Journal of Food Composition and Analysis 111 (2022) 104616

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Interlaboratory exercise for the analysis of carotenoids and related compounds in dried mango fruit (*Mangifera indica* L.)

José Villacís-Chiriboga [a,b,c], Griet Jacobs [a], John Van Camp [b], Kathy Elst [a], Jenny Ruales [c], Verónica Marcillo-Parra [c,d], Volker Böhm [g], Andrea Bunea [k], Martina Cirlini [j], Neal Craft [e], Bruno De Meulenaer [b], M. Graça Dias [f], Giacomo Lazzarino [i], Antonio J. Meléndez-Martínez [n], Pieter Versloot [m], Adriana Z. Mercadante [h], Begoña Olmedilla-Alonso [o], Johana Ortiz-Ulloa [l], Carla M. Stinco [n], Stefan Voorspoels [a,*]

[a] *Flemish Institute for Technological Research (VITO), Unit Separation and Conversion Technology, Boeretang 200, 2400 Mol, Belgium*
[b] *Department of Food Technology, Safety and Health, Ghent University, Coupure Links 653, 9000 Ghent, Belgium*
[c] *Department of Food Science and Biotechnology, Escuela Politécnica Nacional, Campus Rubén Orellana, Ladrón de Guevara E11-253, P.O.BOX 17, 012759 Quito, Ecuador*
[d] *Department of Life Sciences and Agriculture, Universidad de las Fuerzas Armadas ESPE, Av. Gral. Rumiñahui S/N, P.O. BOX 171-5-231B, Sangolqui, Ecuador*
[e] *Eurofins Craft Technologies, Inc., Wilson, NC 27893, USA*
[f] *Departamento de Alimentação e Nutrição, Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P. (INSA, I.P.), Av. Padre Cruz, 1649-016 Lisboa, Portugal*
[g] *Institute of Nutritional Sciences, Bioactive Plant Products Research Group, Friedrich-Schiller-Universität Jena, 07743 Jena, Germany*
[h] *Departamento de Ciência de Alimentos e Nutrição, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas 13083-970, Brazil*
[i] *St. Camillus International University of Medical and Health Sciences, Via S. Alessandro 8, 00131 Rome, Italy*
[j] *Department of Food & Drug, University of Parma, Parma, Italy*
[k] *Department of Chemistry and Biochemistry, Faculty of Animal Science and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Manastur Street, 400372 Cluj-Napoca, Romania*
[l] *Department of Biosciences, Food Nutrition and Health Research Unit, Faculty of Chemical Sciences, Cuenca University, Cuenca, Ecuador*
[m] *Division of Human Nutrition and Health, Wageningen University and Research, Wageningen, The Netherlands*
[n] *Food Colour and Quality Laboratory, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain*
[o] *Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Department of Metabolism and Nutrition, Spanish National Research Council (CSIC), Madrid, Spain*

ARTICLE INFO

Keywords:
Reference material
Isomers
Interlaboratory analysis
Lipophilic compounds
Liquid chromatography
Quality assurance

ABSTRACT

An interlaboratory comparison was done for the analysis of carotenoids in freeze-dried mango. The study was performed from July to September 2018. Mango fruit was freeze-dried, homogenized, and packaged under vacuum conditions in portions of 6 g (test sample). Two test samples were sent to the participating laboratories for analysis. Laboratory results were rated using Z-scores in accordance with ISO 13528 and ISO 17043. The standard deviation for proficiency assessment (also called target standard deviation) was determined using a modified Horwitz function and varied between 10% and 25%, depending on the analyte. Out of 14 laboratories from 10 different countries, 9 laboratories (64%) obtained a satisfactory performance ($Z \leq 2$) for the analysis of β-carotene. While for 7 laboratories that analyzed α-carotene, (9*Z*)-β-carotene, β-cryptoxanthin, and zeaxanthin, 4 laboratories (57%) obtained a satisfactory performance. However, only 2 laboratories out of 7 (29%) obtained a satisfactory performance for lutein. Based on the comparability of the analytical results, this study concludes that freeze-dried mango pulp can be used as a reference material for the analysis of α and β-carotene, (9*Z*)-β-carotene, β-cryptoxanthin, and zeaxanthin by applying different analytical procedures for their extraction and quantification.

Abbreviations: BHT, butylated hydroxytoluene; IS, internal standard; CRM, certified reference material; QC, Quality control; QA, Quality assurance.

* Correspondence to: Flemish Institute for Technological Research (VITO), Business Unit Separation and Conversion Technology, Boeretang 200, 2400 Mol, Belgium.
E-mail address: stefan.voorspoels@vito.be (S. Voorspoels).

https://doi.org/10.1016/j.jfca.2022.104616
Received 3 February 2022; Received in revised form 29 April 2022; Accepted 3 May 2022
Available online 6 May 2022

1. Introduction

Fruit and vegetables are the primary sources of nutrients and other compounds that, isolated or in combinations, positively affect human health. As recommended by the WHO, 400 g of fruits and vegetables per day is necessary to seize their beneficial effects and prevent the onset of cardiovascular diseases, type 2 diabetes, cancer, cardiovascular diseases, obesity, and other health disorders. Information provided by FAO indicates that out of total deaths related to ischemic heart disease, cancer of the gastrointestinal tract, and stroke, 14%, 11%, and 9%, respectively, could be prevented with adequate consumption of fruits and vegetables. Worldwide, around 4 million deaths were attributed to insufficient intake of fruit and vegetables in 2017 (FAO, 2020).

Mango (*Mangifera indica* L.) is one of the most traded and consumed tropical fruits, with a worldwide production of around 45 million metric tons in 2019. The increasing trends in mango consumption are associated with its flavor and nutritional content since it is a rich source of carbohydrates, organic acids, vitamins, phenolic compounds, and carotenoids (Khalid et al., 2020). Of these compounds, particular interest has been given to carotenoids, a versatile group of bioactives that actively participate in the risk reduction of various adverse conditions (*e.g.*, cancer, cardiovascular diseases, metabolic disorders, eye, skin and bone diseases). Additionally, it has been shown that cognition, early development, immunity, and other beneficial activities, are modulated by the intake of carotenoids. With these premises, efforts have been put together to develop supplements, functional foods, nutraceuticals, or nutricosmetics. The socioeconomic importance of these compounds is therefore undeniable (Meléndez-Martínez et al., 2021a, 2021b).

Carotenoids are classified as carotenes and xanthophylls. Carotenes are exclusively formed by carbon and hydrogen (hydrocarbon chains), while xanthophylls have oxygen in the molecule, which can form oxygenated functional groups (*e.g.*, epoxy, ketone, carbonyl) and be present in the matrix as free carotenoids or esterified with fatty acids. Taking into account these characteristics, combinations of polar and non-polar solvents are suitable for the extraction of carotenoids in low-moisture samples. Moreover, for analytical purposes, both polar and non-polar organic solvents are synergistically used to extract different carotenoids from the same biomass in relatively short times. The selection of the solvent and subsequent clean-up process is a key step for the correct characterization of the carotenoid composition of a plant matrix to avoid underestimating their composition and concentration (Saini and Keum, 2018).

After extraction, the separation of the compounds is fundamental for their identification and characterization. C_{18} chromatography columns have been widely applied to separate carotenoids. UHPLC systems with C_{18} columns with smaller particle sizes (sub 2-μm) offer a better separation and shorter run times. In addition, triacontyl-bonded (C_{30}) columns, with higher hydrophobicity, have improved the resolution for carotenoid separation and efficiently resolve isomers (*e.g.*, α-cryptoxanthin and zeinoxanthin) based on the different retention times. However, for this column, longer run times are required (Giuffrida et al., 2020, 2018). Coupling these devices to different types of detectors (Diode Array Detector (DAD), mass spectrometry (MS)) allows both qualitative and quantitative determination of carotenoids. The conjugated double-bond system of carotenoids constitutes the light-absorbing chromophore that facilitates its identification through UV-Vis. However, the carotenoid concentration of plant biomass can be overestimated due to co-extracted interfering compounds (*e.g.*, carbohydrates, phenolic compounds, polyunsaturated fatty acids) (Biehler et al., 2010; Zhou et al., 2022). MS instruments overcome spectral interferences and detect ions according to their mass-to-charge ratio (m/z). The information obtained on the molecular structure depends upon the molecular mass of the analyte and the fragmentation pattern. Although the last is influenced by the mobile phase and the ionization technique, different techniques obtain specific carotenoid fragments. This approach can be helpful to determine carotenoids with the same molecular mass but different fragmentation patterns, *e.g.*, geometrical and structural isomers (Amorim-Carrilho et al., 2014; Hoffmann and Stroobant, 2007). It is clear that such heterogeneity of procedures for carotenoid analysis in plant materials may affect the accuracy and reliability of the information obtained. In this way, the comparison of the analytical outcome obtained by different approaches can be a valuable approach to assess the reliability of the information delivered.

Even if different techniques have been applied to characterize plant matrices in terms of carotenoid content, several steps in the analytical process, including sampling, sample pretreatment, extraction, choice of mobile phases, and the choice of the separation column, will influence the outcomes. Moreover, variations during sample handling (*i.e.*, preparation and extraction or inconsistencies through analyte recovery and injection variability) can be overcome by using an internal standard (IS), which is a compound with a similar structure to the analytes present in the extract. Typically, it is carried out by dividing the analyte peak response (height or area) in the sample by the internal standard peak response in the sample corrected for the theoretical internal standard peak response (Craft and Furr, 2018). Additionally, antioxidants (*e.g.*, BHT, butylated hydroxytoluene (BHT), pyrogallol, or ascorbic acid) may be added to prevent oxidation or isomerization of the target components. Similarly, sodium bicarbonate is added when the extract is performed in acidic fruits as a mild neutralizing agent to prevent epoxy to furanoid carotenoid rearrangement (Saini and Keum, 2018).

Delivering reliable data is the basis for any laboratory. This is achieved after performing strict quality assurance and quality control (QA/QC) protocols to guarantee the production of traceable information with standard protocols for the acquisition of data. In addition to initial and ongoing in-house method validation, the execution of a ring trial for the analysis of homogeneous materials in different laboratories following ISO 17025 is a requirement of the quality management system (FAO and AGES, 2015).

To this end, several studies reported the in-house and interlaboratory analysis of carotenoids in food products. In general, in-house methods provide good results, as Dias et al. (2008) demonstrated. These authors developed an in-house validated protocol for separation and determination of (all-*E*)-α-carotene, (all-*E*)-β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin in tomato. Overall, good repeatability and low relative standard deviations were obtained. Besides in-house method development, an external validation enhances the reliability of the analytical outcomes. Hence, Eriksen et al. (2017) developed an UHPLC-DAD-based method to analyze significant carotenoids in spinach, serum, chylomicrons, and feces. The method developed was further validated by an external assessment of comparable HPLC systems. This external assessment showed no significant differences in the content of lutein or β-carotene in the samples. In contrast to these studies, the interlaboratory comparison by using different methodologies has provided indistinct results, as shown by Luterotti et al. (2013), who identified the critical uncertainty sources (*e.g.*, protocols) and applied statistical analysis to indicate the conditions under which the biases between the results could not be identified in an intra-and interlaboratory spectrophotometric and HPLC study of lycopene, β-carotene and total carotenoids in tomato products and yellow maize flours/grits. The authors concluded that good correlation coefficients could be found together with high biases in the experimental results, which can cause ambiguous conclusions about the reliability of the results. When different techniques are applied to characterize the carotenoid content of specific biomass, the analytical outcomes cannot always be uniform due to unexplained reasons, which ultimately affects the reliability of the conclusions. Therefore, additional studies comparing different methodologies are of utmost importance.

Performing interlaboratory analysis, or in-house validations, could help identify weaknesses and specific critical points during the research. Yet, given the lack of reference materials with known concentrations, evaluating the quality of the data obtained becomes fundamental for any laboratory. Certified reference materials, such as those issued by the

European Joint Research Centre or the National Institute for Standards and Technology (NIST, US) are a cornerstone in any method validation. In addition, interlaboratory exercises are a powerful tool not only to assess comparability of results, but also to help identify method weaknesses, critical points and performance aberrations. Yet, given the lack of (certified) reference materials for the determination of carotenoids in fruits, optimization, evaluation of method performance and proper validation is a challenge for any laboratory. Such reference materials are an important tool in method validation (including measurement uncertainty), internal and external quality assurance (ISO, 2015). However, the high cost of CRM production is a factor that influences the development of new reference materials (Lauwaars and Anklam, 2005; Zakaria and Rezali, 2014). Therefore, it is important to carry out research to produce new stable materials, characterized by their low cost, availability, and high concentration of easily extractable carotenoid components.

Consequently, this work describes an interlaboratory comparison exercise for carotenoids in freeze-dried mango pulp from Ecuador, a plant material chosen since it fulfils the requirements mentioned earlier (cheap material, immediate availability and rich in carotenoids). This exercise was organised by the Flemish Institute for Technological Research (VITO). The general objective was to compare the performance of the analytical techniques and the analytical standards for the correct identification and quantification of carotenoids in mango samples. Additionally, we aimed to provide a potential reference material that can be used in the future as quality control for the analytical measurement of carotenoids.

2. Materials and methods

2.1. Sample preparation

All the samples were prepared by the Department of Food Science and Biotechnology at Escuela Politécnica Nacional in Ecuador. For the analysis, mango (variety Tommy Atkins) was purchased at a local market in Quito, Ecuador. Samples were transported to the lab, hand washed and the mangoes with a maturity index of 5 (12 – 15°Brix) were selected for the experiments. The fractions (peel, flesh and stone) were manually separated, and the pulp was freeze-dried until a final moisture content of 3.5%. The particle size was reduced with a coffee mill, sieved to exclude particle sizes smaller than 0.425 mm, and the particles with a size greater than that were milled and sieved again. The process was repeated until a suitable amount of material was obtained. In total, around 400 g of mango pulp powder was obtained. Then, samples of 6 g each were transferred under vacuum to polyethylene-aluminum bags and sealed airtight for storage at − 20 °C. In order to avoid degradation of compounds with light, the process was carried out under dim light.

2.2. Homogeneity study

Each bag of the whole batch was assigned a specific number. Eight bags were selected using a random number generator and measured in duplicate under repeatability conditions. In the first instance, extraction with acetone:methanol (70:30; $v.v^{-1}$) was performed, followed by extraction with dichloromethane:methanol (50:50; $v.v^{-1}$), for 15 min in an ultrasound bath at 4 °C (solvent:material ratio of 1:10 $w.v^{-1}$) (Villacís-Chiriboga et al., 2021), and the analysis was performed *via* liquid chromatography with ultraviolet absorbance detection (LC-UV) at a wavelength of 450 nm. β-Carotene was selected as a proxy for homogeneity assessment, as this analyte was present in significant quantity. The homogeneity was evaluated according to the procedure described by Fearn and Thompson (2001).

The Cochran test procedure for duplicate results was used to test for homogeneity of the data set. The use of average-normalised data in the homogeneity assessment was carried out according to ISO (1352)8 (2015), with a target acceptable study variation of 5%. The following equation was used [Eq. 1]:

$$S^2_{sam} = \frac{D^2_{max}}{\Sigma D^2_i} \quad (1)$$

Where:
S^2_{sam} = Cochran´s statistic test
$\mathbf{D_{Max}}$ = the largest difference between duplicates
$\mathbf{D_i}$ = difference of each pair of duplicates

2.3. Stability of the samples

The sample bags were stored at − 80 °C until dispatch. Stability data of reference material for these types of matrixes (*e.g.*, BCR-485) has shown that samples stored at − 20 °C are stable for 48–60 months. Measurements were executed within 3 weeks from dispatch. In view of the nature of the samples (dry, no oxygen, airtight containers), and the stability of carotenoids when stored at − 70 °C (Dias et al., 2014), no influence on sample stability was expected for the duration of this study. Therefore, the stability of the materials was not assessed.

2.4. Procedure for the interlaboratory trial

This interlaboratory ring trial was organized by VITO and invitation letters were sent to 27 candidate laboratories. It was indicated that participation would be free of charge (to reach as many participants as possible). The laboratories that subscribed, received the control materials to be analysed. The condition for participation was that test results had to be submitted within the stipulated deadline. Fourteen laboratories (from 10 different countries) agreed to participate and are represented by the current co-authors. For reasons of confidentiality, the datasets are anonymized. The majority of the participating institutions were from Europe (10 laboratories), 3 institutions were from South America and one was situated in North America. These laboratories received an individual laboratory code to report their measurement results.

2.5. Protocol for requesting the data

Test materials were dispatched to the participants under ambient conditions. Each participant received 2 bags containing 6 g of freeze-dried mango pulp. Moreover, a letter was included explaining that samples must be stored in a freezer after arrival at the laboratory. Once open, they must be kept away from light and oxygen to maintain stability. Participants were asked to perform 3 replicate analyses on 2 different days using the same procedure (which was free to choose). An Excel file was used for reporting, with detailed information on the protocol, equipment and reagents, besides the identification and quantification of the analyzed compounds.

2.6. Methods of analysis of the participants

The methods applied by the participants are described in Table 1. The amount of sample used for the extraction varied among the labs, ranging between 10 and 3000 mg. Regarding the solvents used, acetone, methanol (MeOH) and tetrahydrofuran (THF) were mostly applied for the extraction. HPLC was used for the separation of the compounds, except for one laboratory reporting the use of UHPLC. Eight laboratories used C_{30} columns, while five laboratories used C_{18} columns. For the identification and quantification of carotenoids, one laboratory used MS and the other laboratories used a diode-array detector (DAD) (one reported results as UV-Vis).

2.7. Statistical analysis and measurement of standard uncertainty

Intra-day precision was evaluated by analyzing data of three extracts

Table 1
Methods reported by the participants.

Lab code	Sample intake (mg)	Sample pretreatment	Extraction	Clean-up	IS	LC	Column phase	Column dimensions	Detector/ Wavelength	Injection solvent	Conservation	Reference
1	800	Saponification with pyrogallol	THF:hexane	NR	None	HPLC	C_{18}	25 cm × 4.6 mm; 3 μm	DAD/ 450 nm	20 EtAc/80 (90 ACN/ 10 IPA)	Light protection with UV filters	[44]
2	500 – 1500	Saponification	THF:MeOH (1:1; v-v)	PVDF 0.45 μm filters	(All-*E*-)β-apo-carotenal	HPLC	C_{18}	10 cm × 4.6 mm; 3 μm in series with 25 cm × 4.6 mm; 5 μm	DAD/ 450 nm	ACN:MeOH:DCM (7:2:1; v-v:v)	BHT during extraction and light protection	[45]
3	1000	Enzymatic digestion + saponification	Acetone:Pentane (4:6)	Clean-up with 10% NaCl-solution and H_2O	(All-*E*-)β-apo-carotenal	HPLC	C_{30}	25 cm × 4.6 mm; 5 μm	DAD/450 – 470 nm	MeOH:ACN (9:1; v-v) + EtAC + 0.25% triethylamine	NR	[46]
4	500	Saponification of extract	MeOH:THF (1:1; v-v)	None	Echinenone	HPLC	C_{30}	25 cm × 4.6 mm; 5 μm	DAD/ 450 nm	MeOH:MTBE (1:1; v-v)	BHT during extraction and light protection	[47]
5	250	Saponification	Acetone	None	None	HPLC	C_{30}	25 cm × 4.6 mm; 5 μm	DAD /450 nm	MeOH:MTBE (1:1; v-v)	Light protection, nitrogen and low temperature	[48]
6	10 – 15	NR	H_2O:Acetone:ACN (2:4:4; v-v:v)	None	(All-*E*-)β-apo-carotenal	HPLC	C_{18}	15 cm × 4.6 mm; 5 μm	DAD /NR	H_2O:acetone:ACN (1:2:2; v-v:v)	Light protection	[49]
7	3000	Saponification	Acetone + Hexane: Petroleum ether (1:1; v-v)	PTFE 0.45 μm filters	(All-*E*-)β-apo-carotenal	HPLC	C_{30}	25 cm × 4.6 mm; 5 μm	UV-Vis/450	MeOH:MTBE:H_2O (v:v: v)	BHT for standard solutions	[50]
8	500	Saponification	Hexane:EtOH: Acetone (5:2.5:2.5; v-v:v) + hexane	None	(All-*E*-)β-apo-carotenal	HPLC	C_{18}	25 cm × 2.1 mm; 5 μm	DAD /450 nm	ACN:MeOH:EtAc (6:2:2; v-v:v)	NR	[51]
9	1000	NR	Acetone:MeOH (50:50; 1:1) + DCM:MeOH (50:50; 1:1)	None	NR	UPLC	C_{18}	10 cm × 2.1 mm; 1.8 μm	MS/NR	DCM	BHT during extraction	[16]
10	500 – 1000	NR	Light petroleum/ EtAc/MeOH (1:1:1; v-v:v)	PTFE 0.45 μm filters	NR	HPLC	C_{30}	25 cm × 4.6 mm; 5 μm	DAD/NR	EtAc	NR	[52]
11	1000	Saponification	Ethanol:hexane (4:3; v-v)	Clean-up with 10% NaCl-solution and H_2O	(All-*E*-)β-apo-carotenal	HPLC	C_{30}	25 cm × 4.6 mm; 5 μm	DAD/ 450 nm	EtAc + 0.25% (v/v) triethylamine/MeOH/ ACN (50:45:5; v-v:v)	BHT during extraction	[53]
12	1800 – 2000	None	MeOH:THF (1:1; v-v)	None	Retinyl acetate	HPLC	C_{18}	25 cm × 3 mm; 5 μm	DAD/450	MeOH:Butanol (6:4, v-v)	BHT during extraction	[54]
13	120 – 130	NR	Hexane:Acetone (1:1; v-v)	NR	NR	HPLC	C_{30}	15 cm × 4.6 mm; 3 μm	DAD/285 – 450 nm	EtAc	NR	[35]
14	110 – 115	NR	H_2O + Acetone + Diethyl ether: Petroleum ehter (1:1 v:v)	NR	(All-*E*-)β-apo-carotenal	HPLC	C_{30}	25 cm × 4.6 mm; 5 μm	DAD/ 450 nm	MeOH:MTBE (1:1; v-v)	BHT during extraction	[55]

NR = Not reported; MeOH = methanol; ACN = acetonitrile; EtOH = ethanol; THF = tetrahydrofuran; DCM = dichloromethane; EtAc = Ethyl acetate; IPA = isopropyl alcohol; MTBE = Methyl-tert-butyl ether

measured on the same day, while the inter-day precision was evaluated using all the values from the two days. Both results were expressed as the coefficient of variation (% CV).

The standard uncertainty μ_A [Eq. 2] was used to calculate the relative uncertainty of measurement results ($\mu_{A,rel}$) [Eq. 3] as follows (Chen et al., 2021):

$$\mu_A = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \overline{X}_m)^2}{n(n-1)}} \quad (2)$$

$$\mu_{A,rel} = \frac{\mu_A}{\overline{X}_m} \quad (3)$$

Where.

x_i = Average test results of each laboratory.

$\overline{X}_m$ = Average test results of all laboratories.

n = Number of laboratories.

The precision of the mean values was calculated as 2 ×SD (Nübler et al., 2021). The performance of the laboratories was evaluated using the Z-score [Eq. 4] (ISO 8, 1352, 2015), determined by fit-for-purpose standard deviations (FFP), which at the same time were estimated by the Horwitz equation (Horwitz and Albert, 2006).

$$Z_i = \frac{X_i - X_a}{\sqrt{\mu^2(X_i) + \mu^2(X_a)}} \quad (4)$$

Where:

X_i = participants' result.

X_a = Reference value (average of averages for the same compound).

$\mu(X_i)$ = reported standard uncertainty of the result X_i.

$\mu(X_a)$ = standard uncertainty for the assigned value.

3. Results and discussion

In the last years, there has been significant progress in the research on carotenoids in food, feed and plant-based materials. However, the lack of interlaboratory exercises for the analysis of these compounds has hindered compliance with quality requirements during quality control and quality assurance. This is even more evident if we consider that carotenoids are known as unstable bioactives and that certified reference materials (CRM) maintain a relatively high production cost.

3.1. Homogeneity study

β-Carotene concentration was measured to assess the homogeneity of the samples. The Cochran value (C_{CAL}) was obtained by calculating the variability between the replicates (data not shown) and compared against the critical value (C_{CRI}) of 0.68 (95% confidence level). The calculated value was lower than the critical limit (0.56 < 0.68). Hence the whole set was retained since adequate homogeneity was found. Moreover, a test statistic S^2_{sam} < critical value (0.00125 < 0.0058) was obtained, so the test passed the criterium for homogeneity.

3.2. Reported results

All the participants reported concentrations above the limit of quantification (LOQ) for β-carotene in the sample provided. However, large variability in reported results was found for the other carotenoid components. Some laboratories reported up to 16 different compounds while others only focused on 1 compound. Overall, 37 different analytes were reported by all participating laboratories. Based on all data received, performance was evaluated based on the quantification of α-carotene, β-carotene, (9Z)-β-carotene, β-cryptoxanthin, lutein and zeaxanthin (Table 2). In agreement with other reports, β-carotene was the major carotenoid in mango, with measured concentrations ranging from 7.85 to 30.0 μg.g^{-1} dry weight (DW), followed by lower concentrations of (9Z)-β-carotene (1.22 – 6.22 μg.g^{-1} DW), zeaxanthin (0.39 – 2.95 μg.g^{-1} DW), α-carotene (0.40 – 4.50 μg.g^{-1} DW), β-cryptoxanthin (0.16 – 1.98 μg.g^{-1} DW) and lutein (0.58 – 4.09 μg.g^{-1} DW). Despite the differences in carotenoid concentrations, the compounds described in this study have also been previously described as the main carotenoids in the variety Tommy Atkins (Marcillo-Parra et al., 2021; Ruales et al., 2018).

The measured concentration of quantified analytes depends on the extraction and subsequent clean-up. Besides, other processing parameters during extraction also influence the outcome. Some laboratories saponified the extract, which hydrolyzes carotenol esters along with triglycerides and proteins resulting in a simplified chromatogram with only non-esterified carotenoids, while the use of BHT could have stabilized the carotenoids in the extracts due to its antioxidant properties. However, the exposition of the extract to light, high temperature, acids, or the combination of such factors could have induced the *E-Z* isomerization of the carotenoids in the sample, which is not avoided by the

Table 2
Concentration of the reported carotenoids, all expressed in μg.g^{-1} DW as average ± SD (n = 6).

	Compounds [μg.g^{-1} DW]*					
Labcode	α-carotene	β-carotene	(9Z)-β-carotene	β-cryptoxanthin	Lutein	Zeaxanthin
1	0.40 ± 0.03^{a}	18.69 ± 0.30 ^{cd}		0.89 ± 0.07^{b}	1.94 ± 0.09^{c}	1.77 ± 0.05^{b}
2	1.04 ± 0.43^{b}	19.67 ± 1.11 ^{cd}	3.20 ± 0.37^{e}		3.95 ± 0.39^{e}	<LOD (0.019)
3		18.00 ± 1.96^{c}				
4		19.55 ± 0.44 ^{cd}	2.53 ± 0.10^{d}			
5	0.96 ± 0.13^{b}	18.96 ± 1.28 ^{cd}	1.94 ± 0.18^{b}	0.91 ± 0.11^{bc}	3.11 ± 0.12^{d}	1.82 ± 0.13^{b}
6	4.54 ± 0.79^{e}	19.93 ± 1.89 ^{cd}	1.22 ± 0.16^{a}	0.16 ± 0.04^{a}		1.73 ± 0.16^{b}
7		12.09 ± 1.54^{ab}				
8		28.44 ± 1.36 ^{f}				
9		23.23 ± 6.44^{e}		1.98 ± 0.14^{e}	0.78 ± 0.56^{a}	0.39 ± 0.18^{a}
10		13.27 ± 1.73^{b}	1.86 ± 0.12^{b}			
11	2.43 ± 0.15^{d}	9.65 ± 0.93^{a}		1.63 ± 0.12^{d}	1.36 ± 0.47^{b}	
12	0.71 ± 0.11^{ab}	20.99 ± 0.45^{de}	6.22 ± 0.17 ^{f}	1.00 ± 0.03^{c}	3.97 ± 0.12^{e}	1.44 ± 0.10^{b}
13	1.87 ± 0.06^{c}	29.98 ± 0.86 ^{f}	2.22 ± 0.09^{c}	1.89 ± 0.08^{e}	4.09 ± 0.12^{e}	2.95 ± 1.17^{c}
14		7.85 ± 0.70^{a}				
Average	1.71	18.59	2.74	1.21	2.71	1.45
SD	1.32	6.12	1.53	0.61	1.32	0.97
Relative uncertainty (%)	31.67	9.13	22.77	20.47	18.84	19.98
Precision of mean values	2.65	12.24	3.06	1.21	2.52	1.46
CV (%)	77.59	32.91	55.77	50.14	46.14	61.84

LOD = Limit of detection.

DW = Dry weight

Different letters in the same column indicate statistically significant differences among the concentration detected for each compounds in each laboratory ($p < 0.05$).

presence of BHT (Arvayo-Enríquez et al., 2013). On the other hand, the addition of little proportions of ammonium acetate or triethylamine (usually ≤ 0.1%) to solvents in the mobile phase is thought to improve the recovery of carotenoids from the column and the peak shapes (Melendez-Martinez et al., 2013; Rodriguez-Amaya, 2001).

As shown in Table 1, all the laboratories used reversed-phase columns, but of different types (C_{18} or C_{30}), which influence the resolution of the carotenoid molecules. These differences are very likely due to the interaction of carotenoids in the extracts with the stationary phase. C_{18} columns have been widely applied for the analysis of carotenoids in view of their hydrophobicity and suitability for separation under a wide range of polarities and solvents. However, C_{30} columns are characterized by a higher hydrophobicity, and provide enough phase thickness to enhance interaction with carotenoids. C_{30} columns were specially developed for carotenoid analysis, since these columns can separate not only isomers (α-, β-), but also geometric isomers of carotenoids (*Z/E*) (Sander et al., 2002). This phase has also efficiently separated optical isomers of some carotenoids (Meléndez-Martínez et al., 2009). Moreover, the operation of the columns is influenced by the chromatographic packing, which is a function of the column wall, particle migration and arrangement (Dorn et al., 2017). In this sense, Zelenyánszki et al. (2019), showed that commercially available columns are axially heterogeneous. Greater homogeneity was observed in shorter columns.

After separation, different detectors were reported for the identification and quantification of carotenoids. Among the 14 laboratories, 12 used DAD, 1 UV/Vis, while MS was used by one laboratory. Since all the laboratories reported β-carotene, results obtained with DAD and MS detection are very similar (see Tables 1 and 2). However, the laboratory that used a UV-Vis detector reported a concentration significantly lower compared to the other laboratories. As explained by Crupi et al. (2012), if calibrated correctly, the detection limits and reproducibility of the analysis of carotenoids are similar for both MS and DAD. Thus, it could be possible that the variations in the result of the laboratory that used a UV-Vis detector are attributed to the sample handling, preparation, and the prevention of degradation or oxidation of carotenoids.

3.3. Validation of mango sample as reference material for carotenoid analysis

The quantification of the different carotenoid compounds in the mango samples was evaluated by means of both inter- and intra-day precision, expressed as the coefficient of variation (%CV) (Table 3). The variation for the intra-day analysis was, on average, 6.28%, although in specific cases outliers were found (variations up to 69%). In the same line, the inter-day precision was 12.71%, average value. Similarly, variations up to 97% were found. Overall, it can be seen that the participants were characterized by a good precision (CV lower than 10% and values above 30% are considered outliners). These errors could be attributable to sample handling or the presence of impurities (Farias Couto et al., 2013; Kimura and Rodríguez-Amaya, 1999). In a study performed by Stinco et al. (2014), a rapid resolution liquid chromatography (RRLC) method for analysis of carotenoids was developed and in-house validated on 12 commercial fruits and vegetables. The repeatability (%CV on the intra-day assay) ranged between 0.58% and 6.81%, and reproducibility (%CV for the inter-day assay) ranged from 4.66% to 11.87% for the analysis of unsaponified samples. When the samples were saponified, %CV values up to 26.38% and 27.61% were obtained for the repeatability and reproducibility tests, respectively. These results show the negative effect that saponification (which is mainly used to remove unwanted lipids, chlorophylls and/or to simplify the chromatograms when carotenoids are esterified) can have in the quantification of carotenoids, since this step could lead to isomerization or destruction of alkaline labile carotenoids. Although the extent of such effects depends on the saponification conditions, *e.g.*, temperature, reaction time and alkali concentration. In a similar study, an analytical method for the determination of carotenoids *via* RRLC in baby fecal

Table 3
Intra and inter-day precision measurement, all expressed as the average ($n = 3$) of CV (%).

	α-carotene			β-carotene			(9Z)-β-carotene			β-cryptoxanthin			Lutein			Zeaxanthin		
	Day 1	Day 2	Interday	Day 1	Day 2	Interday	Day 1	Day 2	Interday	Day 1	Day 2	Interday	Day 1	Day 2	Interday	Day 1	Day 2	Interday
Labcode																		
1	5.7	4.1	6.7	0.7	1.8	1.6				7.5	3.7	7.7	2.9	3.1	4.8	0.7	2.5	3.0
2	6.0	3.2	40.8	6.5	4.1	5.6	2.6	12.0	10.7				1.1	3.5	10.0			
3				3.9	13.5	10.9												
4				2.4	0.8	2.3	2.4	1.5	3.5									
5	3.5	2.6	13.6	1.1	1.1	6.8	2.1	0.7	8.6	1.2	2.6	11.4	1.5	1.2	3.7	0.6	2.5	2.2
6	6.3	16.2	17.5	9.9	2.1	9.5	1.1	1.9	11.9	15.5	6.9	21.1				9.6	2.5	7.7
7				7.4	12.2	12.7												
8				5.2	4.1	4.8												
9				19.6	17.8	27.7				0.1	9.2	6.6	14.5	68.7	96.9	13.8	2.5	56.9
10				13.8	8.7	13.0	2.1	6.8	5.8									
11	5.6	5.1	5.5	13.2	2.9	9.6							19.0	3.5	34.5			
12	14.1	7.7	14.7	0.9	2.9	2.1	6.3	0.6	2.5	0.3	3.6	2.9	1.8	3.8	3.0	2.6	2.5	12.8
13	2.2	2.7	2.9	3.3	2.3	2.9	2.1	3.3	3.5	3.8	3.2	3.9	3.4	2.6	3.1	60.4	2.5	51.7
14				2.6	3.6	8.9												

samples was in-house validated. In line with previous results, unsaponified samples were evaluated with repeatability values ranging from 0.86% to 6.94% and reproducibility values ranging from 2.36% to 9.92% (Stinco et al., 2019).

On the other hand, the relative uncertainty of the data set (Table 2) was between 9.13% and 31.67% for β-carotene and α-carotene, respectively. These values follow the same trend as the %CV. In a similar way, a lower precision, expressed as a higher deviation from the average value, was obtained due to a large SD for β-carotene (± 12.24 $\mu g.g^{-1}$ DW), and a lower SD for β-cryptoxanthin (± 1.21 $\mu g.g^{-1}$ DW).

As explained above, Z-scores were calculated for all parameters for which more than 6 compounds were reported. The criteria of classification were as follows: if the Z-score is $Z \leq 2$, the performance of the laboratory is satisfactory, while with a value of $2 < Z < 3$ the classification of the laboratory is questionable and at a Z-score ≥ 3 the result was regarded as unsatisfactory. The graphical representation of the Z-score for the analyzed carotenoids is given in Supplementary material SS.1.

The results of the performance of laboratories are displayed in Table 4. It can be seen that for the majority of the compounds, a Z-score lower than 2 was found, meaning a satisfactory result for the laboratories. However, the results for lutein were found to be unsatisfactory for 42% of the laboratories. This variation could have been due to the extraction method used, destruction of this xanthophyll during saponification and deficient separation (Scott et al., 1996). In addition, the separation of geometric and structural isomers demands longer run times and the specific use of a C_{30} column, since C_{18} columns does not resolve geometrical isomers and inefficiently resolves positional isomers (*i.e.*, lutein and zeaxanthin) (Simonovska et al., 2013).

Moreover, regarding β-carotene, there is no correlation between the amount of sample used for extraction and the method performance (data not shown), even though the sample amount ranged between 10 mg and 3 g. For the other carotenoids, for which the content in the sample was lower, applying 10 mg of sample resulted in the largest Z-scores. This indicates that a higher sample amount for carotenoid analysis is advisable. Additionally, a smaller particle size (≈ 50 μm) could be positively related to a better extraction performance in analytical processes (Saini and Keum, 2018). Moreover, as explained above, the solvent used for the extraction can also influence the extraction yield. From the results displayed in Table 1, it can be seen that among all the solvents used (*e.g.*, H_2O, THF, MeOH, pentane, hexane, petroleum ether), most of the laboratories included acetone within the solvent mixture.

The quality of the data generated from each lab was also evaluated on the basis of the prevention of degradation or isomerization of the carotenoids in the presence of antioxidants and the use of an IS. The 4 laboratories that didn't report any conservation method or addition of IS were evaluated with Z-scores lower than 2, meaning that their performance was satisfactory for the evaluation of β-carotene. Of these laboratories, four reported light protection during the procedure, which could provide a good explanation for the satisfactory result obtained. On the other hand, since no addition of IS nor conservation was reported for the other laboratories, it is difficult to establish a reason for the acceptable outcome. It could be due to the relative stability of carotenoids when solubilized in extractants, as has been previously shown (Patel et al., 2019). Hence, results that were the least in agreement were all traceable to laboratories that did not use or report any form of conservation. Carotenoids are unstable molecules that can undergo isomerization after extraction or severe purification steps (Martins and de Rosso, 2016), which can affect the quantification. Moreover, it could be seen that specific laboratories were able to identify different configurations of the same compounds, *i.e.*, (13*Z*)- or (15*Z*)-violaxanthin, (13*Z*)- or (15*Z*)-β-carotene (data not shown). Although some of these isomers might be separated on some C_{18} columns, C_{30} column offers a better separation of geometrical isomers, as already explained. With this information in mind, it is clear that the analysis depends both on the process and on the sample handling. Yet, as mentioned, given the instability of carotenoids, the use of BHT, pyrogallol or ascorbic acid, together with an IS could be used for analytical purposes. Moreover, a mild alkali (sodium bicarbonate) should be added for extraction to avoid epoxy to furanoid rearrangement (due to presence of violaxanthin in mango) (Rodriguez-Amaya, 2001).

3.4. Development of CRM from mango powder for the analysis of carotenoids

The importance of CRM for analytical laboratories could be traced back in time to the 1970's, when the growing concern about the quality of data generated in food analysis led to the formation of specialized organizations (*i.e.*, National Institute of Standards and Technology, Bureau Communautaire de Référence, Institute for Reference Materials and Measurements, among others) and the production of many homogeneous stabilized biological materials both from animal and plant origin (Wise and Phillips, 2019). Over the years, with the progress of analytical technology and laboratory analysis, along with the increasing demand for analytical measurements of good quality, there has been an increase in the production of CRM for such purposes.

However, most analytical laboratories perceive CRM as expensive and prohibitive, depending on the type and the state of the matrix (*e.g.*,

Table 4
Study performance based on Z-score obtained for each analyte.

Labcode	α-carotene	β-carotene	(9*Z*)-β-carotene	β-cryptoxanthin	Lutein	Zeaxanthin
1	-3.1	-0.4		-1.3	-1.9	1.5
2	-1.6	0.1	1.1		3.0	-6.7
3		-0.7				
4		0.1	-0.5			
5	-1.8	-0.2	-1.9	-1.3	0.9	1.7
6	6.6	0.3	-3.7	-4.3		1.3
7		-3.8				
8		4.6				
9		2.0		3.2	-4.8	-4.6
10		-3.2	-2.1			
11	1.7	-5.0		1.7	-3.4	
12	-2.3	0.8	8.5	-0.8	2.8	-0.1
13	0.4	5.4	-1.3	2.8	3.3	6.9
14		-5.8				
Number of participants	7 (54%)[a]	14 (100%)	7 (54%)	7 (54%)	7 (54%)	7 (54%)
Satisfactory	4 (57%)	9 (64%)	4 (57%)	4 (57%)	2 (29%)	4 (57%)
Unsatisfactory	2 (29%)	5 (36%)	2 (29%)	2 (29%)	3 (43%)	3 (43%)
Questionable	1 (14%)	0 (0%)	1 (14%)	1 (14%)	2 (29%)	0 (0%)

$Z \leq 2$ = satisfactory; $2 < Z < 3$ = questionable; $Z \geq 3$ = unsatisfactory
[a] = number of participants (% with respect to the number of laboratories)

solid, powder, liquid or even needs for transport under special conditions, and many other considerations). Yet, the acquisition of a CRM and the performance of a quality control assessment should be viewed as an investment rather than an expense, since the analysis repetition will be reduced, and consequently, the reagents will be used to a lesser extent, while the reliability of the laboratory will be enhanced (Venelinov and Quevauviller, 2003).

Besides price, the stability of the compounds is a factor that is also considered before acquiring and using a specific CRM. In the specific case of carotenoids, which are regarded as unstable compounds, the exact measurement of their concentrations might be influenced by sample handling, specific extraction procedures and other variables that are inherent to each laboratory.

The development of a CRM with mango demands the examination of several points. Although quality control for the analysis of lipophilic compounds can be performed with already available CRM suited for this purpose (BCR-485 mixed vegetables), their availability and high cost (~250 €) hinder their large scale use in carotenoid analysis. As an alternative, mango could be used as CRM, due to its low cost, availability and high concentration of carotenoids. Additionally, the overall cost could be significantly reduced if the peels are used, due to their higher concentration of carotenoids compared to the rest of the fruit fractions and the fact that it is regarded as a waste in the food industry. Regarding the cost of production, it is essential to limit the production cost through cooperation between academia and industry. As Venelinov and Quevauviller (2003) explained, international networks covering the demands and promoting interchanges between users and consumers can be effective to optimize the overall cost of CRM. However, preparing a CRM from mango requires extra technical steps (*e.g.*, freeze-drying, milling). Even though these factors can be considered expensive, studies performed in freeze-drying optimization have shown that the initial investment represents the major part of the overall cost. In contrast, the operational cost represents between 5% and 9% (Stratta et al., 2020). Keeping the material in an airtight environment is essential to avoid moisture absorption. Additionally, freeze-drying avoids overheating the sample, provides stability, minimizes chemical decomposition, avoids contamination during storage, maintains long-term viability, and facilitates product distribution. Under these conditions, it is predictable that carotenoids in the mango matrix will remain stable.

It should be stated that the participation of highly qualified laboratories, with expertise in carotenoid analysis by applying different in-house validated methods, makes us conclude that the use of freeze-dried mango for quality control in analytical laboratories is appropriate for use, not only because of its low cost but also because of the presence of extractable bioactive compounds suitable for analytical purposes. As such, freeze-dried milled mango could be considered a potential vegetal matrix for developing readily available, low-cost CRM for the analysis of carotenoids.

4. Conclusion

The results of this study provide insights on worldwide interlaboratory comparability for the accurate determination of carotenoids in mango. The results have proven that β-carotene, α-carotene, (9*Z*)-β-carotene, β-cryptoxanthin and zeaxanthin were satisfactorily determined, independently from the analytical protocols of each laboratory. In this same line, for most carotenoids the extraction solvent and the detector didn't significantly affect the analytical outcomes, however, the stationary phase, the sample handling and the sample size were important factors for the accurate characterization of the extracted carotenoids. Additionally, this research provided valuable information for the development of reference materials based on mango to be used for quality assessment in analytical laboratories. The economic perspective for the development of these materials seems to be promising, in view of the concentration of carotenoids in mango and their easy extractability. Overall, research involving the analysis of carotenoids could be improved if new CRM material, produced from waste and scientifically tested, is used.

CRediT authorship contribution statement

José Villacís-Chiriboga: Formal analysis, Data curation, Investigation, Validation, Visualization, Writing – original draft. **Griet Jacobs:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing. **John Van Camp:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **Kathy Elst:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. **Jenny Ruales:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. **Verónica Marcillo-Parra:** Investigation, Resources, Writing – review & editing. **Volker Böhm**: Investigation, Writing – review & editing. **Andrea Bunea**: Investigation, Writing – review & editing. **Martina Cirlini**: Investigation, Writing – review & editing. **Neal Craft**: Investigation, Writing – review & editing. **Bruno de Meulenaer**: Investigation, Writing – review & editing. **M. Graça Dias**: Investigation, Writing – review & editing, **Giacomo Lazzarino**: Investigation, Writing – review & editing. **Antonio J. Meléndez-Martínez**: Investigation, Writing – review & editing. **Pieter Versloot**: Investigation, Writing – review & editing. **Adriana Z. Mercadante**: Investigation, Writing – review & editing. **Begoña Olmedilla-Alonso**: Investigation, Writing – review & editing. **Johana Ortiz-Ulloa**: Investigation, Writing – review & editing. **Carla M Stinco**: Investigation, Writing – review & editing, **Stefan Voorspoels:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was performed within the frame of the TEAM EC2017TEA442A103 VLIR-UOS project "Improving Ecuadorian child nutrition by using mango by-products as potential sources of bioactive compounds". JV-Ch wants to acknowledge the quality technical support of Samara Fernández de Souza from VITO. VM-P acknowledges Mayra Anaguano from EPN. AZM acknowledges Fabiane C. Petry for the carotenoid analysis, FAPESP (grant 2018/23752-1) and CNPq (grant 309182/2018-2).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2022.104616.

References

FAO, AGES, 2015. Things to know about the ring test [WWW Document]. FAQ about FAO-IAG Ring Test. URL http://www.fao.org/ag/againfo/home/documents/2015_Announcement_Ring_test.pdf (accessed 7.16.20).

Amorim-Carrilho, K.T., Cepeda, A., Fente, C., Regal, P., 2014. Review of methods for analysis of carotenoids. TrAC - Trends Anal. Chem. 56, 49–73. https://doi.org/10.1016/j.trac.2013.12.011.

Arvayo-Enríquez, H., Mondaca-Fernández, I., Gortárez-Moroyoqui, P., López-Cervantes, J., Rodríguez-Ramírez, R., 2013. Carotenoids extraction and quantification: a review. Anal. Methods 5, 2916–2924. https://doi.org/10.1039/c3ay26295b.

Biehler, E., Mayer, F., Hoffmann, L., Krause, E., Bohn, T., 2010. Comparison of 3 spectrophotometric methods for carotenoid determination in frequently consumed fruits and vegetables. J. Food Sci. 75, C55–C61. https://doi.org/10.1111/J.1750-3841.2009.01417.X.

Chen, Y., Yang, M., Wang, M., Yu, H., Zhou, J., Wang, T., 2021. Development of a new matrix certified reference material for metronidazole in egg powder. Microchem. J. 168, 106379 https://doi.org/10.1016/j.microc.2021.106379.

Craft, N.E., Furr, H.C., 2018. Methods for assessment of vitamin A (retinoids) and carotenoids. In: Harrington, D. (Ed.), Laboratory Assessment of Vitamin Status. Elsevier, UK, pp. 21–47. https://doi.org/10.1016/B978-0-12-813050-6.00002-4.

Crupi, P., Preddy, V., Antonacci, R., 2012. HPLC-DAD-MS (ESI+) determination of carotenoids in fruit. In: Preddy, V. (Ed.), Vitamin A and Carotenoids: Chemistry, Analysis, Function and Effects (Food and Nutritional Components in Focus, Volume 1). RSC Publishing, pp. 282–302.

Dias, M.G., Camões, M.F.G.F.C., Oliveira, L., 2008. Uncertainty estimation and in-house method validation of HPLC analysis of carotenoids for food composition data production. Food Chem. 109, 815–824. https://doi.org/10.1016/j.foodchem.2007.12.026.

Dias, M.G., Camões, M.F.G.F.C., Oliveira, L., 2014. Carotenoid stability in fruits, vegetables and working standards - Effect of storage temperature and time. Food Chem. 156, 37–41. https://doi.org/10.1016/j.foodchem.2014.01.050.

Dorn, M., Eschbach, F., Hekmat, D., Weuster-Botz, D., 2017. Influence of different packing methods on the hydrodynamic stability of chromatography columns. J. Chromatogr. A 1516, 89–101. https://doi.org/10.1016/j.chroma.2017.08.019.

Eriksen, J.N., Madsen, P.L., Dragsted, L.O., Arrigoni, E., 2017. Optimized, fast-throughput UHPLC-DAD based method for carotenoid quantification in spinach, serum, chylomicrons, and feces. J. Agric. Food Chem. 65, 973–980. https://doi.org/10.1021/acs.jafc.6b04925.

FAO, 2020. Fruit and Vegetables – Your Dietary Essentials. FAO, Rome, Italy. https://doi.org/10.4060/CB2395EN.

Farias Couto, M., Peternelli, L., Pereira Barbosa, L., 2013. Classification of the coefficients of variation for sugarcane crops. Ciência Rural 43, 957–961. https://doi.org/10.1590/S0103-84782013000600003.

Fearn, T., Thompson, M., 2001. A new test for "sufficient homogeneity. Analyst 126, 1414–1417. https://doi.org/10.1039/b103812p.

Giuffrida, D., Donato, P., Dugo, P., Mondello, L., 2018. Recent analytical techniques advances in the carotenoids and their derivatives determination in various matrixes. J. Agric. Food Chem. 66, 3302–3307. https://doi.org/10.1021/acs.jafc.8b00309.

Giuffrida, D., Zoccali, M., Mondello, L., 2020. Recent developments in the carotenoid and carotenoid derivatives chromatography-mass spectrometry analysis in food matrices. Trends Anal. Chem. 132, 116047 https://doi.org/10.1016/j.trac.2020.116047.

Hoffmann, E., Stroobant, V., 2007. Mass analysers. In: Mass Spectrometry - Principles and Applications. Wiley, UK, p. 479.

Horwitz, W., Albert, R., 2006. The Horwitz ratio (HorRat): a useful index of method performance with respect to precision. J. AOAC Int. 89, 1095–1109.

ISO, 2015. ISO Guide 33:2015 - Reference materials — Good practice in using reference materials [WWW Document]. URL https://www.iso.org/standard/46212.html (accessed 4.1.22).

ISO 13528, 2015. Stat. Methods Use Profic. Test. Interlab. Comp.

Khalid, F., Nawaz, H., Hanif, M.A., Rehman, R., Al-Sadi, A.M., 2020 (Mango). In: Asif Hanif, M., Nawaz, H., Mumtaz Khan, M., Byrne, H. (Eds.), Medicinal Plants of South Asia. Elsevier, pp. 495–508. https://doi.org/10.1016/b978-0-08-102659-5.00037-9 (Mango).

Kimura, M., Rodríguez-Amaya, D., 1999. Sources of errors in the quantitative analysis of food carotenoids by HPLC. Arch. Latinoam. Nutr. 49, 58-S-66-S.

Lauwaars, M., Anklam, E., 2005. Method validation and reference materials. In: De Bièvre, P., Günzler, H. (Eds.), Validation in Chemical Measurement. Springer Berlin Heidelberg, pp. 128–133. https://doi.org/10.1007/3-540-27034-5_23.

Luterotti, S., Marković, K., Franko, M., Bicanic, D., Madžgalj, A., Kljak, K., 2013. Comparison of spectrophotometric and HPLC methods for determination of carotenoids in foods. Food Chem. 140, 390–397. https://doi.org/10.1016/j.foodchem.2013.02.003.

Marcillo-Parra, V., Anaguano, M., Molina, M., Tupuna-Yerovi, D.S., Ruales, J., 2021. Characterization and quantification of bioactive compounds and antioxidant activity in three different varieties of mango (*Mangifera indica* L.) peel from the Ecuadorian region using HPLC-UV/VIS and UPLC-PDA. NFS J. 23, 1–7. https://doi.org/10.1016/J.NFS.2021.02.001.

Martins, P.L.G., de Rosso, V.V., 2016. Thermal and light stabilities and antioxidant activity of carotenoids from tomatoes extracted using an ultrasound-assisted completely solvent-free method. Food Res. Int. 82, 156–164. https://doi.org/10.1016/j.foodres.2016.01.015.

Melendez-Martinez, A.J., Stinco, C.M., Liu, C., Wang, X.D., 2013. A simple HPLC method for the comprehensive analysis of cis/trans (Z/E) geometrical isomers of carotenoids for nutritional studies. Food Chem. 138, 1341–1350. https://doi.org/10.1016/J.FOODCHEM.2012.10.067.

Meléndez-Martínez, A.J., Escudero-Gilete, M.L., Vicario, I.M., Heredia, F.J., 2009. Separation of structural, geometrical and optical isomers of epoxycarotenoids using triacontyl-bonded stationary phases. J. Sep. Sci. 32, 1838–1848. https://doi.org/10.1002/JSSC.200800717.

Meléndez-Martínez, A.J., Böhm, V., Borge, G.I.A., Cano, M.P., Fikselová, M., Gruskiene, R., Lavelli, V., Loizzo, M.R., Mandić, A.I., Brahm, P.M., Mišan, A.Č., Pintea, A.M., Sereikaitė, J., Vargas-Murga, L., Vlaisavljević, S.S., Vulić, J.J., O'Brien, N.M., 2021a. Carotenoids: Considerations for their use in functional foods, nutraceuticals, nutricosmetics, supplements, botanicals, and novel foods in the context of sustainability, circular economy, and climate change. Annu. Rev. Food Sci. Technol. 12, 433–460. https://doi.org/10.1146/ANNUREV-FOOD-062220-013218.

Meléndez-Martínez, A.J., Mandić, A.I., Bantis, F., Böhm, V., Borge, G.I.A., Brnčić, M., Bysted, A., Cano, M.P., Dias, M.G., Elgersma, A., Fikselová, M., García-Alonso, J., Giuffrida, D., Gonçalves, V.S.S., Hornero-Méndez, D., Kljak, K., Lavelli, V., Manganaris, G.A., Mapelli-Brahm, P., Marounek, M., Olmedilla-Alonso, B., Periago-Castón, M.J., Pintea, A., Sheehan, J.J., Šaponjac, V.T., Valšíková-Frey, M., Meulebroek, L., Van, O'Brien, N., 2021b. A comprehensive review on carotenoids in foods and feeds: status quo, applications, patents, and research needs. Crit. Rev. Food Sci. Food Nutr. https://doi.org/10.1080/10408398.2020.1867959.

Nübler, S., López, M.E., Castaño, A., Mol, H., Schäfer, M., Haji-Abbas-Zarrabi, K., Bury, D., Koch, H.M., Vaccher, V., Antignac, J.-P., Dvorakova, D., Hajslova, J., Thomsen, C., Vorkamp, K., Göen, T., 2021. Interlaboratory comparison investigations (ICI) and external quality assurance schemes (EQUAS) for cadmium in urine and blood: Results from the HBM4EU project. Int. J. Hyg. Environ. Health 234, 113711. https://doi.org/10.1016/j.ijheh.2021.113711.

Patel, A.S., Kar, A., Dash, S., Dash, S.K., 2019. Supercritical fluid extraction of β-carotene from ripe bitter melon pericarp. Sci. Rep. 9, 19266. https://doi.org/10.1038/s41598-019-55481-4.

Rodriguez-Amaya, D.B., 2001. A Guide to Carotenoid Analysis in Food. ILSI Press, USA.

Ruales, J., Baenas, N., Moreno, D.A., Stinco, C.M., Meléndez-Martínez, A.J., García-Ruiz, A., 2018. Biological active Ecuadorian mango 'Tommy Atkins' ingredients—an opportunity to reduce agrowaste. Nutrients 10, 1138. https://doi.org/10.3390/NU10091138.

Saini, R.K., Keum, Y.S., 2018. Carotenoid extraction methods: a review of recent developments. Food Chem. 240, 90–103. https://doi.org/10.1016/j.foodchem.2017.07.099.

Sander, L.C., Sharpless, K.E., Craft, N.E., Wise, S.A., 2002. Development of engineered stationary phases for the separation of carotenoid isomers. Anal. Chem. 66, 1667–1674. https://doi.org/10.1021/AC00082A012.

Scott, K.J., Finglas, P.M., Seale, R., Hart, D.J., De Froidmont-Görtz, I., 1996. Interlaboratory studies of HPLC procedures for the analysis of carotenoids in foods. Food Chem. 57, 85–90. https://doi.org/10.1016/0308-8146(96)00094-5.

Simonovska, B., Vovk, I., Glavnik, V., černelič, K., 2013. Effects of extraction and high-performance liquid chromatographic conditions on the determination of lutein in spinach. J. Chromatogr. A 1276, 95–101. https://doi.org/10.1016/J.CHROMA.2012.12.032.

Stinco, C.M., Benítez-González, A.M., Hernanz, D., Vicario, I.M., Meléndez-Martínez, A.J., 2014. Development and validation of a rapid resolution liquid chromatography method for the screening of dietary plant isoprenoids: carotenoids, tocopherols and chlorophylls. J. Chromatogr. A 1370, 162–170. https://doi.org/10.1016/J.CHROMA.2014.10.044.

Stinco, C.M., Benítez-González, A.M., Meléndez-Martínez, A.J., Hernanz, D., Vicario, I.M., 2019. Simultaneous determination of dietary isoprenoids (carotenoids, chlorophylls and tocopherols) in human faeces by rapid resolution liquid chromatography. J. Chromatogr. A 1583, 63–72. https://doi.org/10.1016/J.CHROMA.2018.11.010.

Stratta, L., Capozzi, L.C., Franzino, S., Pisano, R., 2020. Economic analysis of a freeze-drying cycle. Process 2020 (8), 1399. https://doi.org/10.3390/PR8111399.

Venelinov, T., Quevauviller, P., 2003. Are certified reference materials really expensive? TrAC - Trends Anal. Chem. 22, 15–18. https://doi.org/10.1016/S0165-9936(03)00105-5.

Villacís-Chiriboga, J., Voorspoels, S., Uyttebroek, M., Ruales, J., Van Camp, J., Vera, E., Elst, K., 2021. Supercritical CO_2 extraction of bioactive compounds from mango (*Mangifera indica* L.) peel and pulp. Foods 10, 2201. https://doi.org/10.3390/foods10092201.

Wise, S.A., Phillips, M.M., 2019. Evolution of reference materials for the determination of organic nutrients in food and dietary supplements—a critical review. Anal. Bioanal. Chem. 411, 97–127. https://doi.org/10.1007/s00216-018-1473-0.

Zakaria, O., Rezali, M.F., 2014. Reference materials as a crucial tools for validation and verification of the analytical process. Procedia - Soc. Behav. Sci. 121, 204–213. https://doi.org/10.1016/j.sbspro.2014.01.1121.

Zelenyánszki, D., Lambert, N., Gritti, F., Felinger, A., 2019. The effect of column packing procedure on column end efficiency and on bed heterogeneity – Experiments with flow-reversal. J. Chromatogr. A 1603, 412–416. https://doi.org/10.1016/j.chroma.2019.05.040.

Zhou, X., Ding, W., Jin, W., 2022. Microwave-assisted extraction of lipids, carotenoids, and other compounds from marine resources. In: Garcia-Vaquero, M., Rajauria, G. (Eds.), Innovative and Emerging Technologies in the Bio-Marine Food Sector. Academic Press, pp. 375–394. https://doi.org/10.1016/B978-0-12-820096-4.00012-2.