

ORIGINAL RESEARCH ARTICLE

Green Extraction and Sustainability Assessment of Total Carotenoids from Carrot (*Daucus carota*) Peel Waste using Soybean Oil

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ABSTRACT

The environmental and health impacts of organic solvent extraction for carotenoid recovery highlight the need for a greener, more sustainable process. This study investigates the use of soybean oil as a green, environmentally friendly alternative for recovering carotenoids from carrot (*Daucus carota*) peels, offering a zero-waste approach to conventional, often toxic organic solvents. A two-factor, three-level full factorial experimental design was employed to evaluate and optimize the temperature (30, 45, and 60 °C) and the liquid-solid (L/S) ratio (20, 30, and 40 mL:g). In addition, Total Carotenoid Content (TCC) was quantified with UV-Visible spectrophotometry. Statistical analyses showed that the L/S ratio primarily drove extraction efficiency ($p < 0.01$), thereby improving mass transfer and matrix penetration. Temperature ($p < 0.01$) and its interactions with the L/S ratio ($p < 0.01$) also significantly enhanced cell wall permeability. The model was reduced, as quadratic terms were insignificant ($p > 0.05$). The optimal predicted conditions of the reduced model yielded $77.49 \pm 13.13 \mu\text{g } \beta\text{-carotene/g}$ of dried peel at a 40 L/S ratio and 30 °C, with validation runs yielding $76.04 \pm 3.71 \mu\text{g } \beta\text{-carotene/g}$ of dried peel. A final score of 0.59 based on the *Path2Green* sustainability metrics suggests that the carotenoid extraction process is eco-friendly and low-impact, especially high (+1.00) in biomass utilization, post-treatment, scaling, transport, waste, purification, application, and the use of eco-friendly solvents. These findings demonstrate that soybean oil is an effective and sustainable solvent alternative for carotenoid recovery, offering a practical option for greener industrial processes.

Keywords: Carotenoids, carrot peels, full factorial, soybean oil, green extraction, optimization

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INTRODUCTION

With a continuous rise in demand for natural and synthetic additives in food, pharmaceutical, and textile production, the market value of carotenoids is steadily increasing. Apart from playing a crucial role in various natural processes, such as photosynthesis, these bioactive compounds are noted for their rich yellow, orange, and red pigmentation and for their beneficial antioxidant, anti-inflammatory, anticancer, antibacterial, and neuroprotective properties (Masyita et al., 2025). Due to the wide range of food and health applications of carotenoids, the universal carotenoid market was valued at USD 1.40 billion in 2018 and is forecast to reach USD 1.85 billion by the end of 2026, growing at a compound annual growth rate of 3.57% (Yaqoob et al., 2021). In Southeast Asian (SEA) countries such as the Philippines, natural colorants, including carotenoids, have become more relevant in local markets as consumers' preferences for clean-label products have grown (Hermosura et al., 2025). Their

prominence in manufacturing industries has significantly contributed to the shift towards green and sustainable technologies, particularly as agricultural byproducts have been found to remain abundant sources of carotenoids (Kultys and Kurek, 2022).

Carrots (*Daucus carota*), one of the most commonly consumed vegetables, are an exceptionally rich source of carotenoids and dietary fibers (Mantiniotou et al., 2024). With a high beta-carotene content, these orange-colored crops are considered the best source of vitamin A among all vegetables, offering benefits in reducing the risk of conditions such as eye and heart-related diseases. They are typically used for dishes or eaten raw as snacks or garnish (Antolin and Malanon, 2024). However, processing carrots generates volumes of organic residues, including peels, pomace, cores, and tops. To lessen its environmental toll and further utilize the crop's valuable properties, many have begun practicing waste valorization strategies, including pigment extraction (Butt et al., 2026).

Carotenoid extraction typically involves the use of solvents that penetrate the solid matrix, where solubilization then occurs (Viñas-Ospino et al., 2023). To ensure efficient and effective recovery of target compounds, solvent and compound compatibility is crucial. While organic solvents, such as hexane and ethanol, were noted as effective and efficient for extracting significant amounts of carotenoids, their use in large quantities may eventually lead to negative health, safety, and environmental issues (Menezes Silva et al., 2023; Rini et al., 2022). For instance, hexane solvents were found to have detrimental effects on the nervous, endocrine, and reproductive systems of highly exposed individuals due to their composition of toxic metabolites (i.e., n-hexane, 2, 5-hexanedione) (Cravotto et al., 2022). Additionally, like most volatile organic compounds (VOCs), these chemicals are potential fire hazards and air and water contaminants, posing risks to the safety and well-being of human and animal habitats (Kumar and Deak, 2025). Hence, efficient yet eco-friendly alternatives, such as natural deep eutectic solvents (NaDES) and vegetable oils, that leverage the lipophilic and hydrophobic nature of carotenoids are being explored. (Viñas-Ospino et al., 2023).

While NaDES are currently considered the “golden standard” for green solvents, their efficiency in carotenoid extraction remains limited by their viscosity (Joshi et al., 2025; Vladić et al., 2023). High viscosity NaDES inhibits solute and solvent mass transfer, leading to increased extraction time and decreased efficiency (Joshi et al., 2025; Vladić et al., 2023). Although adding water helps modulate viscosity, excessive water content can negatively affect the solubilization of hydrophobic and lipophilic compounds, such as carotenoids, resulting in decreased extraction efficiency and compound instability (Badea et al., 2025; Dai et al., 2015). Additionally, while NaDES are generally recognized as safe, they are relatively novel to the food industry, leaving gaps in post-processing and purification-free applications (Martins et al., 2025). Meanwhile, vegetable oils are generally non-volatile, safe, renewable, and reasonably priced, making them analogous alternatives to NaDES. Additionally, they mitigate extract degradation by inhibiting the oxidation of

compounds (Viñas-Ospino et al., 2023). By leveraging their complex triglyceride composition, vegetable oil-based solvents exhibit a notable selective solubility for both polar and non-polar bioactive compounds. While NaDES and vegetable oils face similar viscosity-induced kinetic hurdles for pigment recovery, workarounds for NaDES viscosity typically involve the addition of water, which, when not optimized, may result in thermodynamic limitations in terms of target compound solubility and stability due to the modification of hydrogen bond networks (Jauregi et al., 2024). Meanwhile, the viscosity of vegetable oils can be readily modulated via physical methods, such as UAE and MAE, without compromising the thermodynamic affinity and oxidative stability of the target compounds (Panthakant Janepinich et al., 2023). Various studies have shown that the direct or optimized application of vegetable oils as solvents for extraction resulted in the efficient recovery of carotenoids from crops and waste materials (Drosaki et al., 2025; Sachindra and Mahendrakar, 2005, as cited in Yara-Varón et al., 2017).

In particular, soybean oil is considered the most efficient and promising solvent for carotenoid extraction. Soybean oil is inexpensive and widely used. In addition, the solvent offers notable characteristics, such as being non-flammable, non-volatile, and non-toxic, all of which contribute to its high extraction efficiency of carotenoids (Cardoso et al., 2024; Ishikiriya et al., 2024). For instance, in a study by Cardoso et al. (2024), soybean oil paired with UAE was found to be the most effective in extracting various bioactive compounds, such as carotenoids (69% recovery), from *Capsicum chinense* Jacq. among all the vegetable oils tested as solvents for extraction. Due to its lower viscosity, soybean oil facilitates better mass transfer compared to other oils, which are relatively thicker. It was also noted that it helps with maintaining the stability of carotenoid extracts even after months of storage (Ishikiriya et al., 2024). Table 1 shows a summary of related papers on the performance of various solvents when used for carotenoid extraction.

Table 1. Performance of various solvents for the recovery of carotenoids from various biomass.

Study	Biomass	Extraction Solvent	Extraction method	Parameters	Recovery of Carotenoids
Sachindra and Mahendrakar (2005)	<i>Penaeus indicus</i> waste	Sunflower oil	Modified method of (Chen and Meyers, 1982)	L/S 2:1 mL/g, 150 ml n, 70 °C	Carotenoid yield: 26.3 ± 2.31 µg/g shrimp waste
Drosaki et al. (2025)	<i>Prunus</i> sp.	Soybean oil	Ultrasound-assisted Extraction	55 °C; S/L 3 g/10 m; 80% amplitude level, 50 min	TCC: 0.672 mg/100 g of dry peel
Rini et al. (2022)	<i>Daucus carota</i> subsp. <i>sativus</i>	Hexane	Maceration	Sample moisture content ≤ 12 %, 5 h sonication	TCC: 147.06 ± 2.66 mg/100g
Menezes Silva et al. (2023)	<i>Bactris gasipaes</i> Kunth peels	Ethanol	Ultrasound-assisted Extraction	S/L 1:10 w/v, 25 °C, 5 min	TCC: 69.88 ± 0.60 mg/100 g, dry weight
Badea et al. (2024)	<i>Solanum lycopersicum</i> L. waste	1:5 Choline Chloride /1,3-Butanediol	Ultrasound-assisted Extraction	65 °C, 70 W, 12 min, S/L 1:20 w/v	20.69 ± 0.33 β carotene a, 21.51 ± 0.43 lycopene a, 13.09 ± 0.90 tocopherol a, 0.09 ± 0.01 lutein a
Cardoso et al. (2024)	Mature <i>Capsicum chinense</i> Jacq. from Igarapé-Açu, Pará, Brazil.	Soybean oil	Ultrasound-assisted Extraction	constant at 800 W, 20 kHz	TCC: 38.22 ± 0.16 µg lutein/g DW
Ishikiriya et al., (2024)	<i>Musa acuminata</i> 'Dwarf Cavendish'	Soybean oil	Solid-liquid extraction	50 °C, S/L 1:6, 300 rpm agitation	TCC: 756 µg of β-carotene/mL of oil, 55% recovery
Joshi et al. (2025)	<i>Tagetes</i> sp.	3:1 Choline chloride/ Glucose NaDES	Ultrasound-assisted Extraction	Ultrasonication (35 min), incubation (25 °C), incubation time (2 h)	TCC: 971.31 µg/mg DW

Note: L/S – liquid-to-solid ratio; w/v – weight: volume ratio; TCC - Total Carotenoid Content; S/L – solid-to-liquid ratio; a - mg/100 g tomato residue; DW – dry weight

While soybean oil has been noted as an effective solvent for carotenoid extraction, solid-liquid extraction techniques also depend on other factors such as liquid-to-solid mass ratio and temperature (Ling and Hadinoto, 2022). Increasing the liquid-to-solid mass ratio dilutes dissolved carotenoid concentration and increases the concentration gradient (Norshazila et al., 2017). Increasing the amount of solvent results in a more rapid yet thorough extraction, as it increases the solvent's loading capacity, preventing the chemical system from reaching equilibrium too early and allowing greater diffusion of bioactive compounds out of the plant material (Septiani et al., 2021). Moreover, these facilitate non-stationary mass transfer of the solute and improved solvent penetration into the carotenoid components, which aid in the efficient recovery of the bioactive components (Norshazila et al., 2017). Furthermore, temperature plays a key role in oil-based solvent extraction methods. A positive correlation between extraction yield and temperature (30°C to 50°C) was observed after oleic acid, usually found in vegetable oils, was used to recover carotenoids from carrot pomaces (Vo et al., 2023). Performing the extraction at moderate temperatures reduced viscosity and improved the solubility of the solvent, thereby driving cavitation and boosting the mass transfer of carotenoid compounds into oleic acid. However, it was also noted that higher temperatures pose risks in carotenoid degradation and reduced extraction efficiency (Badea et al., 2025; Kultys and Kurek, 2022; Vo et al., 2023). Hence, optimizing extraction parameters is a viable approach to ensuring effective and efficient recovery of carotenoid compounds.

Apart from efficiency, this method of extraction also promotes sustainability with its adherence to the principles of Path2Green—a promising comprehensive framework for assessing the sustainability of any extraction process, comprising 12 principles, namely: biomass, transport, pre-treatment, solvent, scaling, purification, yield, post-treatment, energy, application, repurposing, and waste management. It considers factors such as waste generation, resource depletion, and energy consumption, aiming to provide a more refined and holistic perspective on all aspects of extraction processes, from biomass collection to method completion (de Souza Mesquita et al., 2024).

In line with the need for efficient, effective, and eco-friendly methods of pigment extraction, this study aims to utilize soybean oil as a green solvent for the recovery of carotenoids from carrot peels. To further enhance the extraction process, a full-factorial optimization will be performed on two crucial variables: liquid-to-solid ratio and temperature. Additionally,

the environmental viability of the extraction process was evaluated using the Path2Green metrics. By utilizing materials and techniques that adhere to green chemistry principles, this study contributes to realizing the full potential of eco-friendly solvents, waste valorization, and sustainable technologies.

MATERIALS AND METHODS

Materials and chemicals

Hexane was obtained from RCI Labscan Ltd., Bangkok, Thailand. Soybean oil was acquired from a local chemical supplier in Makati City, Philippines. Carrot (*Daucus carota*) peels were sourced locally around Metro Manila, Philippines. The peels were cleaned and freeze-dried (Scientz-18N, Ningbo Scientz Biotechnology Co., Ltd., China) for 72 hours. The resulting peels were further processed by pulverization in a mixer grinder and filtered through a 60-mesh sieve. It was stored in a dark and moisture-free environment before extraction.

Experimental Design

The study used a two-factor, three-level full factorial design to optimize carotenoid extraction yield from carrot peels (Equation 1). The observed values were then modelled after a second-order polynomial equation:

$$TCC = \beta_0 + \beta_a A + \beta_b B + \beta_{ab} AB + \beta_{aa} A^2 + \beta_{bb} B^2 \quad (1)$$

Where: dependent variable as total carotenoid content (TCC), A and B are the independent variables, liquid-to-solid (mL/g) and temperature (°C), respectively, β_0 as the intercept, β_a and β_b as the linear effect of A and B, and β_{ab} is the interaction relationship between the two variables, and β_{aa}^2 and β_{bb}^2 as the quadratic terms.

The response variable is the total carotenoid content (TCC), and the parameters involved are stated in Table 2. The effects were studied at two experimental levels. The pulverized carrot peels were dispersed in soybean oil based on the parameters of the model. Afterward, the mixtures in covered 250 mL Erlenmeyer flasks were placed in a shaking water bath (VS-1205SW1; Vision Scientific Co. Ltd.) at 100 rpm agitation speed for 20 min at the set temperature (30, 45, and 60 °C). The samples were then filtered through a filter paper and stored away from light and at room temperature until further analysis.

Table 2. Independent variables of the two-factor, three-level full factorial experiment.

Independent variables	Units	Low (-1)	Middle (0)	High (+1)
Liquid-Solid Ratio (L/S) (A)	mL/g	20	30	40
Temperature (B)	°C	30	45	60

Determination of total carotenoid content

Each of the samples was diluted 10-fold in hexane, and the total carotenoid content (TCC) of the carrot peels was subsequently measured at 450 nm using the UV-Visible spectrophotometer (Shimadzu Pharma-Spec UV-1700, Shimadzu Corporation, Japan) (Viñas-Ospino et al., 2024). The TCC was calculated using the hexane absorption extinction coefficient and the Beer-Lambert law, with results expressed as $\mu\text{g } \beta\text{-carotene/g}$ of dried peel (Equation 2) (Viñas-Ospino et al., 2024). The equation is given as follows:

$$TCC (\mu\text{g} \cdot \text{g}^{-1} \text{ dried peel}) = \frac{A_{450} \times V \times DF \times 10^6}{2560 \times m_{dp}} \quad (2)$$

Where: A_{450} as the absorbance at 450 nm; V as the total volume of the extract (mL); DF as the dilution factor; 10^6 as the conversion factor to μg ; 2560 as the extinction coefficient of hexane; and m_{dp} as the mass of the dried peel sample (g).

Greenness assessment using Path2Green metrics

The environmental sustainability of the green extraction process was evaluated using Path2Green (de Souza Mesquita et al., 2024). The 12 principles that govern green extraction for Path2Green assessed the sustainability of the entire study protocol, from biomass sourcing to waste management. These 12 principles were rated on a scale of -1 to 1, with higher scores indicating strong adherence to the green extraction

principles as measured by the Path2Green metric; lower scores indicate poorer adherence and misalignment with the principles. The evaluation was performed using the *Path2Green* mobile application. The application provides a radar chart that highlights areas for improvement based on scores and color coding (i.e., green, yellow, and red), with red indicating areas in need of improvement (Plaza and Marina, 2026). The final weighted score is shown in the middle of the pictogram. It is automatically calculated by the weighted score for each principle, with values ranging from -1.0 (poor rating) to +1.0 (excellent rating).

Statistical analysis

All experiments and validations were done in triplicate ($n = 3$). The model's parameters were estimated and fitted using standard least squares and evaluated using statistical diagnostics, including the Shapiro-Wilk test, the Durbin-Watson test, the maximum Cook's distance, and the maximum Variance Inflation Factor (VIF). Its significance was determined using analysis of variance (ANOVA) to assess the model, linear, interaction, and quadratic terms for each observed parameter. Additionally, Tukey HSD was used to evaluate the pairwise differences at each level for the univariate effects of each parameter, with

a significance threshold of < 0.05 . All statistical analyses, model fitness, and visualizations were performed using JMP Student Edition (JMP Statistical Discovery LLC, United States) and GraphPad Prism (GraphPad Software, LLC, United States). The model was constructed with two independent variables and a response variable (TCC). The model was also evaluated on its fitness using the coefficient of determination (R^2), predicted R^2 , and adjusted R^2 .

RESULTS

Measured responses from experimental runs

The freeze-dried peels retained their orange color throughout the freeze-drying, pulverization process, and extraction (Figure 1a, 1b, and 1c). The dispersion of the pulverized peels in soybean oil (Figure 1c) resulted in a color change in the oil solvent, which indicates the dissolution of oil-soluble pigments from the carrot peel matrix into the soybean oil (Lara-Abia et al., 2022; Sharma et al., 2022). The resulting extract retained its yellow-orange color after filtration, confirming the presence of oil-soluble compounds in the oil phase (Figure 1d).

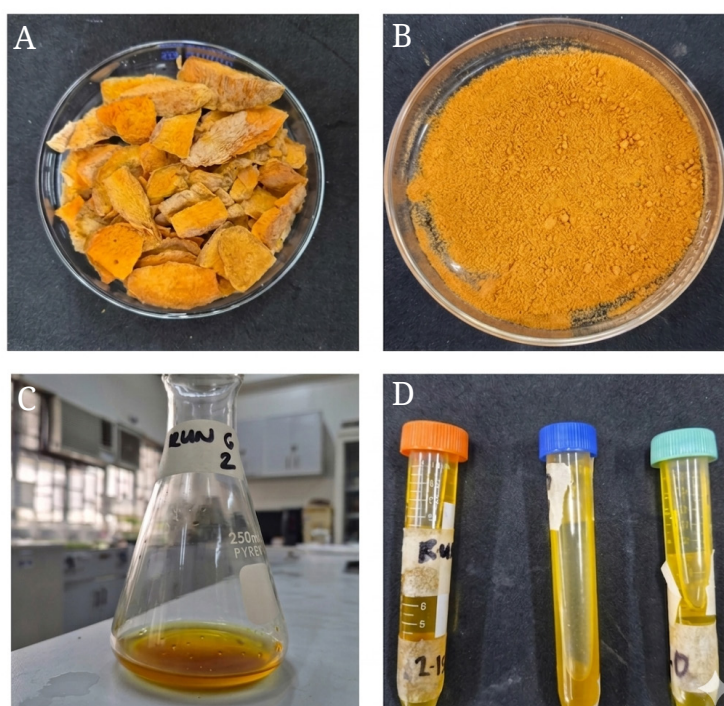


Figure 1. (A) freeze-dried carrot (*Daucus carota*) peels; (B) pulverized peels; (C) pulverized peels dispersed in soybean oil; and (D) resulting filtered mixture of the soybean oil extraction

The total carotenoids extracted at varying L/S ratios and temperatures are shown in Table 3. The observed data showed that 20 L/S and 45 °C produced the lowest total carotenoid content

(TCC) of $6.51 \pm 1.11 \mu\text{g } \beta\text{-carotene/g}$ of dried peel (Table 3). The highest observed extraction of $84.22 \pm 5.56 \mu\text{g } \beta\text{-carotene/g}$ of dried peel was achieved at 40 L/S and 45 °C.

Table 3. Parameters and the response values for total carotenoid content (TCC).

Run	Liquid-solid (L/S) (mL/g)	Temperature (°C)	Total Carotenoid Content ($\mu\text{g } \beta\text{-carotene/g}$ of dried peel)
1	30 (0)	60 (+1)	48.59 ± 5.82
2	30 (0)	30 (-1)	30.19 ± 3.16
3	40 (+1)	30 (-1)	77.71 ± 6.91
4	20 (-1)	60 (+1)	22.89 ± 2.05
5	40 (+1)	45 (0)	84.22 ± 5.56
6	40 (+1)	60 (+1)	70.47 ± 8.73
7	20 (-1)	30 (-1)	6.04 ± 1.46
8	30 (0)	45 (0)	43.63 ± 6.43
9	30 (0)	45 (0)	42.85 ± 3.73
10	20 (-1)	45 (0)	6.51 ± 1.11

Statistical significance and predictive modeling

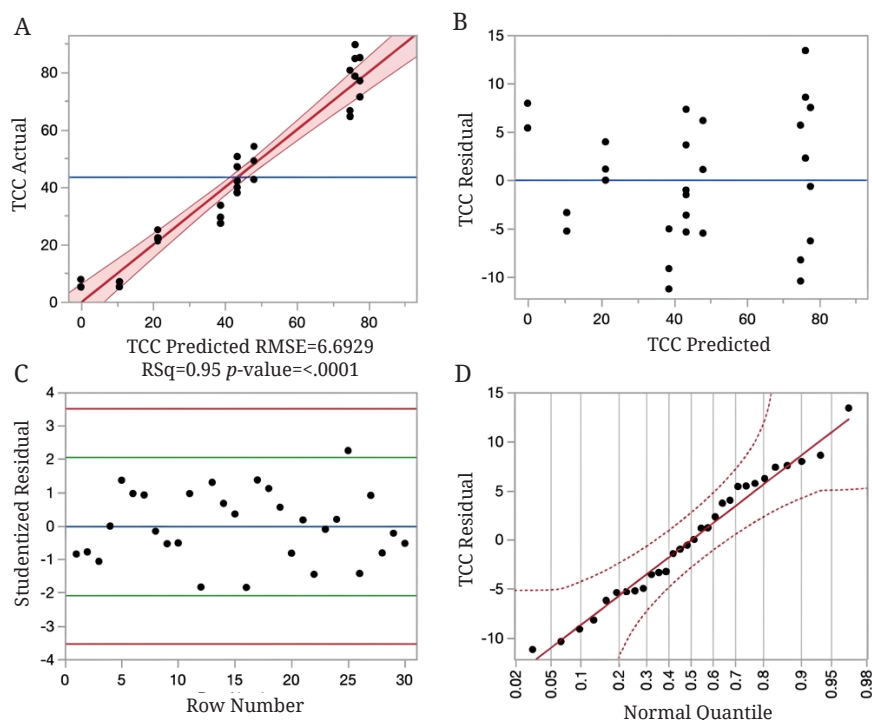


Figure 2. (A) Predicted TCC versus actual; (B) TCC residuals versus TCC predicted; (C) studentized residuals; and (D) TCC residual normal quantile plot.

Diagnostics were performed to verify the model's reliability and assumptions (Figure 2). The Shapiro-Wilk test yielded an insignificant p -value ($p = .07$), supporting the normality assumption for the observed data residuals; Figure 2d illustrates the normal distribution. The maximum Variance Inflation Factor (VIF) of 1.02 supports this by indicating that there is no multicollinearity between the factors, so that all parameters are independent. A maximum Cook's distance of 0.23 also confirms that the model is not biased by any outliers that overly influence the observations.

Analysis of variance (ANOVA) was performed to evaluate the observed experimental results, as shown in Table 4. A p -value < 0.05 with a 95% confidence interval was considered statistically significant for all the terms. The overall model was found to be highly significant in representing the observed data ($p < 0.0001$), supported by a high coefficient of determination ($R^2 = 0.95$),

indicating a strong positive correlation between recovered carotenoids and liquid-to-solid ratio and temperature. The adjusted R^2 value (Adj. $R^2 = 0.94$) and predicted R^2 value (Pred. $R^2 = 0.93$) validate the model's reliability. However, a significant lack of fit ($p = .002$) was observed, suggesting that the model may not fully capture the system's behavior. All univariate parameters were consistent with the model being significant. The liquid-to-solid (A) and temperature (B) variables were significant ($p < 0.0001$ and $p = 0.006$, respectively). The interactions between the two parameters (AB) were also statistically significant ($p = 0.004$). However, the quadratic terms were not significant for either variable: A2 ($p = 0.15$) and B2 ($p = 0.37$). Hence, the model was reduced to the main-effects model, including only significant effects and interactions and omitting non-significant quadratic terms to improve predictive power.

Table 4. ANOVA results showing significant values for the full model.

Source	SS	DF	Mean square	F-value	p -value
Model	20339.70	5	6741.38	93.06	<0.0001
A	19396.62	1	19396.62	443.72	<0.0001
B	392.28	1	392.28	8.97	0.006
A*B	435.25	1	435.25	9.96	0.004
A*A	95.84	1	95.84	2.19	0.15
B*B	36.12	1	36.12	0.83	0.37
Residuals	1049.12	24	43.71		
Cor. total	21388.82	29			
Lack of Fit	524.97	3	174.99	7.01	0.002
Pure error	524.15	21	24.96		
SD	6.61		R^2	0.95	
Mean	43.31		Adj. R^2	0.94	
C.V.%	15.28%		Pred. R^2	0.92	

The reduced model yielded results similar to those of the full model (Table 5). It was highly significant ($p < 0.0001$), along with the liquid-to-solid ratio (A) ($p < 0.0001$). This was followed by the temperature ($p < 0.007$) and the interaction effect ($p < 0.004$). The reduced model had a decreased R^2 of 0.95; yet, the predicted R^2 increased to 0.93. Given that the full model has an $R^2 = 0.95$ and $\text{Pred. } R^2 = 0.92$; the narrower gap between the two metrics of the reduced model indicates

that the reduced model is parsimonious and less susceptible to overfitting. Despite the improvement, the reduced model still exhibited a significant lack of fit ($p = 0.003$), indicating that it did not fully account for all systemic behaviors. The final reduced regression equation (Equation 3) for estimating the optimum coded conditions is as follows:

$$TCC=43.31+32.83 A + 4.67 B - 6.02 AB \quad (3)$$

Table 5. ANOVA results of the reduced model.

Source	SS	DF	Mean square	F-value	p-value
Model	20224.15	5	6741.38	150.49	<0.0001
A	19396.62	1	19396.62	433.006	<0.0001
B	392.28	1	392.28	8.76	0.007
A*B	435.25	1	435.25	9.72	0.004
Residuals	1164.68	26	44.80		
Cor. total	21388.82	29			
Lack of Fit	640.53	5	128.11	5.13	0.003

Validation of optimum parameters

The maximum desirability of the reduced model was identified using the JMP Prediction Profiler, which identifies

the optimum condition to be at an L/S ratio of 40 and 30 °C, $77.49 \pm 13.13 \mu\text{g } \beta\text{-carotene/g}$ of dried peel, with a desirability score of 0.82 (Figure 3). These optimal conditions were validated, which yielded a TCC of $76.04 \pm 3.71 \mu\text{g } \beta\text{-carotene/g}$ of dried peel.

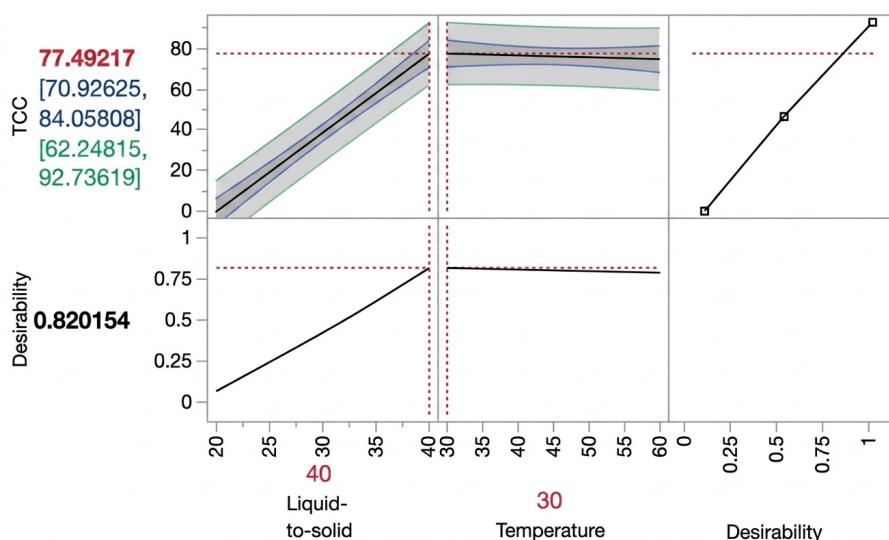


Figure 3. Validation of the predicted optimum parameters.

Table 6 presents the validation runs ($n = 3$) of the predicted optimal parameters given at an L/S ratio of 40 and a temperature of 30. The predicted yield was determined using the JMP Prediction Profiler and compared with experimental values from three independent validation runs to assess the model's

predictive performance. The mean experimental yield was $76.04 \pm 3.71 \mu\text{g } \beta\text{-carotene/g}$ of dried peel. The experimental validation runs yielded relative errors (RE) of 6.65, 3.13, and 2.68%, respectively, with an overall mean RE of 1.91% and an average RE of 4.15%.

Table 6. Validation of the predicted optimum parameters.

Validation run	Liquid-solid ratio (mL/g)	Temperature (°C)	Predicted yield ^a	Experimental yield ^a	RE (%)
1	40	30	77.49	72.66	6.65
2	40	30		80.00	3.13
3	40	30		75.47	2.68
Mean ($n = 3$)				76.04 ± 3.71	

Note: a - $\mu\text{g } \beta\text{-carotene/g}$ of dried peel

Effects of extraction parameters on carotenoid recovery

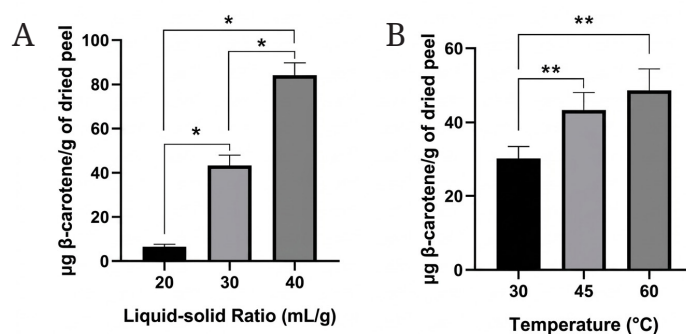


Figure 4. Univariate effects of (A) liquid-solid ratio; and (B) temperature on total carotenoid content.

Note: significant increase in carotenoid recovery based on Tukey HSD: * $p > 0.0001$ and ** $p < 0.001$

The univariate effects of liquid-solid ratio and temperature are shown in Figure 4. A significant positive trend can be observed for the liquid-solid (L/S) ratio, as evidenced by the significant Tukey HSD for all ($p < 0.0001$) (Figure 4a). Moving from a lower L/S ratio of 20 to 30 shows a significant increase in extraction recovery from 6.51 ± 1.11 µg β-carotene/g of dried peel to 43.63 ± 6.43 µg β-carotene/g of dried peel while maintaining a temperature of 45 °C. Moving from an L/S ratio of 30 to 40 was also observed to significantly increase carotenoid recovery, achieving 84.22 ± 5.56 µg β-carotene/g of dried peel while maintaining 45 °C, the highest concentration observed. The univariate effects of temperature also exhibited a

positive trend, albeit to a much lesser extent (Figure 4b). The differences between the levels of temperature were statistically significant, particularly when moving from 30 to 45 °C and from 30 to 60 °C ($p = 0.009$ and $p = 0.003$, respectively). The highest yield of 48.59 ± 5.82 µg β-carotene/g of dried peel was achieved at +1 temperature (60 °C).

The contour plot (Figure 5) shows that the lowest TCC recovery is concentrated at an L/S ratio of 20, and the highest recovery at an L/S ratio of 40. The prominent verticality of the contour gradients suggests that liquid-to-solid (A) has the greatest influence in extracting carotenoids than temperature.

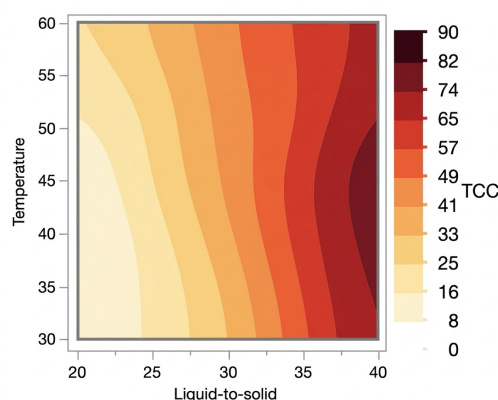


Figure 5. Contour plot of total carotenoid content.

The effect summary results show a highly significant impact of the parameters A, B, and their interaction (A*B) on the response variable, total carotenoid content, for the reduced model (Figure 6). The statistical significance threshold was set at $p < 0.05$ (Logworth > 1.30). The univariate effect of liquid-to-solid (LS) (A) was found to be the most dominant and the most prominent. It is the most significant parameter in driving the recovery of total carotenoid, as confirmed by ANOVA ($p < 0.0001$, Logworth = 17.007). The ANOVA shows

that the interaction effect (A*B) ($p = .00$, Logworth = 2.35) and the temperature (B) ($p = 0.006$, Logworth = 2.19) were significant. However, their impact on carotenoid recovery was smaller than that of parameter A (Figure 6). The quadratic terms, A2 and B2, were not included in the reduced model because they did not reach the statistical threshold; hence, all the terms, excluding the quadratic, have significant effects, with parameter A primarily driving the greatest effect on total carotenoid recovery from carrot peels.

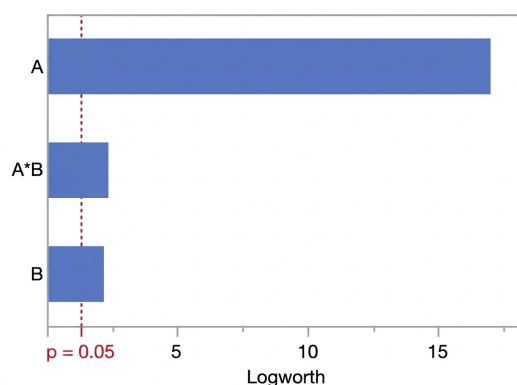


Figure 6. Pareto chart of standardized effects of the parameters and their interactions in logworth.

The highest extraction recovery of $84.22 \pm 5.56 \mu\text{g } \beta\text{-carotene/g}$ of dried peel was achieved at a liquid-to-solid ratio of 40 and an extraction temperature of 45°C . The highest predicted recovery of TCC was also observed at a 40 liquid-to-solid ratio and 30°C , yielding $77.49 \mu\text{g } \beta\text{-carotene/g}$ of dried peel. The effect of temperature on carotenoid recovery showed a positive, significant

linear relationship as temperature increased (Table 2 and Figure 4). Increasing the temperature from 30 to 60°C while maintaining a 20 liquid-to-solid ratio (A) yields TCCs of $6.04 \pm 1.46 \mu\text{g } \beta\text{-carotene/g}$ of dried peel and $22.89 \pm 2.05 \mu\text{g } \beta\text{-carotene/g}$ of dried peel, respectively. Although its effect was lower than that of the liquid-to-solid ratio (A), it still significantly affected carotenoid recovery.

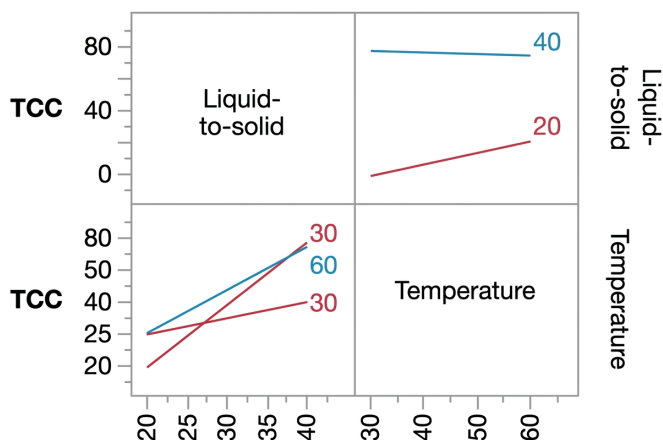


Figure 7. Interaction profiles of liquid-to-solid and temperature.

The interaction between liquid-to-solid (LS) ratio and temperature (A*B) exhibited a statistical significance in the recovery of carotenoids (Table 4 and Figure 6). Likewise, for temperature (B), the interaction effect is minimal compared to that of the L/S ratio (A) on TCC extraction. The interaction profiles are illustrated in Figure 7, in which both parameters (A*B) influence TCC recovery. As evidenced by the effect size in Figure 7, there is a substantial increase in TCC when moving from a low L/S ratio (20) to a high L/S ratio (40) (Figure 7), $6.04 \pm 1.46 \mu\text{g } \beta\text{-carotene/g}$ of dried peel and $22.89 \pm 2.05 \mu\text{g } \beta\text{-carotene/g}$ of dried peel, respectively; likewise, the interaction profile suggests that increasing temperature from 30°C to 60°C leads to an increase in TCC recovery at a low L/S ratio of 20. Conversely, a higher L/S ratio (40) leads the TCC response to

remain constant, then decrease slightly as temperature increases to 60°C . The non-parallel, steeply positive trend in Figure 7 likely indicates that liquid-to-solid (A) has a highly significant effect on TCC recovery, as evidenced by the ANOVA ($p < 0.0001$) and Figure 6. At lower temperatures (30°C), the slope of the line is steeper as the L/S ratio increases from 20 to 40. It is also worth noting that higher temperatures (60°C) at a lower L/S ratio (20) yield higher carotenoid recovery than lower temperatures (30°C); however, the total concentration of TCC remains the same at both temperatures as the L/S ratio increases to 40. Increasing L/S ratio and temperature would therefore indicate a positive trend in TCC recovery; however, temperature becomes secondary to L/S ratio in determining extraction efficiency, as evidenced by the large effect and ANOVA ($p < 0.0001$).

Sustainability assessment of soybean oil extraction of carotenoids using Path2Green



Figure 8. Path2Green assessment based on 12 green extraction principles.

The sustainability of the carotenoid extraction process was evaluated and assessed using the Path2Green assessment, which has been used as a metric for sustainable and greener extraction methods and viability of various biomass for industrial application (de Souza Mesquita et al., 2024; Ishikiriya et al., 2024; Li et al., 2024; Rodrigues and Silva, 2026). Figure 8 shows a final score of 0.593 across all 12 principles, with a maximum score of 1.00. The favorable scores indicate a moderately green profile for the entire extraction process of using soybean oil for carotenoid recovery. The majority of the assessed principles (i.e., 1, 2, 4, 5, 6, 8, 10, and 12) showed the maximum values (score +1.00), as denoted by a green color: biomass selection, transport, type of solvent, scaling, purification, post-treatment, application, and waste. However, Path2Green also

identified limitations in the extraction process. It flags the yield and energy consumption with a low score of -0.5, indicated by the red color. The process has inherent environmental costs due to the use of high temperatures and the semi-exhaustive valorization of the biomass. Moreover, the pre-treatment strategy was also observed to be a limitation, as the carrot peels underwent freeze-drying and pulverization, which required physical processing before use as extracts; hence, it is denoted in red and assigned a score of -0.20. A closed-loop extraction system was also not tested in the study (denoted in yellow), as the performance of the solvent was observed; hence, the score obtained was 0.00, as the material was only ever proposed and implemented. The summary of each principle, weight, and score is presented in Table 7.

Table 7. Path2Green scores for the extraction of total carotenoids from carrot peels using soybean oil.

Principle	Weight	Score	Observation
Biomass	6	+1.0	The carrot peels are agricultural wastes and a by-product of many processes
Transport	5	+1.0	Sourcing the biomass would be located near industries
Pre-treatment	2.5	-0.20	The carrot peels were only pre-treated with physical methods prior to extraction
Solvent	6	+1.00	Soybean oil was used as a green solvent
Scaling	5	+1.00	The method employs an in-flow process
Purification	2.5	+1.00	The extract is ready to use
Yield	4	-0.50	It may still contain bioactive compounds, hence semi-exhaustive extraction
Post-treatment	2.5	+1.00	There is no need for post-treatment
Energy	5	-0.50	The shaking water bath is energy-dependent
Application	4.5	+1.00	The resulting extracts can be used in all domains: pharmaceutical, nutrition, well-being, cleaning and hygiene, feed & preservatives, and cosmetics
Repurposing	6	0.00	The solvent is only being proposed and implemented
Waste management	6	+1.00	High extraction yield

DISCUSSION

The highest observed concentration in the study was at a 40 L/S ratio and 45 °C, with $84.22 \pm 5.56 \mu\text{g } \beta\text{-carotene/g}$ of dried peel. These results were consistent with the carotenoid recovery reported in a study using pumpkins, which yielded $80.71 \mu\text{g } \beta\text{-carotene/mL}$ oil at a solid-liquid ratio of 1:20, 40 °C, and 400 rpm agitation (60% recovery) (Ishikiriya et al., 2024). The same study also reported the highest yield (72%) at a solid-liquid ratio of 1:74, 50 °C, and 300 rpm agitation. This increase in carotenoid recovery can be attributed to larger concentration differences between phases; hence, increasing the solvent-to-solid ratio facilitates better matrix penetration, thereby improving mass transfer and higher carotenoid recovery (Viñas-Ospino et al., 2024). Having more solvent would therefore increase the concentration gradient and prevent the system from becoming too saturated, allowing more carotenoids to interact with the solvent (Portillo-López et al., 2021). This would explain why the liquid-to-solvent parameter (A) has a greater effect on carotenoid recovery, due to improved mass transfer and an increase in the solvent's diffusion coefficient.

Increasing the L/S ratio from 20 to 40 at 60 °C also results in a positive trend. The TCC recovery is much higher at an L/S ratio of 20 because thermal energy increases solubility and facilitates solvent-induced cell wall rupture (Portillo-López et al., 2021). However, increasing the L/S ratio further to 40 results in similar TCC recovery across temperatures from 30 to 60 °C; hence, increasing the solvent would increase the concentration gradient, yet the effect on temperature decreases as the system reaches equilibrium. A positive relationship between the L/S ratio and the concentration gradient aligns with the idea that a greater amount of solvent results in a steeper concentration gradient between the solute's internal and external surfaces, thereby enhancing mass transfer (Norshazila et al., 2017). It was also found

that increasing the extraction temperature from 15 to 30 °C results in higher carotenoid yields, as heat enhances the solubility and cell wall permeability of carotenoids to the solvent. Using a higher solvent concentration increases contact between the solid and solvent, promoting better solvent penetration and greater solute release from the solid (Shu et al., 2025).

Temperature was also noted as a significant factor influencing extraction efficiency, as increasing the temperature within optimal processing conditions induces energy-driven vibrations that weaken intermolecular bonds and release target bioactive compounds into the solvent. Yet, the dominance of the L/S ratio alone in the recovery of TCC largely overshadows the effects of temperature and its interaction with the L/S ratio. This can be explained by the fact that the mixture solutions were not heated directly; rather, the solvent's internal temperature may be slightly lower than the set temperature of the water bath due to indirect heating and thermal lag. Even so, this does not directly explain why temperature has not affected carotenoid extraction efficiency, given the prolonged 20 min extraction time across all experimental trials. An increase in temperature affects cell wall permeability, thereby improving carotenoid solubility in the solvent; it leads to cell wall ruptures and the release of intracellular products into the solvent (Portillo-López et al., 2021). The optimal extraction yield of carotenoids from peach peels was reported to be at 40 °C, with diminishing efficiency as the temperature increased further (Drosaki et al., 2025). Other studies have reported that temperatures above 60 °C led to the degradation of compounds and a reduction in carotenoid content (Portillo-López et al., 2021). This may suggest that the temperature increase becomes redundant because the system has already saturated and reached its maximum recovery (Portillo-López et al., 2021). This finding is consistent with the results from the extraction of peach peel from vegetable oils; the study noted that the

solvent-to-solid ratio is the most influential parameter, with temperature exerting only a limited effect on carotenoid recovery, especially in soybean oil extraction, where the optimal yield is limited to 40 °C (Drosaki et al., 2025). Temperature would, therefore, act as a facilitating parameter that accelerates the solubility of the compounds in the solvent because it cannot overcome the constraints of solvent concentration, especially in a saturated system.

Path2Green analysis for greenness evaluation culminated in a compounded score of 0.59, with the extraction process attaining a maximum score of +1.00 in 8 of the 12 categories. As the carotenoid extracts were derived from commonly discarded carrot peels rather than freshly produced carrots, the extraction performed scored the maximum for the first Path2Green principle of valorizing naturally sourced biomass rather than purposely mass-producing sources for the sole objective of compound extraction. By repurposing agricultural residues such as fruit processing waste, the extraction finds value in what is often deemed unusable, helping reduce their environmental impacts; locally sourcing this waste would also reduce the environmental impact of biomass transportation (de Souza Mesquita et al., 2024). By procuring inputs for extraction from nearby, accessible sources, the environmental impacts of product processing and transportation are reduced. The extraction also posits itself to be eco-friendly in terms of solvent selection; by utilizing soybean oil, a biodegradable, non-toxic, and non-volatile solvent, as an alternative for conventional volatile organic solvents, the extraction showed careful consideration of the overall sustainability of the process without compromising efficiency and effectiveness (de Souza Mesquita et al., 2024). Its straightforward, cost-effective, and efficient extraction method would position it as a strong candidate for scalability, given its continuous extraction approach, which generates minimal waste by fully utilizing all inputs and outputs (de Souza Mesquita et al., 2024; Ishikiriyama et al., 2024). The soybean oil solvent itself does not contain harmful organic substances; hence, solvent removal is unnecessary, allowing for their direct integration in food products and the elimination of the need for additional and residue-intensive purification (de Souza Mesquita et al., 2024; Ishikiriyama et al., 2024; Viñas-Ospino et al., 2023). With the soybean oil solvent's inherent biodegradability, non-volatility, and lack of need for extensive chemical processing, the extraction process also establishes itself as a safe and promising method for applications in various domains concerning pharmaceutical, cosmetic, nutritional, and wellness industries (de Souza Mesquita et al., 2024; Li et al., 2024; Mussagy et al., 2023).

The extraction process remains subject to refinement in certain areas. As the extraction used mechanical pretreatments (e.g., freeze-drying, grinding), it involved high-energy processes for extended periods to induce the release of the target compounds. In terms of yield, the extraction process remains semi-exhaustive. Subsequent studies may explore a wider range of parameters and additional optimization techniques to improve efficiency and achieve complete valorization. The suggested approach for refining the method should consider using low-energy techniques to increase its sustainability, in line with the ninth Path2Green principle of utilizing clean yet highly efficient energy for extraction (de Souza Mesquita et al., 2024). Moreover, the recovery and repurposing of the inputs and outputs of the extraction process may be explored to achieve greater sustainability in line with the 11th Path2Green principle on closed-loop extraction systems. In essence, amidst the aforementioned gaps, the extraction process can be regarded as moderately sustainable and highly viable based on the 12 Path2Green principles for green and sustainable extraction methods.

These results were found to be on par with Zobot and Silva (2026), who explored and compared different extraction methods for annatto seed oil, bixin from annatto seeds, and depigmented starch from annatto byproducts. They found that the choice of solvent used in the extraction process matters significantly for the sustainability outcome; more organic, environmentally compromising solvents often yielded a lower Path2Green evaluation score. This was highlighted by comparisons across multiple extraction methods, including lesser green processes such as Soxhlet extraction, which yielded a Path2Green score of 0.139; ultrasound-assisted extraction, which yielded a score of 0.243; and low-pressure solvent extraction, which yielded a score of 0.334. On the contrary, they also explored more viable and sustainable options, such as supercritical CO₂ extraction, which yielded a Path2Green score of 0.74, and aqueous extraction, which yielded a score of 0.57. They found that more environmentally friendly extraction processes yielded higher Path2Green scores than their less environmentally friendly counterparts. Therefore, the high Path2Green evaluation score, combined with the use of a more environmentally friendly solvent, such as soybean oil, makes the extraction process more sustainable and viable. Aside from solvent selection, biomass choice also has a significant bearing on the sustainability evaluation of an extraction process. A study that used unripe *Genipa americana* L. fruits and whey protein and employed thermal and ultrasound extraction techniques reported a Path2Green evaluation score of 0.65 (Martins Strieder and Silva, 2025). A study using orange peels to recover phenolic antioxidants also reported a similar Path2Green evaluation score of 0.543 using pressurized liquid extraction (Plaza et al., 2025). These results suggest that the use of environmentally viable biomasses enhances the sustainability of an extraction process. However, the evaluation also identified improvements in the extraction process, particularly in the principles of repurposing and yield, indicating the need to explore a closed-loop extraction process and to maximise extraction efficiency for complete valorization.

CONCLUSION

The study employed a two-factor, three-level full factorial design. The model was reduced to a linear interaction model because the quadratic terms were insignificant. With the reduced model, the most optimal predicted TCC was found at a 40 L/S ratio and 30 °C, with a predicted TCC of 77.49 ± 13.13 µg β-carotene/g of dried peel. The validation runs yielded 76.04 ± 3.71 µg β-carotene/g of dried peel with an overall mean relative error of 1.91%. The L/S ratio proved to be the most significant parameter for driving carotenoid recovery. Ultimately, this research study underscores the profound potential of waste valorization and the adherence to the green principles of chemistry. With a final score of 0.593 across all 12 principles of Path2Green, this study demonstrates its green and sustainable nature. Utilizing agricultural byproducts with eco-friendly solvents, such as soybean oil, sustainable extraction of carotenoids for direct integration in food, pharmaceutical, and cosmetic industries, among others, paving the way for health-conscious manufacturing processes. Further studies can consider optimizing yield and solvent repurposing while expanding the design space in which quadratic effects become significant and minimizing limitations from lack of fit.

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AUTHOR CONTRIBUTIONS

H. C. F. P, H. M. I. B, P. S. B. R, C. F. M, and J. R. C. E: Conceptualization; H. C. F. P, H. M. I. B, P. S. B. R, and J. R. C. E: Introduction; H. C. F. P, H. M. I. B, P. S. B. R, and J. R. C. E: Methodology; H. C. F. P, H. M. I. B, P. S. B. R, and J. R. C. E: Data Gathering; H. C. F. P, H. M. I. B, P. S. B. R, and J. R. C. E: Formal Analysis; H. C. F. P, H. M. I. B, P. S. B. R, and J. R. C. E: Investigation; H. C. F. P, H. M. I. B, P. S. B. R, and J. R. C. E: Visualization; C. F. M and J. R. C. E: Writing - Review and Editing. All authors have read and agreed to the publication of this manuscript.

DECLARATIONS

Informed consent statement

None is needed in the study.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

AI Declaration

The authors declare that no Artificial Intelligence (AI) or AI-assisted technologies were used in the preparation of this manuscript.

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