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Comparison of quantitative carotenoid analysis using UV-Vis spectrophotometry and high-performance liquid chromatography

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Abstract

The quantification of β-carotene in agricultural by-products is crucial for their valorization. However, commonly used analytical methods, such as UV–Vis spectrophotometry and high-performance liquid chromatography (HPLC), present critical differences in specificity and cost. This study compared β-carotene quantification using both methods, employing ethanol and hexane as extraction solvents, to determine β-carotene in peels from four mango varieties (Kent, Edward, Haden, and Criollo), following a 2×2×4 factorial design. Proximate analysis revealed significant differences in composition among the varieties. The results demonstrated a significant effect of the triple interaction (method × solvent × variety), indicating that all factors must be considered when selecting a β-carotene quantification protocol. Spectrophotometry systematically overestimated β-carotene content (up to 60% higher with ethanol) due to interference from other pigments. In contrast, HPLC provided specific quantification, revealing that hexane was significantly more efficient than ethanol for extraction. The Kent variety showed the highest β-carotene content. It is concluded that the HPLC–hexane combination is the optimal protocol for accurate quantification, whereas spectrophotometry provides only a useful screening estimate that should be interpreted with caution. The use of HPLC is recommended for studies requiring high accuracy, as well as for the validation of rapid methods against this standard for each new matrix.

Keywords: analytical methods, β- carotene, mango, peel, solvents

Introduction

Carotenoids are pigments of major biological and nutritional relevance, belonging to the class of provitamin A carotenoids. Their physiological importance lies in their role as the main dietary precursors of vitamin A (retinol), an essential micronutrient involved in vital processes such as vision, immune function, reproduction, and cell growth (Grune et al., 2010). In addition, β-carotene exhibits remarkable antioxidant activity, acting as an effective scavenger of reactive oxygen species, which confers a potential role in the prevention of chronic diseases associated with oxidative stress (Eggersdorfer & Wyss, 2018). This combination of functions has driven its widespread use as a coloring additive (E160a) in the food industry, as an active ingredient in the cosmetic industry, and as a nutritional supplement.

Accurate and reliable determination of β-carotene concentration is therefore a fundamental requirement in diverse fields such as food quality control (juices, dairy products, oils), evaluation of dietary supplement composition, clinical research on nutritional status, and the development of new fortified food matrices (Rodriguez-Amaya, 2015). However, the analytical quantification of β-carotene presents inherent challenges derived from its chemical nature: it is a highly unsaturated, lipophilic molecule that is sensitive to light, heat, and oxidation, which may lead to degradation and isomer formation during sample processing and analysis (Rivera-Madrid et al., 2016). Moreover, in complex biological or food matrices, β-carotene usually coexists with other carotenoids and chromophoric compounds with similar structures, hindering its individual identification and quantification without adequate separation.

To address these challenges, the scientific community and analytical laboratories have developed and standardized various methodologies, which

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can be broadly classified into two main categories: spectroscopic methods and chromatographic methods. UV–Vis spectrophotometry represents the historical and most widely used technique for estimating total carotenoids or specific groups, such as provitamin A carotenoids. Its principle is based on the ability of these compounds to absorb light in the visible region (generally between 450–480 nm for β-carotene), following the Beer–Lambert law (Saini & Keum, 2018). This method is notable for its instrumental simplicity, low cost, rapid analysis, and minimal operator training requirements, making it ideal for routine analyses, large-scale screening, or settings with limited resources. Nevertheless, its main limitation is the lack of specificity. Measuring absorbance at a characteristic wavelength in a crude extract does not allow discrimination of β-carotene from other co-extracted carotenoids (such as lutein or lycopene) or from degradation products that may absorb in the same spectral region, potentially leading to systematic overestimation (Luterotti et al., 2013; Marcillo-Parra et al., 2021).

In contrast, high-performance liquid chromatography (HPLC), especially when coupled with diode array detectors (DAD) or mass spectrometry, is currently considered the "reference method" or gold standard for the analysis of individual carotenoids (FAO/WHO, 2016). Its strength lies in its high resolution, specificity, and sensitivity. The chromatographic phase enables physical separation of β-carotene from interfering compounds prior to detection, while the DAD provides absorption spectra that serve as a "fingerprint" for confirmatory identification. This allows precise and selective quantification of β-carotene, even in the presence of its cis and trans isomers and other pigments (Qian et al., 2012; Sanchez-Camargo et al., 2019). However, this analytical superiority entails significant drawbacks: the equipment is considerably more expensive, requires specialized maintenance, analyses are more time-consuming, solvent consumption is higher, and highly trained personnel are needed.

This dichotomy between the accessibility and speed of UV–Vis spectrophotometry and the specificity and accuracy of HPLC creates an analytical scenario in which method selection is not trivial and depends critically on the study objective, matrix characteristics, and available resources (Hu et al., 2021). Consequently, researchers and quality control laboratories require a quantitative and detailed understanding of the degree of agreement or discrepancy between the results generated by both techniques. A rigorous comparison should not only assess numerical correlation between obtained values but also investigate the factors contributing to divergences, such as the presence of specific interferents in different matrices, the effect of extraction protocols, and the magnitude of uncertainties associated with each method (Galanakis, 2020).

Therefore, the main objective of this study is to perform a comparative evaluation of the analytical performance of UV–Vis spectrophotometry and HPLC for β-carotene quantification. This evaluation was conducted using mango peel samples from different varieties commonly consumed in Peru. The results of this research aim to provide an evidence-based practical guide to assist analysts in selecting the most appropriate method according to their specific needs, critically interpreting the data generated by each technique, and understanding the inherent limitations of each approach, thereby contributing to more robust and reliable measurement of this critical nutrient.

Materials and Methods

Raw material

Mango peels used in this study were obtained from four varieties: Kent, Edward, Haden, and Criollo. Fruits at commercial ripeness were purchased from the central market of Lambayeque, Peru, and selected based on the absence of physical damage and phytopathological defects. After acquisition, fruits were washed with running water and manually peeled using a stainless-steel peeler, separating the peel from the pulp. The peels were first frozen (ILSHIMBIOBASE, DF3517S, South Korea) at −45 °C and subsequently freeze-dried (BioBase, BK-FD10S, South Korea) at a pressure of 4.5 Pa for 20 h. Afterwards, the temperature was gradually increased until reaching room temperature (24–25 °C) (Saini & Keum, 2018). The dried material was then ground using a universal mill (IKA WERKE, M20, Germany) and sieved to obtain a particle size <150 µm. The resulting flour from each variety was stored in vacuum-sealed aluminum bags and kept at −20 °C in darkness until analysis.

Solvents used for extraction were HPLC-grade ethanol (99.9%, Sigma-Aldrich) and HPLC-grade hexane (95%, Merck). For HPLC analysis, β-carotene standard (≥93%, Sigma-Aldrich) was used. All other reagents and solvents were of analytical grade.

Proximate analysis of mango peels

Proximate analysis of the peel flour from each mango variety was performed according to the official methods of the Association of Official Analytical Chemists (AOAC), with all determinations carried out in triplicate.

Moisture content was determined by oven drying at 105 °C until constant weight (AOAC 950.46), while ash content was obtained by incineration in a muffle furnace at 550 °C for 6 h (AOAC 942.05). Total lipid content was quantified by continuous extraction using a Soxhlet apparatus for 6 h with hexane as the solvent (AOAC 991.36). Protein content was determined by the Kjeldahl method, applying a nitrogen-to-protein conversion factor of 6.25 (AOAC 960.52). Total dietary fiber was evaluated using the enzymatic–gravimetric method (AOAC 985.29). Total carbohydrates were calculated by difference, according to the equation: 100 − (% moisture + % ash + % fat + % protein + % fiber), as described by Rodriguez-Amaya (2015).

Preparation of the extracts

Carotenoid extraction was carried out following the methodology adapted from Rivera-Madrid et al. (2016). Briefly, 0.5 g of mango peel flour was suspended in 5 mL of extraction solvent (ethanol or hexane) and homogenized using a multi-rotator (Boeco RS-24, Germany) for 15 min at 99 rpm. The mixture was then subjected to ultrasonic treatment (Ultrasonic Cleaner, UCP10, USA) in an ice bath for 10 min to facilitate cell disruption, while protecting the samples from light using aluminum foil. Subsequently, the extract was centrifuged (Centurion14 Scientific, Pro-Analytical CR4000R, Turkey) at 4500 rpm and 4 °C for 15 min. The supernatant was collected, and the pellet was re-extracted twice with 10 mL of fresh solvent. Combined supernatants were filtered through a 0.22 µm PTFE syringe filter and adjusted to a final volume of 50 mL with the corresponding solvent. Extracts were analyzed immediately or stored at −80 °C in darkness for a maximum of 24 h.

Total carotenoid content by UV–Vis spectrophotometry

Spectrophotometric quantification of total carotenoid content was performed according to the method described by Rodriguez-Amaya (2015). Absorbance of the extracts was measured at 450 nm using a UV–Vis spectrophotometer (Thermo Scientific, Genesys 30, USA) with a 1 cm path-length quartz cuvette. The extraction solvent (ethanol or hexane) was used as the blank. Total carotenoid concentration was calculated using an equation based on the Beer–Lambert law and the average specific absorption coefficient for carotenoids: Carotenoids (mg/L) = [(A × V × 10□)/(ε × P)], where A is the measured absorbance, V is the total volume of the extract (mL), P is the weight of the sample (mg), and ε is the specific absorption coefficient (for carotenoid mixtures, a value of 2592 in hexane and 2500 in ethanol was used as a standard reference). Each sample was analyzed in triplicate.

Carotenoid content by HPLC

Separation and quantification of individual carotenoids were performed following the validated method described by Qian et al. (2012), with slight modifications. An HPLC system (SHIMADZU, LCMS-2020, Japan) equipped with a quaternary pump, autosampler, column oven, and diode array detector (DAD) was used. Separation was achieved on a reversed-phase C18 column (Zorbax Eclipse Plus, 4.6 × 150 mm, 3.5 µm) maintained at 25 °C. The mobile phase consisted of ultrapure water (A) and acetonitrile/2-propanol (2:1, v/v) (B), using the following linear gradient: 0–0.7 min, 70% B; 0.7–6.1 min, 100% B; 6.1–10.4 min, 100% B; 10.4–15.7 min, 100% B; and 15.7–22.2 min, 70% B for column re-equilibration. The flow rate was set at 1.34 mL/min, and the injection volume was 7 µL. Detection was carried out at 540 nm, and full spectra were recorded between 250 and 550 nm. β-Carotene was identified by comparing its retention time and UV–Vis absorption spectrum with those of the corresponding standard. Quantification was performed using external calibration curves ($r^2 > 0.999$) prepared with β-carotene standard.

Factorial design A × B × C

A completely randomized 2 × 2 × 4 factorial design was employed, considering analysis method, solvent type, and mango variety as experimental factors. The method factor included two levels: UV–Vis spectrophotometry (M1) and high-performance liquid chromatography (HPLC) (M2). The solvent factor comprised ethanol (S1) and hexane (S2), while the variety factor included Kent (V1), Edward (V2), Haden (V3), and Criollo (V4). The combination of these factors resulted in 16 experimental treatments (2 × 2 × 4), each evaluated in triplicate (n = 3), yielding a total of 48 experimental units (**Table 1**).

Statistical analysis

Data from proximate composition and carotenoid content analyses were expressed as mean ± standard deviation. The effects of the main factors and their interactions on carotenoid content were evaluated using a three-way analysis of variance (three-way ANOVA), and contour plots were generated to visualize interaction effects. In addition, a one-way analysis of variance (one-way ANOVA) was performed to assess differences among the mango peel varieties studied. Mean comparisons were carried out using Tukey's post

Table 1. Trials obtained for the AxBxC factorial design of the factors: method, solvent and variety. Experimental runs obtained for the A × B × C factorial design considering the factors: analytical method, solvent, and mango variety.

Factors		Method							
		Spectrophotometry				HPLC			
		Variety				Variety			
		Kent	Edward	Haden	Criollo	Kent	Edward	Haden	Criollo
Solvent	Ethanol	Y_{1111} Y_{111n}	Y_{1121} Y_{112n}	Y_{1131} Y_{113n}	Y_{1141} Y_{114n}	Y_{1211} Y_{121n}	Y_{1221} Y_{122n}	Y_{1231} Y_{123n}	Y_{1241} Y_{124n}
	Hexane	Y_{2111} Y_{211n}	Y_{2121} Y_{212n}	Y_{2131} Y_{213n}	Y_{2141} Y_{214n}	Y_{2211} Y_{221n}	Y_{2221} Y_{222n}	Y_{2231} Y_{223n}	Y_{2241} Y_{224n}

hoc test at a 95% confidence level ($p < 0.05$) (de Oliveira et al., 2015). All statistical analyses were conducted using R Project software (version 4.2.3) and RStudio (version 3.6.0).

Results and Discussion

Proximate composition of mango peels

The results of the proximate analysis of the four mango peel varieties are presented in **Table 2**. Proximate composition varied significantly among the different varieties, with pronounced differences observed in dietary fiber and carbohydrate contents. The Kent variety exhibited the highest dietary fiber content, followed by Edward and Haden, whereas the Criollo variety showed the lowest value. These findings are consistent with previous studies reporting mango peel as an important source of dietary fiber, predominantly insoluble, whose levels may vary widely depending on cultivar and degree of ripeness (Ajila et al., 2010). The high fiber content observed in mango peels, particularly in the Kent and Edward varieties, suggests their potential use as functional ingredients for food fortification, contributing to improved digestive health and modulation of the glycemic response. In contrast, the Criollo variety exhibited a lower fiber content but the highest proportion of total carbohydrates, calculated by difference, which is in agreement with previous reports (Rodriguez-Amaya, 2015; Marçal & Pintado, 2021).

Regarding lipid and protein contents, the Kent variety exhibited the highest values, being significantly higher than the other varieties for both components, whereas the Criollo variety showed the lowest levels. Ash content was significantly higher in the Haden and Criollo varieties (approximately 4.45%) compared with Kent and Edward. These differences in lipid, protein, and mineral composition can be attributed to genetic factors, cultivation conditions, and variations in the biosynthesis and accumulation of metabolites in peel tissues (Díaz-Vela et al., 2013; Adilah et al., 2018). Moisture content in the peel flour was relatively low in all varieties (<7.5%), which is crucial to ensure microbiological and chemical stability during storage (Saini & Keum, 2018). The Kent variety exhibited the highest moisture content, which may be related to its higher proportion of hydrophilic components such as dietary fiber. This variability is not only relevant for the nutritional valorization of mango peels but may also have direct implications for the extraction processes of bioactive compounds, such as carotenoids. For instance, a matrix with higher lipid content (as observed in Kent peels) may enhance the solubilization and extraction of lipophilic carotenoids, whereas a matrix with a dense fiber structure may retain these compounds more strongly, thereby affecting extraction efficiency (Rivera-Madrid et al., 2016).

β-Carotene quantification

The results of β-carotene content (expressed as µg/g of peel, dry basis), obtained under a 2 × 2 × 4 factorial design (Solvent × Method × Variety), are presented in **Table 3**. Data analysis revealed a significant third-order interaction among extraction solvent, analytical method, and mango peel variety ($p < 0.05$) according to the factorial ANOVA. The highest β-carotene content was observed in the Kent variety when hexane was used as the extraction solvent and quantification was performed

Table 2. Proximate composition (% dry basis) of peels from four varieties of mango (*Mangifera indica L.*).

Variety	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	Carbohydrates*(%)
Kent	7.29 ± 0.21^{a}	3.69 ± 0.15^{b}	5.47 ± 0.18^{a}	3.36 ± 0.12^{a}	35.12 ± 1.05^{a}	45.07 ± 1.32^{c}
Edward	5.36 ± 0.17^{c}	3.17 ± 0.12^{c}	5.18 ± 0.14^{a}	2.47 ± 0.10^{b}	29.60 ± 0.98^{b}	54.22 ± 1.41^{b}
Haden	5.89 ± 0.19^{bc}	4.44 ± 0.16^{a}	4.67 ± 0.16^{b}	2.25 ± 0.09^{b}	27.62 ± 0.87^{b}	55.13 ± 1.28^{b}
Criollo	6.71 ± 0.20^{b}	4.49 ± 0.14^{a}	3.03 ± 0.11^{c}	1.46 ± 0.07^{c}	15.32 ± 0.65^{c}	68.90 ± 1.55^{a}

*Calculated by difference. Values expressed as mean ± SD (n=3). Different letters in the same column indicate significant differences ($p < 0.05$).

Table 3. β-Carotene content (µg/g dry basis) in mango peels determined by UV–Vis spectrophotometry and HPLC using ethanol and hexane as extraction solvents.

Factors	Variety	Spectrophotometry	HPLC
Ethanol	Kent	316.1±0.563^{aB}	196.87±5.95^{aG}
	Haden	210.65±0.114^{cF}	93.075±0.814^{dN}
	Edward	240.43±0.429^{bE}	115.4±4.71^{bL}
	Criollo	186.1±0.413^{dH}	104.91±0.652^{cM}
Hexane	Kent	309.61±0.601^{aB}	355.57±2.45^{aA}
	Haden	201.19±0.306^{cG}	132.34±7.65^{dK}
	Edward	251.15±0.436^{bD}	271.47±0.638^{bC}
	Criollo	157.95±0.239^{dJ}	167.15±1.15^{cI}
Source of Variation		Value F	Value p
Method		4522.67	<0.05
Solvent		3481.34	<0.05
Variety		6536.30	<0.05
Method x Solvent		4800.84	<0.05
Method x Variety		274.19	<0.05
Solvent x Variety		518.82	<0.05
Method x Solvent x Variety		266.30	<0.05
R^2		99.91	<0.05

NOTE: Values expressed as mean ± SD (n=3). Different uppercase letters (A to N) throughout the table indicate significant differences ($p < 0.05$) among the 16 treatments (three-way factorial ANOVA). Different lowercase letters (a-d) within the same column for a given method and solvent indicate significant differences ($p < 0.05$) among mango peel varieties (one-way ANOVA).

by HPLC, whereas the lowest value corresponded to the Haden variety extracted with ethanol and quantified by UV–Vis spectrophotometry. Furthermore, when evaluating the varieties by fixing both method and solvent, the Kent variety consistently exhibited the highest β-carotene content in all cases (one-way ANOVA). These findings indicate that the perceived efficiency of a given solvent for β-carotene extraction depends critically on the quantification technique employed and on the specific biological matrix analyzed (Feder et al., 2019).

The most pronounced discrepancy was observed when comparing values obtained by UV–Vis spectrophotometry versus HPLC. Consistently across all varieties extracted with ethanol, the spectrophotometric method yielded significantly higher values—up to 60% higher in the case of Kent—than those obtained by chromatographic analysis. This phenomenon can be directly attributed to the lack of specificity of the UV–Vis method. By measuring absorbance at 450 nm, this technique quantifies not only β-carotene but also other co-extracted carotenoids (such as lutein and β-cryptoxanthin), as well as possible degradation products or chromophoric interferents absorbing in the same spectral region, leading to systematic overestimation (Luterotti et al., 2015).

These results confirm the main limitation traditionally attributed to spectrophotometric techniques in carotenoid analysis (Scott, 2001; Rodriguez-Amaya, 2015). In contrast, when hexane was used as the extraction solvent, the relationship between methods was variable and depended on the mango variety. For the Kent and Edward varieties, HPLC reported significantly higher β-carotene values than UV–Vis spectrophotometry. This suggests that hexane efficiently extracted relatively pure β-carotene, which was accurately quantified by HPLC, whereas the UV–Vis extract may have contained compounds that, despite being present, absorbed less proportionally at 450 nm, or that the average absorption coefficient used for spectrophotometric calculations was not optimal for these specific extracts. For the Haden and Criollo varieties, the trend was reversed, with higher values obtained by UV–Vis, reinforcing the strong influence of matrix composition on the generation of interfering signals.

The choice of extraction solvent proved to be a critical factor, particularly for the reference method (HPLC). Hexane, a nonpolar solvent, was significantly more efficient than ethanol (polar) for extracting lipophilic β-carotene when quantification was performed by HPLC. This behavior is consistent with the "like dissolves like" principle and with previous studies recommending nonpolar or slightly polar solvent systems for optimal carotenoid recovery (Saini & Keum, 2018; Rivera-Madrid et al., 2016). However, the solvent effect was attenuated and less consistent when spectrophotometry was employed. For some varieties, such as Kent, differences between solvents were minimal, further supporting the hypothesis that this method measures a "composite signal" of multiple pigments, whose relative extraction efficiencies with ethanol and hexane may not differ as markedly as those of pure β-carotene. The Kent variety consistently stood out as having the highest β-carotene content, regardless of method or solvent, followed by Edward. This pattern correlates with its higher lipid content (Table 2), which may facilitate the solubilization and extraction of carotenoids.

Evaluation of the studied factors

Figure 1 presents the influence of main effects and interactions on β-carotene content (expressed as µg/g dry peel) obtained by UV–Vis spectrophotometry and HPLC using hexane and ethanol as extraction solvents across four mango varieties (Kent, Haden, Edward, and Criollo). The results highlight the methodological differences discussed previously. For all varieties, spectrophotometry consistently reported higher β-carotene values than HPLC when ethanol was used as the extraction solvent, confirming the systematic overestimation associated with the lack of specificity of this method and the interference of other co-extracted

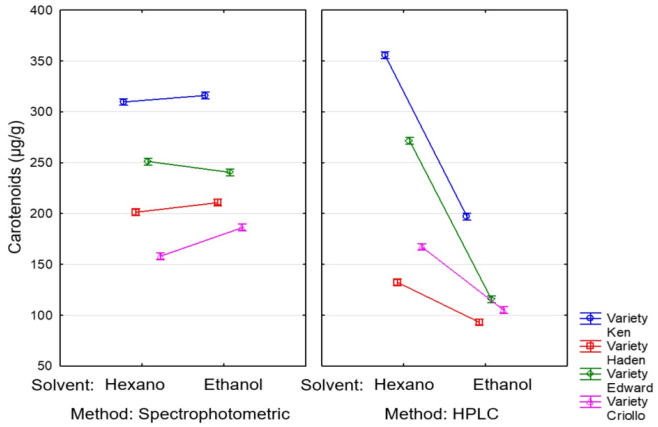

Figure 1 - Influence of the main effects and their interactions on the quantification of β-carotene

carotenoids and pigments absorbing in the same spectral region (Luterotti et al., 2013; Rodriguez-Amaya, 2015). Conversely, when hexane a solvent with higher affinity for lipophilic carotenoids such as β-carotene was employed (Saini & Keum, 2018; Rivera-Madrid et al., 2016), the values obtained by HPLC exceeded or closely matched those from spectrophotometry in the Kent and Edward varieties, indicating a more efficient and selective extraction. Inter-varietal variability was also evident, with Kent consistently exhibiting the highest apparent β-carotene content with both methods and solvents, followed by Edward, Haden, and Criollo. This pattern can be associated with differences in matrix composition, particularly lipid content. Overall, these findings demonstrate that solvent choice and analytical method are not independent factors; rather, their interaction determines the accuracy and precision of β-carotene quantification. This underscores the necessity of employing HPLC in combination with a nonpolar solvent such as hexane to obtain specific and reliable β-carotene values in complex matrices such as mango peels.

In **Figure 2**, contour plots illustrate the interaction between two factors—analytical method and extraction solvent—for each mango peel variety with respect to β-carotene content. For the Kent variety (Fig. 2a), a region of maximum concentration is observed corresponding to the HPLC–hexane or UV–Vis spectrophotometry–ethanol combinations, confirming that these conditions are optimal for β-carotene extraction and quantification in this lipid-rich matrix by minimizing interferences and maximizing extraction efficiency (Saini & Keum, 2018). For the Haden variety (Fig. 2b), the maximum concentration region is observed within the spectrophotometric method regardless of whether ethanol or hexane is used, reflecting the strong influence of specific matrix composition on

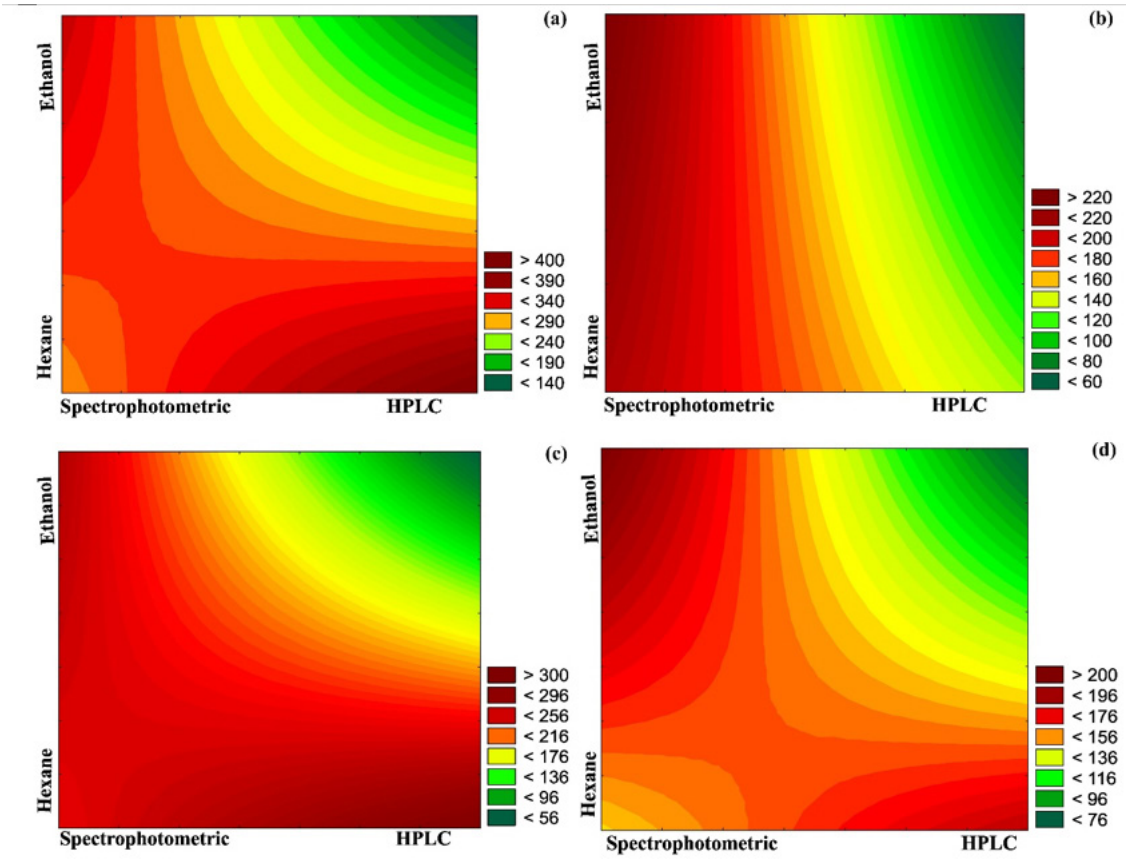

Figure 2. Contour graph for each variety studying the influence of the method and solvent for the mango peel of the Kent (a), Haden (b), Edward (c) and Criollo (d) varieties.

the effectiveness of the extraction and quantification protocol (Rivera-Madrid et al., 2016). In the Edward variety (Fig. 2c), maximum β-carotene concentrations are observed across both methods and solvents; however, values are comparatively lower for the HPLC–ethanol interaction. Finally, for the Criollo variety (Fig. 2d), a pattern similar to that observed for Kent is evident, although it exhibited the lowest overall β-carotene content in the analysis, which can be attributed to its particular carotenoid profile and the presence of co-extracted compounds (Rodriguez-Amaya, 2015). Overall, the contour plots provide an integrated visualization demonstrating that the HPLC–hexane combination represents the most analytically robust operating point for accurate β-carotene quantification in mango peels. Nevertheless, quantification efficiency is strongly variety-dependent, highlighting the importance of considering cultivar-specific matrix effects when standardizing analytical protocols for the valorization of agricultural by-products (Luterotti et al., 2013).

Conclusion

The determination of β-carotene content in mango peels is an analytically complex process in which the significant interaction among matrix variety, extraction solvent, and quantification method is the key determinant of result accuracy and precision. Proximate analysis revealed marked compositional variability among the four varieties studied, with the Kent peel exhibiting the highest contents of dietary fiber, lipids, and protein. This differential composition, particularly the higher lipid content, was positively correlated with enhanced β-carotene extraction, positioning the Kent variety as the most promising source for recovering this bioactive compound from agro-industrial by-products. The methodological comparison clearly demonstrated the fundamental limitation of UV–Vis spectrophotometry, which, due to its lack of specificity, led to systematic overestimation of β-carotene content, especially when ethanol was used as the extraction solvent. This discrepancy, attributable to interference from other co-extracted carotenoids and pigments, confirms that this technique provides only an estimation of "total carotenoid potential" rather than an accurate quantification of individual β-carotene. In contrast, high-performance liquid chromatography coupled with diode array detection (HPLC–DAD) was validated as the reference method for precise and specific quantification. Its ability to separate and identify individual compounds made it possible to discern that solvent efficiency is method-dependent. Hexane, a low-polarity solvent, proved to be significantly more effective than ethanol for extracting lipophilic β-carotene when quantification was performed by HPLC. Consequently, the HPLC–hexane combination was established as the optimal analytical protocol, yielding the most reliable and reproducible values. Finally, the differential response of each mango variety to the evaluated treatments underscores a crucial analytical principle: there is no single universally superior method. The selection of an analytical protocol must be guided by the study objective. For applications focused on quality control, ingredient development, or bioavailability studies requiring high analytical accuracy, the use of HPLC combined with hexane extraction is the most appropriate approach.

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