Indonesian Journal of Pharmaceutical Education (e-Journal) 2025; 5 (3): 302– 310
ISSN: 2775- 3670 (electronic)
Journal Homepage: http://ejurnal.ung.ac.id/index.php/ijpe/index
DOI: 10.37311/ijpe.v5i3.32374

UV–Vis Quantification of β-Carotene in Spinach under Raw and Boiled Conditions

Sumayyah Damanik[1], Anny Sartika Daulay[2*], Ridwanto[3], Fathur Rahman Harun[4]
[1,2,3,4] *Department of Pharmacy, Faculty of pharmacy, Universitas Muslim Nusantara Al Washliyah, Medan, Indonesia, Indonesia*
[*]*E-mail: annysartika@umnaw.ac.id*

Article Info:
Received: 2 June 2025
in revised form: 5 September 2025
Accepted: 2 October 2025
Available Online: 14 October 2025

Keywords:
β-carotene;
Green spinach;
Red spinach;
Boiling;
UV-Vis spectrophotometry

Corresponding Author:
Anny Sartika Daulay
Faculty of Pharmacy
Alwashliyah Muslim Nusantara University
Medan
Indonesia
E-mail:
annysartika@umnaw.ac.id

ABSTRACT

β-Carotene is a provitamin A with strong antioxidant activity but is unstable to heat and oxidation. This study aimed to determine the β-carotene content of green spinach (*Amaranthus viridis* L.) and red spinach (*Amaranthus tricolor* L.) under fresh and boiled conditions using UV–Vis spectrophotometry. The assay was performed at 451 nm after maceration extraction with n-hexane:acetone:ethanol (2:1:1, v/v). Calibration in the range of 4–10 µg/mL showed excellent linearity (y = 0.064x – 0.003, r = 0.999). The results demonstrated that red spinach contained substantially higher β-carotene than green spinach. Boiling slightly increased the content in green spinach (17.16 → 18.76 ± 0.21 mg/g), while it caused a small decrease in red spinach (50.23 → 47.76 mg/g). These findings suggest that boiling has different effects depending on the spinach type, possibly due to a balance between heat-induced degradation and enhanced release from softened plant tissues. In conclusion, red spinach is a richer dietary source of β-carotene, but thermal processing reduces its stability. The validated UV–Vis spectrophotometric method is suitable for routine determination of β-carotene in leafy vegetables.

How to cite (APA 6th Style):
Damanik,S.,Daulay,A.S., Ridwanto.,Harun,F.R. (2025). *UV–Vis Quantification of β-Carotene in Spinach under Raw and Boiled Conditions*. *Indonesian Journal of Pharmaceutical Education (e-Journal)*, *5*(3), 302-310.

ABSTRAK

β-karoten merupakan provitamin A yang berperan sebagai antioksidan kuat, tetapi tidak stabil terhadap panas dan oksidasi. Penelitian ini bertujuan untuk menentukan kadar β-karoten pada bayam hijau (*Amaranthus viridis* L.) dan bayam merah (*Amaranthus tricolor* L.) dalam kondisi segar dan setelah perebusan menggunakan metode spektrofotometri UV–Vis. Analisis dilakukan pada panjang gelombang 451 nm setelah ekstraksi maserasi dengan n-heksan:aseton:etanol (2:1:1, v/v). Kurva kalibrasi pada rentang 4–10 µg/mL menunjukkan linearitas yang sangat baik (y = 0,064x – 0,003; r = 0,999). Hasil penelitian menunjukkan bahwa bayam merah memiliki kadar β-karoten lebih tinggi dibanding bayam hijau. Perebusan sedikit meningkatkan kadar pada bayam hijau (17,16 → 18,76 ± 0,21 mg/g), tetapi menurunkan kadar pada bayam merah (50,23 → 47,76 mg/g). Temuan ini mengindikasikan bahwa perebusan memberi efek berbeda tergantung jenis bayam, kemungkinan akibat keseimbangan antara degradasi termal dan pelepasan β-karoten dari jaringan yang melunak. Kesimpulannya, bayam merah merupakan sumber β-karoten yang lebih kaya, namun pengolahan panas dapat menurunkan stabilitasnya. Metode spektrofotometri UV–Vis ini valid dan layak digunakan untuk penetapan rutin β-karoten pada sayuran berdaun.

Kata Kunci: β-karoten; Bayam hijau; Bayam merah; Perebusan; Spektrofotometri UV–Vis

1. Introduction

Vitamins are essential micronutrients; within this group, β-carotene functions as a provitamin A and antioxidant due to its conjugated double-bond system, yet it is susceptible to oxidation and heat-induced degradation during processing [1],[2],[3]. Leafy vegetables provide practical dietary β-carotene sources. In Indonesia, green spinach (*Amaranthus viridis* L.) and red spinach (*Amaranthus tricolor* L.) are widely consumed, and their agronomic and compositional traits can influence β-carotene extractability and measured content [4],[5],[6].

Processing conditions—particularly temperature, light, and exposure time—can shift the balance between loss processes (oxidation and trans-to-cis isomerization that reduce the strongly absorbing species) and release processes (matrix softening that improves solvent access). Consequently, the net effect of boiling may differ by spinach type due to differences in leaf microstructure, pigment localization, and lipid environment reported for *Amaranthus* leaves [6],[7],[8].

UV–Vis spectrophotometry at 451 nm is a practical approach for β-carotene quantification when extraction and calibration are controlled, enabling rapid measurement suitable for food matrices [9],[10],[11]. However, comparative data that directly contrast *A. viridis* and *A. tricolor* under a simple household treatment (boiling) using a uniform extraction protocol remain scarce in the local context [4],[9],[11].

Accordingly, this study quantifies β-carotene in green and red spinach under fresh and boiled conditions by UV–Vis at 451 nm, and interprets boiling effects mechanistically as the interplay between thermal degradation/isomerization and matrix-driven release. The results aim to inform cooking practices that better preserve β-carotene in leafy vegetables.

2. Methods

Materials and samples

Fresh leaves of green spinach (*Amaranthus viridis* L.) and red spinach (*Amaranthus tricolor* L.) were obtained from a traditional market, sorted, rinsed, and drained. For the boiled condition, leaves were immersed in boiling water for 6 min, drained, cooled to room temperature, and homogenized; fresh samples were homogenized without heating [4],[5].

Chemicals and reagents

β-Carotene analytical standard; n-hexane, acetone, ethanol, petroleum ether, and benzene (all p.a. grade); and distilled water were used as received [1],[6].

Extraction

For each condition (fresh/boiled × green/red), 100 g of leaf homogenate was macerated for 48 h at room temperature using n-hexane:acetone:ethanol (2:1:1, v/v). The filtrate was washed with distilled water in a separatory funnel; the organic phase was collected and evaporated on a water bath to obtain a viscous extract [6],[7].

Instrumentation and analytical wavelength

UV–Vis measurements were performed at 451 nm (ethanol blank). The analytical wavelength was confirmed from the β-carotene absorption maximum within 410–465 nm, yielding λmax = 451 nm for the standard solution [6],[12].

Calibration and Method Performance (UV–Vis, 451 nm)

Calibration standards of 4, 5, 6, 8, and 10 µg/mL (ethanol) were prepared from a 1000 µg/mL stock. Absorbance at 451 nm produced a linear regression $y = 0.064x - 0.003$ with $r = 0.999$ over 4–10 µg/mL [2],[12]. Routine performance checks included: linearity (slope, intercept, r), repeatability at a mid-level extract solution reported as %RSD, and sensitivity estimated from calibration statistics (LOD = 3.3·(Sy/x)/slope, LOQ = 10·(Sy/x)/slope). Measurements were made against ethanol blanks with baseline stability verified per batch [13],[14].

Sample assay

Approximately 50 mg extract was brought to 25 mL with ethanol, filtered if necessary, and read at 451 nm against an ethanol blank. Concentrations were interpolated from the calibration curve and expressed as mg β-carotene per g fresh leaves. Each condition was analyzed in sextuplicate (n = 6) to match the statistical treatment in Results [5],[12].

3. Results and Discussion

Calibration and Method Performance (UV–Vis, 451 nm)

Measurements were carried out at 451 nm, the visible absorption maximum of β-carotene under our solvent system (ethanol). A standard scan of the reference solution provided λ_max = 451.4 nm (A = 0.499), confirming the analytical wavelength used for quantification. Routine wavelength checks, blank/baseline verification, and cuvette cleanliness were implemented in accordance with good UV–Vis practice and compendial guidance [2],[14].

To minimize handling bias, extractions and absorbance readings were performed under reduced light to limit photo-oxidation of carotenoids [3],[11]. The reference spectrum is shown in **Figure 1**.

Linearity was established over 4–10 µg/mL using β-carotene standards prepared in ethanol. At 451 nm, the calibration produced $y = 0.0642x - 0.0027$, $r = 0.9996$, and $s_{y/x} = 0.0072$ A, indicating excellent adherence to the Beer–Lambert law in the working range. The observed Mean ± SD absorbances (n = 3 reads/level) were 0.243 ± 0.004 A (4 µg/mL), 0.319 ± 0.003 A (5 µg/mL), 0.391 ± 0.005 A (6 µg/mL), 0.510 ± 0.004 A (8 µg/mL), and 0.638 ± 0.006 A (10 µg/mL). These behaviors are consistent with commonly reported responses for β-carotene in plant matrices when calibration and extraction are properly controlled [5],[13]. A compact summary of the calibration data is presented in **Table 1**.

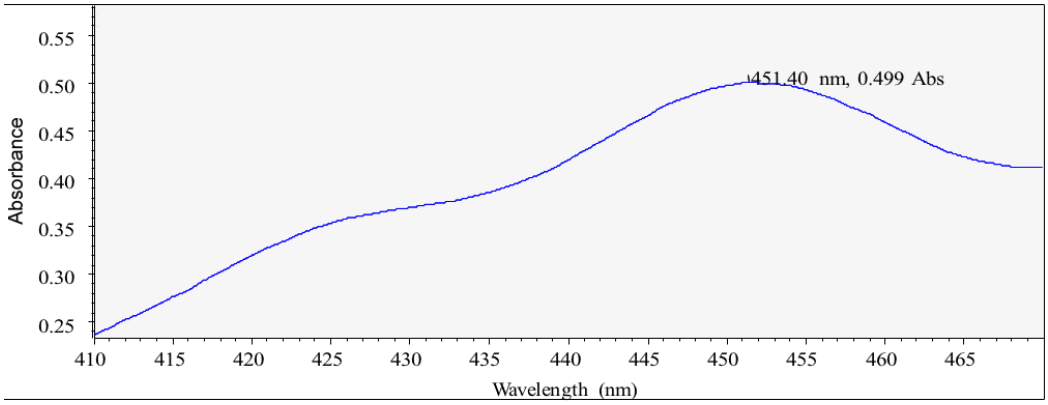

Figure 1. UV–Vis spectrum of β-carotene in ethanol with λ_max at **451.4 nm** (A = 0.499); **451 nm** was used for quantification

Blank and baseline performance supported quantitative use at a single wavelength. The ethanol blank measured ≈ 0.000 ± 0.001 A (n = 5) with baseline drift ≤ 0.002 A across runs, indicating negligible background contribution and stable instrumental conditions [2],[14]. Short-term precision was evaluated at the mid-level (6 µg/mL) using n = 6 consecutive readings, yielding 0.391 ± 0.004 A (%RSD ≈ 1.0%). This repeatability meets typical expectations for spectrophotometric assays and supports day-to-day usability for routine determinations [2],[10].

Table 1. Method performance data for β-carotene by UV–Vis at **451 nm** (Mean ± SD). Calibration levels (n = 3/level), mid-level repeatability at **6 µg/mL** (n = 6), ethanol blank, and sensitivity (LOD, LOQ)

Category	Parameter	Result (Mean ± SD)
Wavelength	λ_max (n = 3 scans)	451.4 ± 0.6 nm
Linearity (Absorbance)	4 µg/mL (n = 3 reads)	0.243 ± 0.004 A
	5 µg/mL (n = 3 reads)	0.319 ± 0.003 A
	6 µg/mL (n = 3 reads)	0.391 ± 0.005 A
	8 µg/mL (n = 3 reads)	0.510 ± 0.004 A
	10 µg/mL (n = 3 reads)	0.638 ± 0.006 A
Precision (repeatability)	6 µg/mL (n = 6 consecutive reads)	0.391 ± 0.004 A *(%RSD 1.0%)*
Baseline control	Ethanol blank (n = 5)	0.000 ± 0.001 A
Sensitivity	LOD	0.37 ± 0.05 µg/mL
	LOQ	1.12 ± 0.14 µg/mL

Note: *Calibration/performance at 451 nm in ethanol (ethanol blank). Working range 4–10 µg/mL; linear fit y = 0.0642x − 0.0027 (r = 0.9996), s_y/x = 0.0072 A. LOD = 3.3σ/S and LOQ = 10σ/S (σ = regression standard error; S = slope), consistent with USP <857> and ICH Q2(R2); food-matrix context may refer to AOAC carotenoid methods [14],[15],[16].*

Sensitivity was estimated from the regression standard error (σ = s_y/x) and the slope (S) using widely applied validation relations, LOD = 3.3 ·σ/S and LOQ = 10 ·σ/S [15]. The calculated values were LOD ≈ 0.37 µg/mL and LOQ ≈ 1.12 µg/mL, aligning with performance expected for β-carotene UV–Vis assays provided that extraction and handling minimize light-induced changes [14],[16]. Taken together verified λ_max,

linear response with tight residuals, stable blanks, low mid-level %RSD, and sub-µg/mL sensitivity the method demonstrates fitness-for-purpose for routine β-carotene quantification in spinach extracts.

β-Carotene Content Across Conditions (Green vs Red; Fresh vs Boiled)

On a fresh-weight basis (n = 6), measured β-carotene contents were 17.16 mg/g (green–fresh), 18.76 ± 0.21 mg/g (green–boiled), 50.23 mg/g (red–fresh), and 47.76 mg/g (red–boiled) (Table 2). In both conditions, red spinach contained markedly more β-carotene than green spinach, consistent with documented differences in pigment composition and leaf traits among *Amaranthus* species that can influence extractability and apparent content [4],[7],[8].

For external context, spectrophotometric literature for *Amaranthus* leaves reports substantially lower absolute values on a fresh-weight basis—approximately 0.24 mg/g for fresh *A. viridis* (decreasing to 0.12 mg/g after blanching) and around 0.015 mg/g (fresh) to 0.0085 mg/g (boiled) for *A. hybridus*—reflecting differences in species, reporting basis, solvent systems, extraction kinetics, and time–temperature parameters [17],[18]. Such methodological and matrix factors should be considered when comparing studies using visible spectrophotometry [2],[5],[10].

Table 2. β-carotene content (mg/g, fresh-weight basis) in green and red spinach under fresh and boiled conditions

Sample (species)	Treatment	Content (mg/g FW)	Δ% (Boiled vs Fresh)	Literature (mg/g FW)
Green spinach (*Amaranthus viridis* L.)	Fresh	17.16	—	0.240 (24.01 mg/100 g) [18]
	Boiled	18.76 ± 0.21	+9.3%	0.123 (12.28 mg/100 g; blanched 6 min) [18]
Red spinach (*Amaranthus hybridus* L.)	Fresh	50.23	—	0.0146 ± 0.000006 (14.6 ± 0.00575 mg/kg) [17]
	Boiled	47.76	−4.9%	0.0085 ± 0.000002 (8.50 ± 0.001703 mg/kg) [17]

Note: *Reported on a fresh-weight basis (mg/g). Values are means; SD shown where available (n = 6). Δ% = ((Boiled − Fresh)/Fresh) × 100. Literature values come from spectrophotometric studies on Amaranthus leaves and are converted to mg/g FW; original units (mg/100 g or mg/kg FW) are given in parentheses [17],[18]. Differences from literature likely reflect species, extraction, and cooking conditions.*

Effect of Boiling Within Each Spinach Type

Within-type comparisons (see **Table 2**) showed opposite responses to boiling: green spinach increased (Δ% = +9.3), whereas red spinach decreased (Δ% = −4.9). The percent change was calculated as ((Boiled − Fresh)/Fresh) × 100 on a fresh-weight basis using the reported means. These results indicate that the direction and magnitude of the boiling effect depend on spinach type.

Analytical conditions were controlled and verified at 451 nm (λ max, linearity, blanks, repeatability; **Figure 1**, **Table 1**), supporting confidence in the comparative trends across conditions. While SD is available for green–boiled (18.76 ± 0.21 mg/g), the fresh and red–boiled entries are currently reported as means; the contrasts are nevertheless informative at the descriptive level [2],[10].

Boiling was applied under a single standardized protocol, so the divergent outcomes more likely reflect intrinsic matrix features of the two spinach types than short-term analytical variability. Variables known to influence quantitative outcomes—

leaf maturity, moisture/fiber, chromoplast integrity, and extraction kinetics—were kept consistent within each type during sampling and processing [2],[5],[10].

In summary, boiling produced a small gain in measured β-carotene for green spinach and a modest loss for red spinach. The underlying reasons are discussed in the mechanistic rationale that follows, to avoid over-interpreting the descriptive contrasts here [3],[5],[11].

Mechanistic Rationale: Degradation/Isomerization vs Matrix Softening

Two competing processes can account for the opposite boiling responses. First, thermal reactions during moist heating reduce the strongly absorbing β-carotene species detected at 451 nm, lowering the UV–Vis signal (loss pathway). Oxygen exposure and heating-induced disruption of chromoplast structures facilitate formation of less-absorbing products and altered isomer populations [3],[11].

Second, heating softens tissues and can enhance extractability (release pathway). Loosening of cell-wall polymers, membrane phase changes, and plastid disintegration increase solvent access to pigment-containing compartments, allowing more β-carotene to partition into the extraction solvent when loss processes are comparatively modest [2],[5].

Which pathway dominates is matrix-specific. Spinach types differ in leaf microstructure, pigment localization, and lipid environment, all of which govern susceptibility to loss versus release under a given time–temperature profile [4],[7],[8]. Handling practicesn light protection, prompt measurement, and a consistent solvent system further tilt this balance in visible-spectrophotometric assays [2],[5],[11].

Viewed through this lens, a loss-dominated regime explains the net decrease observed for red spinach, whereas a release-favored regime explains the small increase for green spinach under identical boiling conditions. Similar matrix-dependent outcomes have been documented for *Amaranthus* leaves and related vegetables analyzed by UV–Vis methods, emphasizing the need to control cooking parameters and extraction kinetics when comparing datasets across studies [2],[5],[18].

Mechanistic Context, Comparison with Prior Reports, and Practical Implications

Opposite boiling responses in the two spinach types can be understood as the net result of two competing pathways: moist heat can reduce the strongly absorbing β-carotene species detected at 451 nm (loss), while tissue softening enhances solvent access and releases pigments into the extract (release). Which pathway dominates depends on leaf microstructure, pigment localization, lipid environment, and handling (light protection, prompt reading) [3],[11].

When set against prior spectrophotometric reports on *Amaranthus* leaves, our fresh-weight values are markedly higher. Literature benchmarks are about 0.24 mg/g for fresh *A. viridis* (≈0.12 mg/g after blanching) and ~0.015 mg/g (fresh) to ~0.0085 mg/g (boiled) for *A. hybridus*, illustrating how species, reporting basis, and processing can shift absolute levels [17],[18].

Methodological choices further contribute to variability. Solvent system, extraction time, and the time–temperature profile during cooking modulate both isomerization/oxidation and pigment release, while calibration/blank handling at 451 nm influences the readout in visible spectrophotometry; careful standardization and transparent reporting are therefore essential for meaningful cross-study comparisons [2],[10].

For household preparation, brief boiling may reduce quantifiable β-carotene in red spinach, whereas green spinach can show minimal losses or small gains—an outcome consistent with a matrix-specific balance between destruction and release. Practical comparability improves when cooking and extraction parameters are kept consistent or at least reported in sufficient detail [2],[5].

This interpretation should be viewed alongside several limitations: reliance on a single spectrophotometric technique (UV–Vis at 451 nm) without orthogonal confirmation, one cooking method and duration, and no cultivar genotyping or continuous internal temperature logging. Future work should address accuracy, intermediate precision, robustness, and specificity ideally with an orthogonal method on a subset of samples to strengthen external comparability.

4. Conclusion

Using a calibrated UV–Vis procedure at 451 nm, β-carotene in spinach was quantified with acceptable linearity, repeatability, and sensitivity. Across the four conditions, red spinach (*Amaranthus tricolor* L.) consistently contained more β-carotene than green spinach (*A. viridis* L.). Boiling produced opposite within-type effects, with a small increase in green spinach and a modest decrease in red spinach, indicating a matrix-specific balance between thermal degradation/isomerization and improved extractability from softened tissues. Absolute contents in this study differed from several external reports, plausibly due to differences in species/cultivar, reporting basis, solvent and extraction kinetics, and heating parameters. Overall, the method is fit-for-purpose for routine screening of β-carotene in spinach, and the findings underscore the need to standardize cooking and extraction conditions for cross-study comparisons. Future work should include recovery (accuracy), intermediate precision, robustness testing, and orthogonal specificity (e.g., HPLC) to strengthen external comparability.

Acknowledgements

The authors gratefully acknowledge the Department of Pharmacy, Faculty of Pharmacy, Universitas Muslim Nusantara Al Washliyah, Medan, Indonesia, for academic support and laboratory facilities during this study. We also thank our colleagues and the anonymous reviewers for their constructive feedback that improved the manuscript.

Conflict of Interest:

The authors declare no conflict of interest related to this study.

References

[1] D. S. Purwanto, D. F. Guntarti, and R. Hidayah, "Effect of purification on active compounds and antioxidant activity of 50% ethanol extract of *Moringa oleifera* L. leaves," *Jurnal Farmasi Indonesia*, vol. 18, no. 2, pp. 97–108, 2021. [Online]. Available: https://doi.org/10.31001/jfi.v18i2.1232

[2] A. Kusbandari and S. Hari, "β-Carotene content and DPPH free-radical scavenging activity of cantaloupe (*Cucumis melo* var. *cantalupensis* L.) fruit extract by UV–Vis spectrophotometry," *Jurnal Farmasi Sains dan Komunitas*, vol. 14, no. 1, pp. 37–42, 2017. [Online]. Available: https://doi.org/10.24071/jpsc.00562

[3] D. S. Rejeki, A. N. Cahyanta, and I. A. Arfiyani, "Effect of steaming on β-carotene content of purple variety sweet potato (*Ipomoea batatas* L.) leaves by UV–Vis spectrophotometry," *Jurnal Kimia Saintek dan Pendidikan*, vol. 5, no. 2, pp. 46–54,

2021. [Online]. Available: https://e-journal.sari-mutiara.ac.id/index.php/KIMIA/article/view/2480

[4] E. S. Ponggele and K. D. Jayanti, "Growth and yield of spinach (*Amaranthus spinosus* L.) in various growing media," *Agropet*, vol. 12, no. 2, pp. 17–22, 2020. [Online]. Available: http://dx.doi.org/10.71127/2828-9250.174

[5] F. Kondororik, M. Martosupono, and A. Susanto, "The role of β-carotene in the immune system for cancer prevention," *Jurnal Biologi & Pembelajarannya*, vol. 4, no. 1, pp. 1–8, 2017. [Online]. Available: https://doi.org/10.29407/jbp.v4i1.667

[6] A. N. Fajri and Suparti, "Hydroponic growth of green spinach (*Amaranthus hybridus* L.) using shallot-waste extract," in *Proc. SNPBS (National Seminar on Biology and Science Education)*, 2022, pp. 232–237. [Online]. Available: https://proceedings.ums.ac.id/snpbs/article/view/1764

[7] B. Chandra, Zulharmita, and A. D. H. Handayani, "Analysis of β-carotene content in red spinach (*Amaranthus hybridus* L.) leaves by visible spectrophotometry," *Jurnal Farmasi Higea*, vol. 9, no. 2, pp. 149–158, 2017. [Online]. Available: http://dx.doi.org/10.52689/higea.v9i2.170

[8] A. N. Rahayu, S. N. N. Yuliani, and D. Mutiasari, "Effect of boiling on vitamin C decrease in *Stenochlaena palustris*," *Indonesian Journal of Clinical Nutrition Physician*, vol. 5, no. 1, pp. 82–93, 2022. [Online]. Available: https://doi.org/10.54773/ijcnp.v5i1.102

[9] S. Misfadhila, Rusdi, B. Chandra, and A. Yunita, "Determination of β-carotene in several dried and fresh chili varieties by UV–Vis spectrophotometry," *Jurnal Farmasi Higea*, vol. 12, no. 1, pp. 75–80, 2020. [Online]. Available: http://dx.doi.org/10.52689/higea.v12i1.266

[10] M. A. D. Putra, S. Y. Lumbessy, and D. N. Setyowati, "Addition of red spinach (*Amaranthus tricolor* L.) flour in koi fish (*Cyprinus carpio* L.) feed to improve color quality," *Samakia: Jurnal Ilmu Perikanan*, vol. 13, no. 2, pp. 134–146, 2022. [Online]. Available: https://doi.org/10.35316/jsapi.v13i2.1317

[11] N. Johar and M. Mustikaningrum, "Evaluation of β-carotene extraction rate constants based on acetone and diethyl ether solvent mixtures," *REACTOR: Journal of Research on Chemistry and Engineering*, vol. 4, no. 1, pp. 19–25, 2023. [Online]. Available: https://doi.org/10.52759/reactor.v4i1.69

[12] A. Agustina, N. Hidayati, and P. Susanti, "Determination of β-carotene in raw and boiled carrots (*Daucus carota* L.) by visible spectrophotometry," *Jurnal Farmasi Sains dan Praktis*, vol. 5, no. 1, pp. 6–10, 2019. [Online]. Available: https://doi.org/10.31603/pharmacy.v5i1.2293

[13] A. F. Gombart, A. Pierre, and S. Maggini, "A review of micronutrients and the immune system—working in harmony to reduce the risk of infection," *Nutrients*, vol. 13, no. 11, pp. 1–41, 2021. [Online]. Available: https://doi.org/10.3390/nu13114180

[14] R. C. Moreton, "United States Pharmacopeia–National Formulary," *International Journal of Pharmaceutical Excipients*, vol. 6, no. 3, 2016. [Online]. Available: https://jefc.scholasticahq.com/article/925.pdf

[15] ICH, *Q2(R1): Validation of Analytical Procedures – Text and Methodology*. Geneva, Switzerland: International Council for Harmonisation, 2005. [Online]. Available: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf

[16] AOAC International, *Official Methods of Analysis of AOAC International*, 22nd ed. Rockville, MD, USA: AOAC International, 2023. [Online]. Available: https://doi.org/10.1093/9780197610145.001.0001

[17] U. Sarker and S. Oba, "Leaf pigmentation, its profiles and radical scavenging activity in selected Amaranthus tricolor leafy vegetables," Scientific Reports, vol. 10, no. 1, 18617, 2020. [Online]. Available: https://doi.org/10.1038/s41598-020-66376-0

[18] A. Chib, A. Bhat, J. D. Bandral, and M. Trilokia, "Effect of thermal processing on nutritional and anti-nutritional factors of amaranthus (*Amaranthus viridis* Linn.) leaves," *The Pharma Innovation Journal*, vol. 11, pp. 385–389, 2022. [Online]. Available: https://www.thepharmajournal.com/archives/?year=2022&vol=11&issue=4&ArticleId=11785