Shrimp Paste (*Terasi*), and *Cincalok*

Cincalok Oil Samples Extracted Using Different Methods	IC_{50} ABTS	IC_{50} FRAP
Maceration	137.33	147.66
Soxhlet	115.93	125.21
Ultrasonication	149.23	202.60

CONCLUSION

This study demonstrated that Soxhlet extraction produced the highest oil yield, whereas maceration was more effective in preserving astaxanthin stability. *Cincalok* exhibited the highest astaxanthin content (10.943 mg/g) and the strongest antioxidant activity based on the DPPH assay (IC_{50} = 54.58 mg/L). However, its response in the ABTS and FRAP assays was relatively low, indicating that its dominant antioxidant mechanism primarily involves free radical scavenging. The main strength of this study lies in the comprehensive comparison of three extraction methods and three types of processed rebon products. Nevertheless, its limitations include the absence of detailed pigment stability evaluation and in-depth identification of astaxanthin isomers. Further research is therefore recommended to focus on astaxanthin purification, stability assessment, and its potential application in food and health-related products based on local raw materials.

ACKNOWLEDGMENTS

This research was supported by the Ministry of Higher Education, Science, and Technology through a research grant (Grant No. 115/C3/DT.05.00/PL/2025).

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