



HAL
open science

Feasibility of Individual Carotenoid Quantification in Mixtures Using UV-Vis Spectrophotometry with Multivariate Curve Resolution Alternating Least Squares (MCR-ALS)

Nawel Achir, Adrien Servent, Marvin Soto, Claudie Dhuique-Mayer

► To cite this version:

Nawel Achir, Adrien Servent, Marvin Soto, Claudie Dhuique-Mayer. Feasibility of Individual Carotenoid Quantification in Mixtures Using UV-Vis Spectrophotometry with Multivariate Curve Resolution Alternating Least Squares (MCR-ALS). *Journal of Spectroscopy*, 2022, 2022, pp.1-13. 10.1155/2022/4509523 . hal-03892105v1

HAL Id: hal-03892105

<https://institut-agro-montpellier.hal.science/hal-03892105v1>

Submitted on 9 Feb 2023 (v1), last revised 7 Nov 2023 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Feasibility of individual carotenoid quantification in mixtures using UV-Vis**
2 **spectrophotometry with multivariate curve resolution alternating least squares (MCR-ALS)**

3

4 Nawel Achir^{a*}, Adrien Servent^{a,b}, Marvin Soto^{a,c}, Claudie-Dhuique-Mayer^{a,b},

5

6

7 ^aQualisud, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, Université de La
8 Réunion, Montpellier, France.

9

10 ^bCIRAD, UMR Qualisud, F-34398 Montpellier, France.

11

12 ^cCentro Nacional de Ciencia y Tecnología de Alimentos (CITA), Universidad de Costa Rica
13 (UCR), Ciudad Universitaria Rodrigo Facio, código postal 11501-2060, San José, Costa Rica.

14

15 *Corresponding author: Nawel Achir. 1101, avenue Agropolis, 34093 Montpellier Cedex 5,
16 France. E-mail: nawel.achir@supagro.fr

17

18

19 **Abstract**

20

21 A fast and low-cost analytical method to determine the concentrations of carotenoids (β -
22 carotene, lutein and lycopene) from mixed standard solutions or from fruit extracts (kiwi
23 fruits, tomato paste, pink grapefruit juice, kiwi-pineapple smoothie and apricot nectar) was
24 tested. The methodology was based on UV-Vis spectrophotometry and Multivariate Curve
25 Resolution-Alternating Least Squares (MCR-ALS). Results showed that relative concentration
26 of β -carotene, lutein and lycopene in solvent were successfully determined by this technique
27 with an error inferior to 6 %. In real extracts, the procedure succeeded well in identifying the
28 major carotenoid type of the fruit samples but also a more complex profile of a fruit mixture.
29 The results also showed that accuracy of carotenoids determination by UV-Vis
30 spectrophotometry-MCR-ALS in fruit extracts was conditioned by their spectral characteristics
31 (III/II ratios and λ_{\max}), their relative proportion, and the extract purity.

32

33 **Key words:** Multivariate data analysis; molecular spectroscopy; fruits; pigments; carotenoids

34

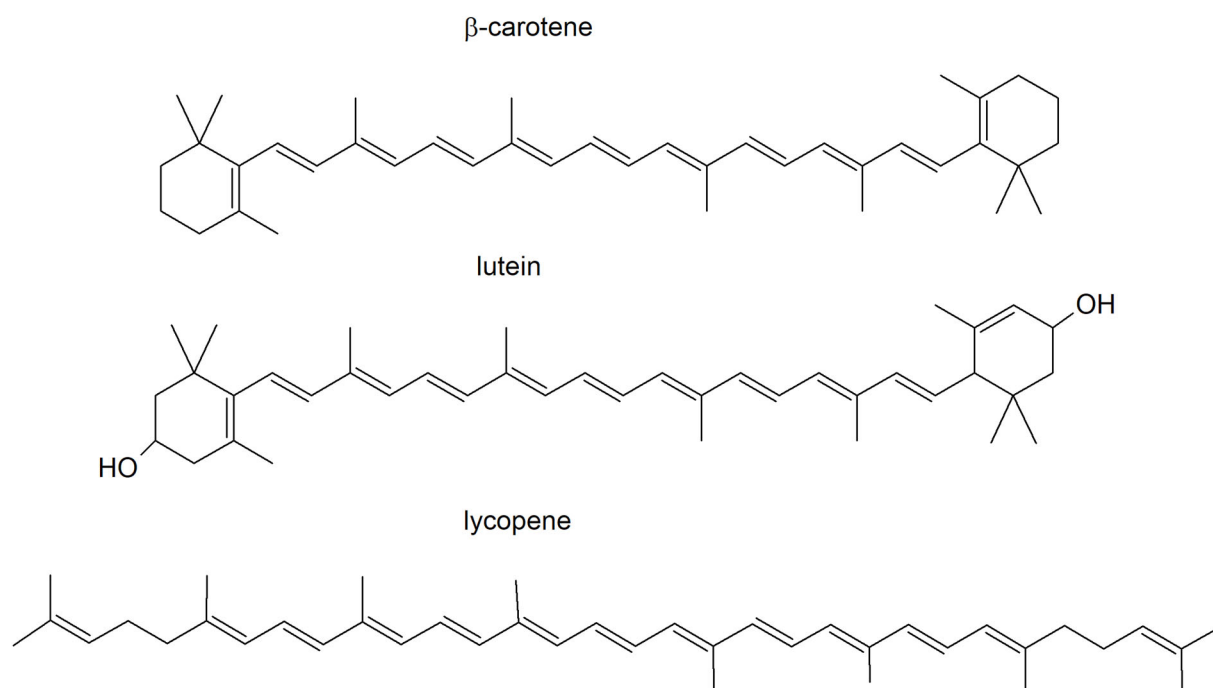
35

36 INTRODUCTION

37

38 Carotenoids are a family of lipophilic light-absorbing chromophores responsible for the
39 yellow, orange, or red color of many fruits and vegetables ¹⁻³. They can be found in very
40 various amounts and profiles as a function of the crop ⁴⁻⁶. These molecules can be classified
41 as a function of their structure and biological role. Carotenes are non-oxygenated carotenoids
42 and oxygenated carotenoids are xanthophylls ³. The most common carotenes are β -carotene
43 and lycopene. The bicyclic β -carotene is the most widespread in vegetables and fruits and can
44 be found in carrot, orange-fleshed sweet potato, apricot, mango, palm fruits, etc. ⁷. Of the
45 acyclic carotenes, lycopene is the most prevalent and is the major carotenoid in tomato, pink
46 grapefruit and watermelon^{2,8}. Xanthophylls are widely distributed in fruits and vegetables.
47 Lutein is present in high levels in leaves, green fruits and vegetables, and yellow flowers ^{3,9}.
48 Carotenoids are valuable natural colorants that are added to many processed foods, in the
49 form of natural extracts or as pure chemically synthesized compounds, in order to give the
50 desired coloring properties ¹⁰.

51 Carotenoids also exhibit numerous health promoting properties. Close to 90% of carotenoids
52 in the human diet that are recovered in human plasma are represented by β -carotene, α -
53 carotene, lycopene, lutein, and cryptoxanthin ^{11,12}. β -carotene is a source of vitamin A with an
54 equivalency ratio for β -carotene to vitamin A estimated as 12:1 ¹³. Lutein is a macular pigment
55 that plays an important role against cataracts and age-related macular degeneration ¹⁴. Lastly,
56 lycopene is the most potent antioxidant among carotenoids, and dietary intake has been
57 shown to be associated with a decreased risk of chronic diseases, such as cancer and
58 cardiovascular disease ¹⁵.



59
60

61 **Figure 1.** Chemical structures of β-carotene, lutein, and lycopene

62

63 **Table 1.** Carotenoid absorption data from literature ^{3,16}

	Molecular weight	ϵ (mM ⁻¹ . Cm ⁻¹)	λ max (nm)	Solvent	III/II (%)
β-carotene	537	139	450	Hexane	25
Lutein	569	145	445	Hexane	60
Lycopene	537	185	470	Hexane	65

64

65 Because these compounds are of nutritional and sensory importance, and because they
 66 cannot be synthesized by humans so they have to be provided by food, many techniques were
 67 developed to analyze and quantify them. Their fine separation is for instance useful to gain
 68 insights into their biological role in nutritional studies ¹⁷. Their precise quantification is
 69 necessary to provide reliable data for food databases. However, many carotenoid-rich plants
 70 are found in developing countries where expertise and/or analytical resources are still
 71 insufficient ¹⁸. In parallel, there is a growing demand for healthy and phytonutrient -rich food
 72 in developed countries so modern agriculture will gain in producing non only high-yield but
 73 also high-nutritional potential crops. This new strategy implies high throughput quantification
 74 of carotenoids. Therefore, phytonutrient analysis in general and carotenoids analysis in

75 particular must meet both precision and material (time, technicity) constraints^{19–21}. The two
76 main protocols used for carotenoid quantification are UV-Vis spectrophotometry and the
77 HPLC methods^{22,23,23–25}. The first one uses the characteristic visible light absorption spectrum
78 of carotenoids. Due to their system of conjugated double bonds, their spectrum consists of a
79 three-peak shape (namely I, II, III) between 400 and 500 nm. The three λ_{\max} vary as a function
80 of the carotenoid structure (Figure 1 and 2A and Table 1). In addition to λ_{\max} , the 3-peak-
81 absorption shape of the carotenoid spectra enable the calculation of the %III/II ratio which is
82 another important parameter to identify them as it is different as a function of the molecule (Figure
83 2A). This parameter is the ratio of the height of the longest-wavelength absorption peak (III), and that
84 of the middle absorption peak (II), taking the minimum between the two peaks as baseline, multiplied
85 by 100^{3,26,27}.

86 Carotenoids in solution obey the Beer-Lambert law. Their absorbance is directly proportional
87 to the concentration which is usually determined using the molar extinction coefficient (ϵ_{mol})
88 in an appropriate solvent²⁸. Therefore, absorbance measurements can be used to quantify
89 the concentration of a pure carotenoid (standard) or to estimate the total carotenoid
90 concentration in a mixture or extract of carotenoids from plants^{29,30}. This technique is rapid
91 and low cost and has been optimized by much research. The main improvements consist in
92 removing interference and particularly that of chlorophylls^{22,24} and optimizing the solvent
93 extraction procedure²³ in order to increase accuracy and rapidity. Other spectrophotometric
94 techniques were tested as near Infrared spectroscopy but proved to be less robust than UV-
95 VIS spectrophotometry for quantification purposes²⁵. However, despite its convenience, the
96 main drawback of this method is the lack of quantification information compared to analyses
97 by HPLC. Indeed, the UV-VIS method does not enable the distinction between carotenoids in
98 a given mixture as the most common protocols use a mean absorption coefficient and a mean
99 absorption wavelength^{22,31}. This problem limits its use for nutritional or adulteration control
100 purposes. HPLC analysis is a more accurate and appropriate method to get individual
101 concentrations of the different carotenoids in a fruit, a dish, or a formulated product^{1,10,32}.
102 This method is however time and solvent-consuming and requires higher costs to establish
103 and maintain this higher technicity. In addition, HPLC analyses sometimes require a long
104 saponification step involving the use of a hazardous substance, potassium hydroxide solution
105 in methanol, to remove the xanthophyll esters that can make quantification difficult³³.
106 Indeed, xanthophylls can be esterified by various fatty acids resulting in a hundred possible

107 combinations of different molecular weights and polarities and unidentified and unresolved
108 chromatographic peaks. The saponification step is not useful in the UV-Vis method since
109 esterification does not significantly affect carotenoid spectra³. In addition, it was proven that
110 saponification leads to significant carotenoid loss²².

111 Since physical separation is a difficult task, it is interesting to explore mathematical separation
112 on the basis of the UV-VIS spectra. The main mathematical treatment on spectra used up till
113 now for carotenoid analysis was the subtraction of absorbance to a certain wavelength to
114 remove interference^{22,24}. For instance, Lichtenthaler (2001) developed formulas in different
115 organic solvents to get a more reliable global carotenoid content in green tissue extract
116 solutions by removing the contribution of chlorophyll b that also absorb at the carotenoids
117 λ_{\max} ³⁴. However, further and more specific information could be obtained from the spectrum
118 by using chemometric tools. Among them, Multivariate Curve Resolution-Alternating Least
119 Squares (MCR-ALS) is a powerful tool in solving mixture analysis problems. Indeed, MCR-ALS
120 can provide a bilinear description of the observed data. As the total absorbance is the sum of
121 the individual absorbance for each species according to the Beer Lambert law, MCR-ALS can
122 dissociate the spectral contribution of pure compounds involved in the mixture and also give
123 their relative concentration. Therefore, MCR-ALS is not a statistical method like partial least
124 square regressions which is usually used with near infrared spectroscopy, where footprint
125 spectra (a hundred) are correlated to chemical features. MCR-ALS has, until then, mainly been
126 used in the pharmaceutical or chemical engineering fields and proved to be efficient to
127 monitor evolving reactions during processes³⁵⁻³⁷. However, this mathematical procedure is
128 now used in the domain of food science to analyze complex natural samples. Indeed, MCR-
129 ALS was successfully tested on voltametric signals to quantify organic acids in fruit juices and
130 on UV-Vis spectra to discriminate polyphenols in a hibiscus extract³⁸⁻⁴¹. It was also employed
131 for quantification and spatial distribution of salicylic acid in film-coated tablets using Raman
132 spectrophotometry, or to get insight into conformational changes and protein folding^{42,43}.

133 MCR-ALS was also used for β -carotene with transient absorption spectroscopy to monitor its
134 excited state as a function of time and also to automatize isoprenoids analysis by HPLC^{44,45}.

135 However, to our knowledge it has never been tested on mixtures of carotenoids for
136 quantification purpose with a conventional UV-Vis spectroscopy protocol. It would however
137 be very informative to test the potential of this technique on these interesting pigments both
138 from a nutritional and organoleptic point of view because they exhibit quite remarkable

139 spectral characteristics in the UV-Vis domain. These properties were interestingly exploited
140 by Küpper *et al.* (2007) who showed that by fitting with Gauss-peak spectra (GPS) it was
141 possible to quantify certain individual carotenoids in crude extracts of leaves of higher plants,
142 brown algae, and cyanobacteria³¹. However, the GPS treatment required a mathematical
143 treatment with complex equation and coding skills. In addition, GPS is based on modeling
144 spectra by a set of Gaussian functions which is not as chemically meaningful as MCR-ALS.
145 Indeed, MCR-ALS assumes that the observed spectra are a linear combination of the spectra
146 of the pure components in the system which is the multiwavelength extension of Lambert-
147 Beer's law⁴⁶. Another advantage of MCR-ALS is that a graphical user-friendly interface was
148 developed in MATLAB and is freely available⁴⁷. This tool enables constraints to be
149 implemented easily, i.e., additional chemical knowledge, such as pure spectra to give even
150 more sense to the resolution.

151 In this paper, we studied the use of a UV-Vis method for analyzing mixed carotenoids with the
152 implementation of spectra treatment by MCR-ALS, to obtain the relative concentration of
153 individual carotenoids from a mixture in solvents or a plant sample extract. To best analyze
154 the possibilities, robustness and limitations of this direct quantification method, we analyzed
155 the spectra of mixed carotenoid standards (β -carotene, lutein and lycopene) in an appropriate
156 solvent and afterwards on three different fruit or processed fruit product extracts. The
157 accuracy and precision of this method were tested by comparison with HPLC results.

158

159 **MATERIAL AND METHODS**

160

161 **Chemical products**

162 β -carotene, lutein, and lycopene standards were purchased from Extrasynthèse (Genay,
163 France). Hexane, dichloromethane, ethanol, methyl-tert-butyl ether, methanol, potassium
164 hydroxyde and pyrogallol were of analytical grade and obtained from Merk (Germany).

165

166 **Raw fruits**

167 Fresh kiwi fruits (FK), pink grapefruit juice (PGJ), apricot nectar (AN), kiwi-pineapple smoothie
168 (KPS), and tomato paste (TP) were purchased in local supermarkets (Montpellier, France).
169 Contrary to PGJ and AN, KPS was flash pasteurized. KPS also contained apple juice (47%) and

170 spirulina extract. These products were chosen to represent a diversity of matrices (species,
 171 raw or processed), and of carotenoid profile.

172

173 **Standard solution preparation**

174 Four individual 100 mL-standard solutions of β -carotene, lutein, and lycopene were made at
 175 a concentration of 1.98, 5.45, 4.18 mg.L⁻¹ respectively in hexane. To properly dissolve lutein
 176 and lycopene, 1 mL of ethanol and dichloromethane was added respectively before hexane
 177 and the mixture was put in an ultrasound bath for 30 s. The concentrations of each solution
 178 were determined using molar extinction coefficient (ϵ mol) in the appropriate solvent checked
 179 by spectrophotometry (Table 1).

180 **Standard solution mixture tests:** Standard carotenoid solutions of β -carotene, lutein,
 181 lycopene were mixed with different volume proportions leading to a total concentration of \sim 5
 182 mg.L⁻¹. The different relative concentrations are presented in Table 2 before UV-Vis analysis.
 183 Solutions 1 to 3 correspond to pure compounds. Solutions 4 to 13 were prepared with β -
 184 carotene lutein and lycopene at different concentrations to estimate the sensitivity of the
 185 MCR-ALS procedure.

186

187 **Table 2.** Relative composition of carotenoids in standard solution mixtures

188

	Standard solution	β -carotene	Lutein	Lycopene
Pure standards	1	100%	0%	0%
	2	0%	100%	0%
	3	0%	0%	100%
Mixed standards	4	0%	57%	43%
	5	27%	73%	0%
	6	32%	0%	68%
	7	17%	47%	36%
	8	9%	51%	39%
	9	22%	31%	47%
	10	21%	57%	22%
	11	4%	54%	41%
	12	27%	16%	57%
	13	24%	65%	11%

189

190

191 **Preparation of fruit carotenoid extracts**

192 Extraction procedures and conditions for analysis were described previously ^{48,49}. Briefly,
193 samples were ground if necessary and then weighed (200 mg for tomato paste, and 1000 mg
194 for pink grapefruit juice, apricot nectar, kiwi-pineapple smoothie and kiwi fruit) in 20 mL glass
195 tubes. 2 mL of an ethanol solution containing 1 % pyrogallol were added in each tube. For each
196 product, 3 samples were prepared in tubes for UV-VIS and 3 for HPLC.

197

198 **UV-VIS sample preparation:** 2 ml of distilled water, and 5 mL of hexane were added in the
199 tubes. After mixing in a tube rotator (Boekel Scientific, USA) during 15 min and decantation,
200 the organic phase was recovered with a Pasteur pipette, and the aqueous phase was extracted
201 once more with 5 mL of hexane. The organic phase was pooled and protected from light prior
202 to UV-Vis spectrophotometry analysis. Dilution (1/10) in hexane was made only for tomato
203 paste extract to reach an absorbance intensity of between 0.7 and 0.9 in the 450-500 nm
204 range.

205 **HPLC sample preparation:** Only tubes containing kiwi were subjected to saponification to
206 remove esters from xanthophylls for 30 min in a water bath at 70 °C by adding 1.5 mL of
207 saturated **potassium hydroxide** (12 N). After incubation, the tubes were cooled in an ice bath.
208 For all tubes (kiwi and the others) 2 ml of distilled water and 5 mL of hexane were added to
209 samples. Then, after mixing and decantation, the organic phase was recovered, and the
210 aqueous phase was extracted two more times with 5 mL of hexane.

211 For all tubes, the organic phases were combined and evaporated to dryness using a vacuum
212 evaporation system (GeneVac EZ-2, SP Scientific, UK). Finally, the residue from samples for
213 HPLC analysis was re-dissolved in 500 µL of methyl tert-butyl ether/methanol (80/20) and
214 placed in an amber vial prior to HPLC analysis

215

216 **Determination of carotenoid concentrations**

217

218 **UV-Vis spectrophotometry measurements and MCR-ALS analysis:** The spectra of pure
219 standard and standard mixtures as well as fruit extracts were acquired in the same conditions.

220 For each solution, 3 spectra were acquired from 250 to 600 nm (every 0.5 nm) with a diode
221 array spectrophotometer (Specord S600 Analytik Jena, Germany).

222

223 Total carotenoids were calculated using the Beer-Lambert law from the absorbance at 450 nm
224 using a mean absorption coefficient of $135310 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ and converted into $\text{mg}\cdot\text{kg}^{-1}$ using
225 a mean molecular weight of $548 \text{ g}\cdot\text{mol}^{-1}$ ²².

226

227 To obtain the relative proportion of the different carotenoids, MCR-ALS was done on the
228 whole spectra. Pre-processing steps consisted in removing the short-wavelength region (250-
229 350 nm) and in normalization. MCR-ALS analysis was done with Matlab (The MathWorks, Inc.,
230 Natick, MA, USA) using the toolbox built by Jaumot *et al.* (2005) ^{41,50}. Briefly, Multivariate
231 Curve Resolution aims to achieve a bilinear decomposition in 2 matrices of an experimental
232 absorbance matrix $D(m,n)$ which corresponds to a combination of spectrophotometric
233 measurements. The two matrices correspond to the concentrations $C(m,k)$ of the individual
234 compounds and their normalized spectra $S^T(k,m)$. The underlying law that links the two-way
235 data matrix $D(m,n)$ to $C(m,k)$ and $S^T(k,n)$ is a generalization of the Beer-Lambert law applied
236 to all wavelengths (Eq. 1).

$$237 \quad D(m, n) = C(m, k) S^T(k, n) + E(m, n) \quad (1)$$

238 with k , the number of chemical species in the unknown mixture and $E(m,n)$, the error matrix.
239 Estimation of the number of components k is done with singular values of the data matrix D .
240 Initial estimations of spectra is obtained from pure variable detection methods. Resolution of
241 Eq (1) is carried out by an iterative process using the ALS algorithm that stops when the error
242 (E) is minimum. Results were improved by the addition of optimization constraints with
243 chemical sense: the non-negativity constraint algorithm “fast non-negative least squares” and
244 spectra were put at the same height (no closure constraint).

245 The goodness of fit of the results to the experimental data is estimated with the residues, or
246 the residual sum of squares calculated as follows:

$$247 \quad \text{residues} = \sum(SRC - ERC)^2 \quad (2)$$

248 with SRC the simulated relative concentration obtained with the MCR procedure from the C
249 matrix of Eq. 1 and ERC the experimental relative concentration of individual carotenoids
250 (provided by the known concentration of standards incorporated in the mixture or using the
251 HPLC results for fruit extracts).

252 Mean error of the model expressed in percentage, providing the idea of accuracy of
253 concentration prediction, was calculated as follows:

$$254 \quad E = \frac{\frac{\sqrt{\sum(SRC-ERC)^2}}{n}}{\frac{\sum ERC}{n}} \times 100 \quad (3)$$

255
256 **HPLC carotenoid analysis:** Carotenoid identification and quantification of the fruit extracts
257 was performed on a reverse-phase HPLC-DAD Agilent 1100 system (Massy, France) with a
258 diode array detector described in Dhuique-Mayer *et al.* (2017)³³. Carotenoids were separated
259 using a C30 column (250 x 4.6 mm i.d., 5 μm) (YMC EUROP GmbH, Germany) with a guard
260 column, and the mobile phase was water as eluent A, methanol as eluent B, and methyl tert-
261 butyl ether as eluent C. Operation temperature was set at 25 °C. The flow rate was set at
262 1mL/min and the injection volume was 20 μL. A solvent gradient was programmed as follows:
263 0-2 min, isocratic 40% A-60% B (initial conditions); 2-5 min, 20% A-80% B; 5-10 min, 4% A-81%
264 B-15% C; 10-60 min, 4% A-11% B- 85% C; 60-70 min, isocratic 4% A-11% B-85% C; 70-71 min,
265 100% B; 71-72 min, with a return to the initial conditions for rebalancing. All-E-β-carotene and
266 their isomers, and all-E-lutein were detected at 450 nm, and all-E-lycopene and their isomers
267 were detected at 470 nm. Z-lycopene content was expressed as the sum of all Z-lycopene
268 isomers. Isomers were identified according to their relative retention times, i.e. elution order
269 and the combined use of their spectral data. The identifications were based on previously
270 published data obtained with the same mobile phase (water/methanol/ methyl tert-butyl
271 ether) and the same detection wavelength range^{51,52}

272 273 **Statistical analysis**

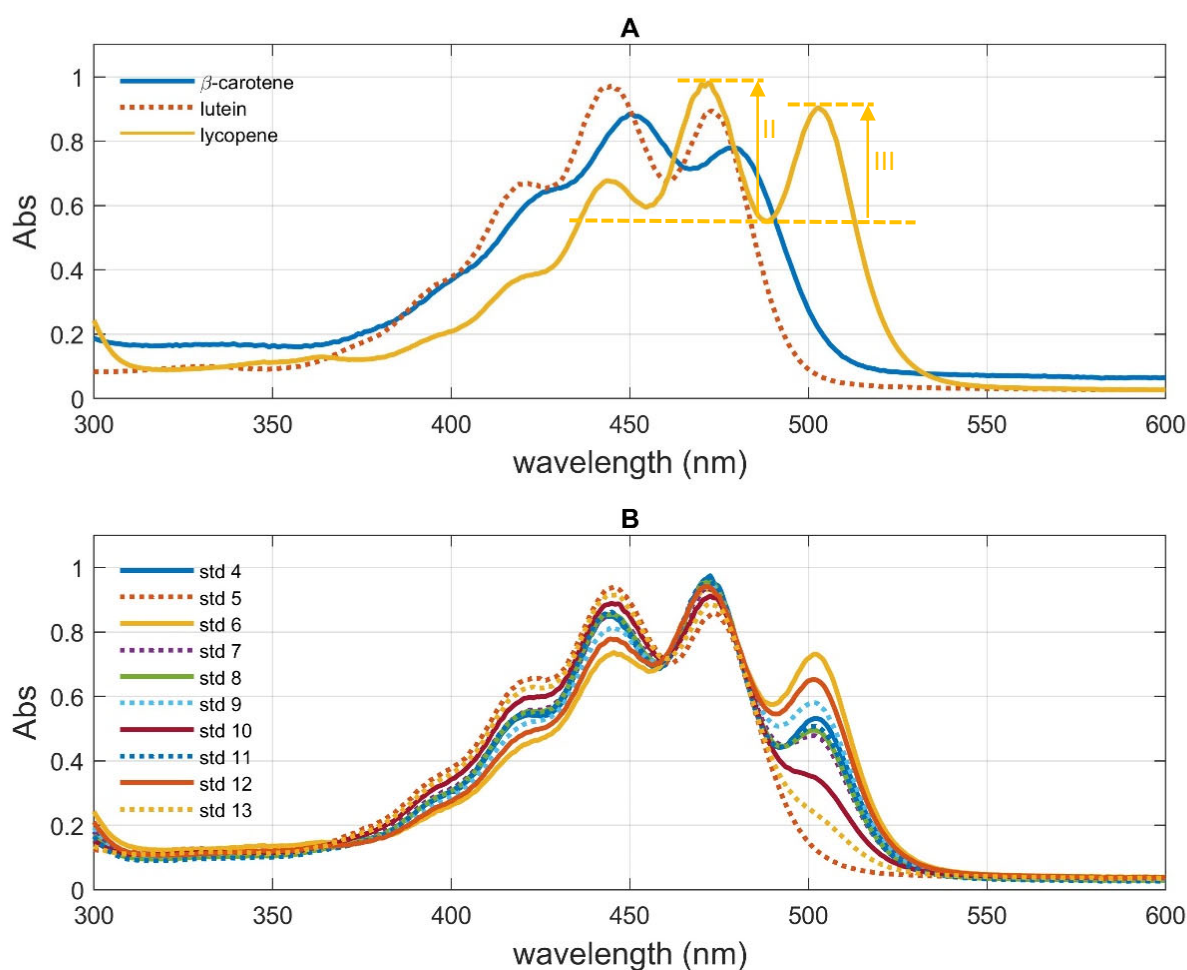
274 Spectrophotometric and HPLC analyses were performed in triplicate for each sample. One-
275 way ANOVA was used to analyze the difference in carotenoid compositions between the
276 different products (difference considered significant when $p < 0.05$). If significantly different,
277 means were further compared using Tukey's test. Statistical analyses were performed with
278 Statistica® (StatSoft Inc., USA).

279 280 **RESULTS AND DISCUSSION**

281 282 **Concentration of individual carotenoids of mixed standard solutions**

283
284
285
286
287
288
289

Spectra of pure standards and their mixture are presented in Fig. 2A and B respectively according to their composition shown in Table 2. The spectra of pure compounds, (solution 1 to 3) and their typical three-peak shape, are illustrated in Fig. 2A. Lutein and lycopene spectra exhibited significant differences with a maximal absorption intensity at 440 and 470 nm respectively. They differed from β -carotene with their high III/II ratio of 60%.

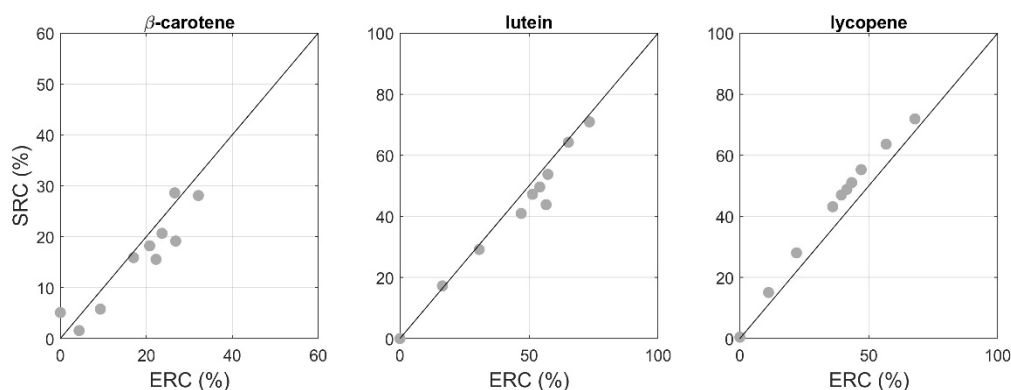


290
291
292
293
294
295
296

Figure 2. Spectra of A) pure standards of β -carotene, lutein, and lycopene (solutions 1-3 of table 2 and B) mixtures of standards (solutions 4-13 of table 2). Calculation of %III/II as indication of spectral fine structure is presented for lycopene spectra ($\%III/II = III/II \times 100$).

Fig. 2B shows the variability of spectra shapes obtained from the standard mixture with relative concentrations for individual carotenoids ranging from 5 to 55%. Two MCR-ALS

297 procedures were applied to these spectra to obtain simulated concentrations of individual
 298 carotenoids. Firstly, we applied the mathematical procedure directly on spectra of Fig. 2B and
 299 secondly on spectra of Fig. 2B augmented with pure standard spectra of Fig. 2A. In both cases,
 300 the number of components k (Eq. 1) was logically chosen equal to three, since it is the number
 301 of the different carotenoids put in the mixed standard solutions. Results of the first simulation
 302 showed that the error was very high and discrepancy between simulated and real values too
 303 great (error above 20% for β -carotene and lutein). These results were explained by the fact
 304 that the pure spectra obtained (not shown here) exhibited confusions, particularly between
 305 β -carotene and lutein. Addition of the spectra of standards added constraints that improved
 306 markedly the resolution of MCR-ALS. Results of this simulation are presented in Fig 3 that
 307 shows real values plotted against simulated ones. Plain lines represent the first bisector of the
 308 plan and stand for a perfect accuracy of the model, that is to say simulated results equal to
 309 the real values of relative concentrations of the table 2.
 310



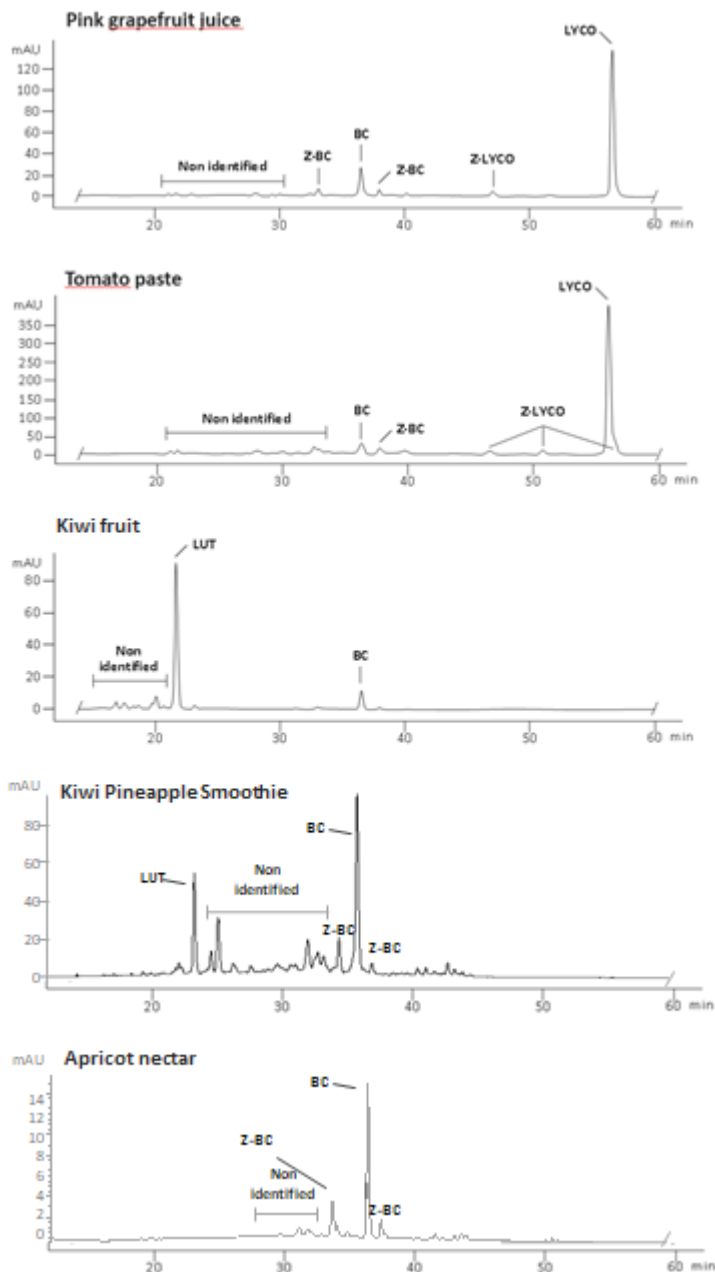
311
 312 **Figure 3.** Simulated relative concentrations of individual carotenoid (SRC) obtained by MCR-
 313 ALS compared to experimental relative concentrations (ERC)
 314

315 As can be seen, the relative proportions of β -carotene, lutein and lycopene obtained by MCR-
 316 ALS were very close to the experimental ones. Final error for individual carotenoid
 317 concentration determination was 6, 3, and 4% for β -carotene, lutein and lycopene
 318 respectively. Therefore, in the case of a pure carotenoid mixture, discrimination of β -carotene,
 319 lutein and lycopene was good providing addition of pure spectra.
 320

321 **Concentration of individual carotenoids in fruit extracts**

322

323 **HPLC results**



324

325

326

327 **Figure 4.** Chromatograms of the 5 fruit extracts measured at 450 nm. BC: all-E- β -carotene;

328 LYC: all-E-lycopene; LUT: lutein; Z-BC: Z- β -carotene isomers; Z-LYC: Z-lycopene isomers

329

330 The chromatograms of the 5 fruit extracts tested are presented in Fig. 4: pink grapefruit juice

331 (PGJ), tomato paste (TP), fresh kiwi (FK), apricot nectar (AN), and kiwi and pineapple smoothie

332 (KPS). Lycopene (all-E- and Z-forms) was the major type in PGJ and TP (~70-80%) which was
 333 accordance with literature ^{48,53,54}. Lutein was the major xanthophyll in FK ⁵⁵ and KPS, and for
 334 apricot it was β -carotene ⁵⁶. β -carotene was also present in KPS, because both pineapple and
 335 spirulina contain it, as well as in low concentrations in TP, KF, and PGJ of 5, 9 and 18%
 336 respectively.

337 Non-identified carotenoids represented a non-negligible proportion. Assuming a
 338 spectrophotometric response coefficient close to that of common carotenoids, non-identified
 339 species could represent about 2 and 6% in AN and PGJ, but 19% in TP and about 26 % in KF
 340 and till 50% in KPS certainly due to its more complex composition (kiwi, pineapple and
 341 spirulina as additive). These non-identified carotenoids may consist of residual esterified
 342 xanthophylls. In addition, in processed food (PGJ, TP, AN) these peaks can correspond to
 343 degradation products of carotenoids. The first indicator of carotenoid deterioration by
 344 thermal treatment is the percentage of isomerization as can be seen in the HPLC
 345 chromatogram of grapefruit juice, apricot nectar or tomato paste. Total carotenoids obtained
 346 from HPLC are presented in table 3. β -carotene and lycopene were calculated as the sum of
 347 all isomers. Logically as it is a concentrated product, total carotenoids accounted for 218
 348 mg.kg⁻¹ in tomato paste and were about 20, 40 80 and 1000-fold lower in PGJ, AN, KF and KPS
 349 respectively.

350

351 **Table 3.** Total carotenoids in mg.kg⁻¹ of fruit extracts obtained with HPLC, spectrophotometry
 352 and spectrophotometry + MCR-ALS.

	Pink Grapefruit juice (PGJ)	Tomato paste (TP)	Fresh Kiwi (FK)	Apricot nectar (AN)	Kiwi-Pineapple Smoothie (KPS)
TC by HPLC	12.5 (0.9) ^b	218.3 (19.4) ^a	2.7 (0.1) ^b	5.5 (0.5) ^b	0.21 (0.1) ^c
TC by spectrophotometry	11.8 (1.9) ^b	226.8 (46.2) ^a	6.4 (1.5) ^b	8.4 (0.4) ^b	0.74 (0.1) ^c
TC by spectrophotometry + MCR-ALS	11.8 (1.9) ^b	218.8 (38.9) ^a	4.2 (1.1) ^b	7.1 (0.3) ^b	0.17 (0.1) ^c

353 Values bearing different letters for the same parameter are significantly different (p<0.05).

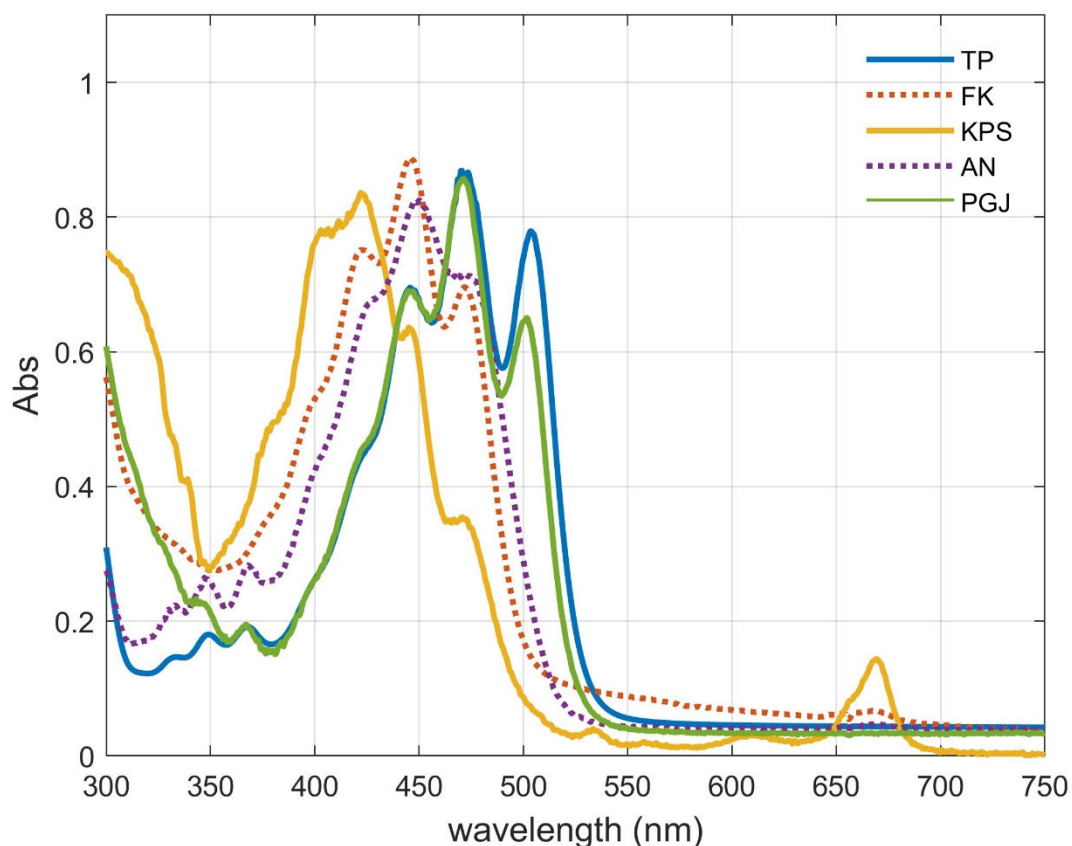
354 Values in brackets are standard errors

355 'TC' for total carotenoids

356

357 Spectrophotometric results

358



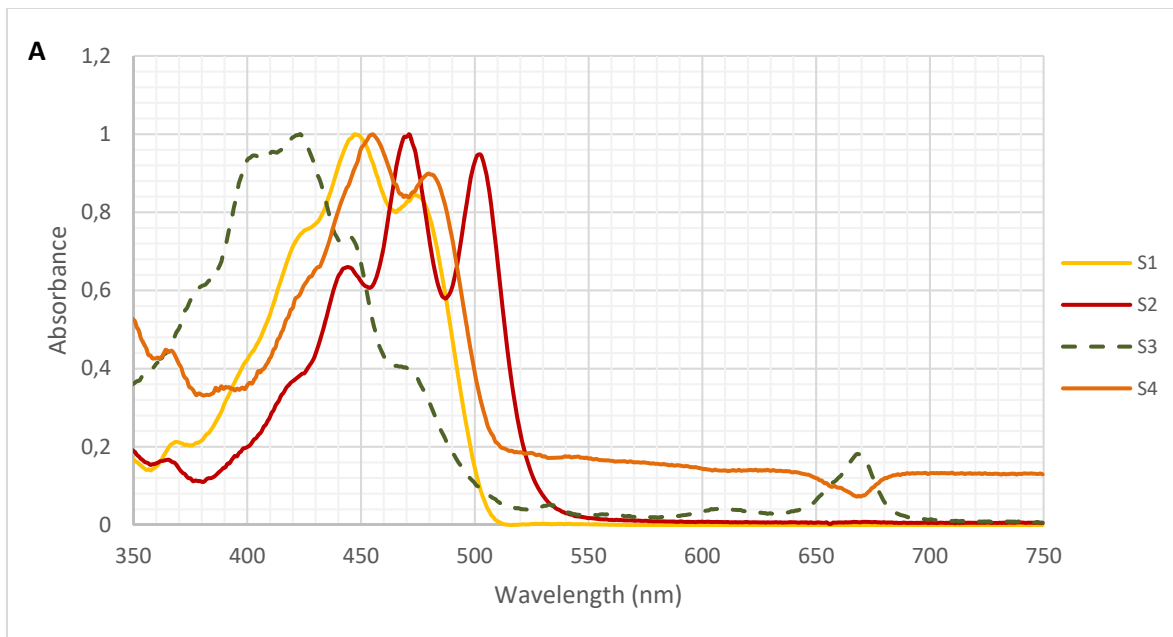
359
 360 **Figure 5.** Spectra of fruit carotenoid extracts: tomato paste (TP), fresh kiwi (FK), kiwi-pineapple
 361 smoothie (KAS), apricot nectar (AN), and pink grapefruit juice (PGJ))

362
 363 Fig. 5 shows the spectra of fruit extracts obtained without saponification. This kind of
 364 extraction was much more rapid by comparison with the saponification-HPLC-procedure (cf
 365 section 2.5.2 vs 2.5.3.). The saponification step is not mandatory since esterification does not
 366 change the spectra of esterified carotenoids³. As can be seen on Fig. 5 spectra of extracts
 367 were much noisier than those of the standards particularly in the short-wavelength range 300-
 368 420 nm. This phenomenon may be due to several reasons. Firstly, the fruit matrix is complex
 369 and variable which can be seen on the spectra of kiwi fruit. The other fruit samples had similar
 370 spectra since they were homogenized during the process of juice or paste making. Fruit
 371 extracts were also more complex because in addition to the main carotenoids, small quantities
 372 of other non-identified carotenoids were extracted together as can be seen in chromatograms
 373 of Fig. 4. In addition, we can assume a possible co-extraction of small quantities of compounds
 374 that may absorb light at the same wavelength as carotenoids, like chlorophylls. This is
 375 obviously visible on the spectra of KF and KPS in the region 650-700 nm of the spectra. Finally,

376 in real matrices, carotenoids undergo chemical changes such as isomerization as can be also
377 seen in Fig. 4. These changes affect the spectrum of the original carotenoid with a λ_{\max} shift
378 and apparition of a *cis* peak³. These artifacts were even more visible in the UV-region of the
379 spectra from 300-350 nm but a slight background noise may remain at the highest wavelength.
380 From the absorbance at 450 nm, total carotenoids were calculated and reached 226 mg.kg⁻¹
381 in tomato paste and were about 20, 25, 35 and 300-fold lower in PGJ, AN, KF and KPS
382 respectively. Therefore, carotenoid content in HPLC results, were equal or 1.5 to 3 times below
383 the spectrophotometric results. These differences were also observed in literature. For
384 instance, Biehler and al. (2010) also reported a concentration of total carotenoids in tomato
385 paste by spectrophotometric higher than that of the HPLC results. The underestimation of
386 HPLC results was attributed to the extraction step where about 20% of carotenoids is lost. This
387 loss can be even high when a saponification is required in case of xanthophyll-rich fruits. In
388 addition, Biehler et al. also reported the possible overestimation in spectrophotometric
389 results due to other compound interference and particularly chlorophylls.

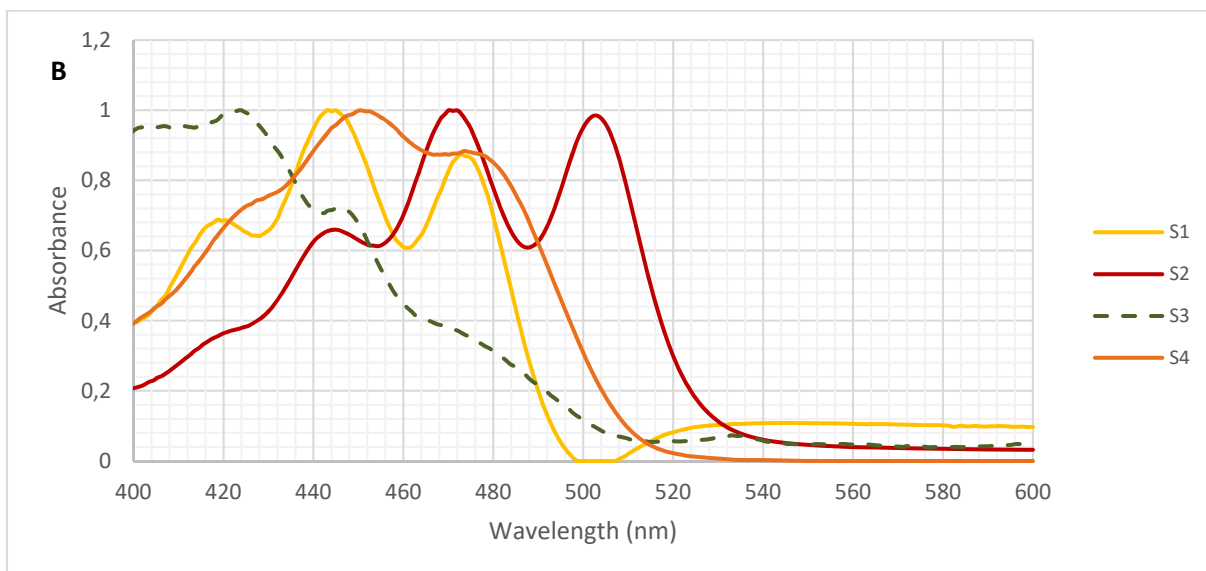
390
391 To get the individual proportion of carotenoid from the UV-VIS data but also of unknown
392 compounds, MCR was applied to the fruit extract spectra of Fig 5 augmented with standard
393 solution spectra of Fig. 2A and B to improve the resolution. As said before, pre-treatments
394 consisted in removing the UV region 250-350 nm and to normalize spectra. Both treatments
395 were essential to reduce spectra noise for the fruit extracts and to perform properly MCR-ALS.
396 Two options of initialization were tested: 3 and 4 compounds (named k in Eq. 1). This number
397 corresponds to the supposed number of pure compounds present in the mixture and was used
398 for MCR-ALS initialization. Indeed, conversely to standard mixture, the number of compounds
399 in the fruit extract was unknown. The first test of 3 was chosen because it corresponded to
400 the number of the major carotenoids. We also tested 4 components to create an unknown
401 compound that artificially gathered all the noise of the other constituents of the extract. This
402 last solution helped to lower the error of the relative quantification of carotenoids. The results
403 after MCR-ALS resolution in terms of spectra of pure compounds are presented in Fig. 6. Pure
404 spectra obtained after resolution based on the experimental spectra from 350 to 750 nm are
405 presented in Fig. 6A and from 400 to 600nm in Fig. 6B.

406



407

408



409

410

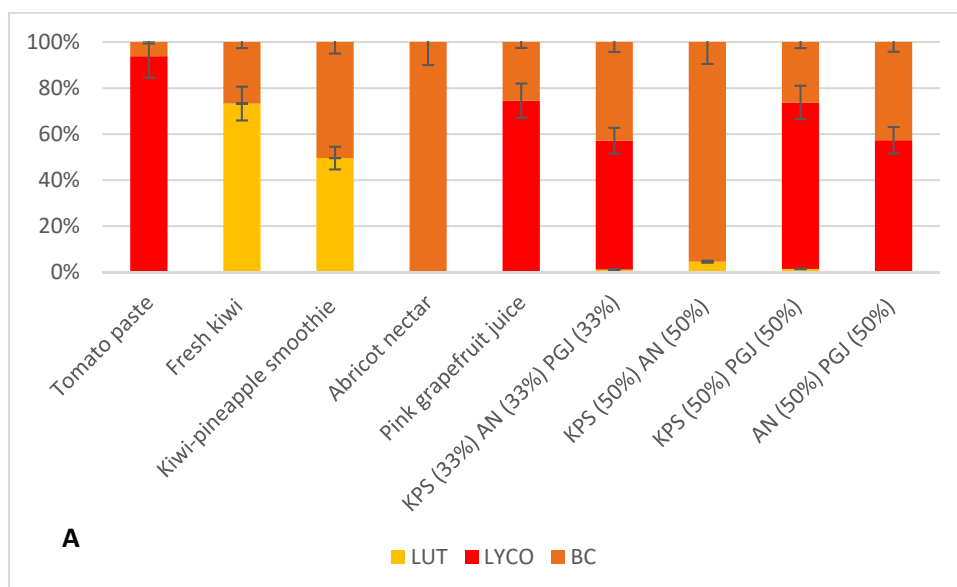
411 **Figure 6.** 4 pure spectra obtained after MCR-ALS procedure on standards and fruit extract
 412 spectra A) from 350-750nm and B) from 350-600 nm

413

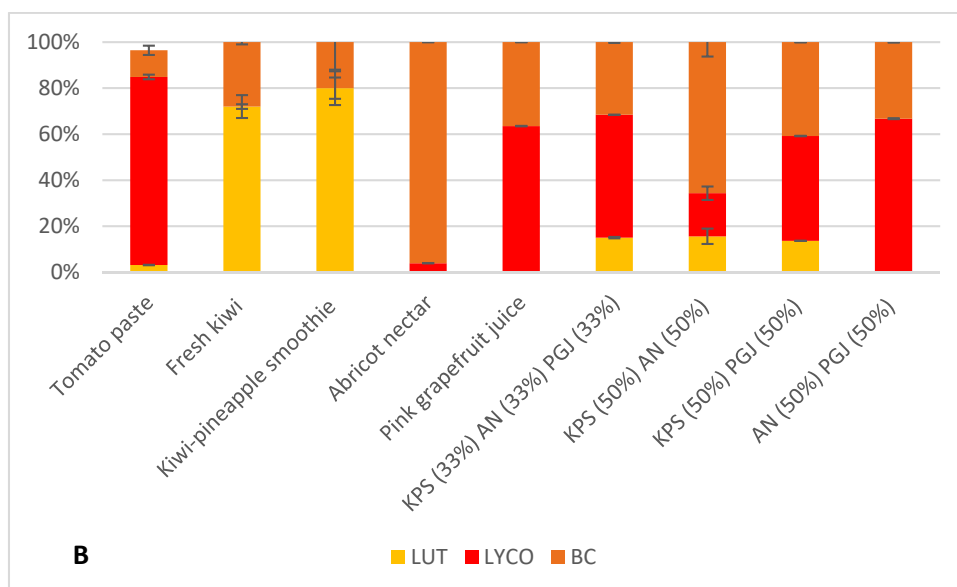
414 From the comparison with spectra of standards, we could easily identify lutein (S1), lycopene
 415 (S2), β -carotene (S4) and the unknown (S3). Fig. 6A shows that it was possible to differentiate
 416 an unknown compound exhibiting absorbance in the 350-425 and 650-700 nm region which
 417 is typical of chlorophylls. From the result of MCR-ALS relative proportions, unknown
 418 contribution could be estimated at 5% for TP and PGJ, 15% for AN, 30% for FK, and nearly 80%

419 for KPS which is consistent with the expected content of other extractable compounds,
 420 particularly chlorophylls, in the different samples. Contribution of chlorophylls was very high
 421 in the commercial KPS, probably because of the addition of spirulina (percentage of this
 422 ingredient not given). The content of non-carotenoid compounds that were removed from the
 423 total carotenoids are given in Table 3 in the line "TC by spectrophotometry + MCR". By doing
 424 this, the overestimation of spectrophotometric estimation was significantly reduced and was
 425 at most 1.3-fold and 1.5-fold for AN and KF respectively.

426 Relative proportion of lutein, lycopene and β -carotene were obtained after resolution in the
 427 range 400-600 nm (with pure spectra obtained in Fig. 6B) for TP, FK, KPS, AN, PGF and 4 different
 428 mixtures of KPS, AN and PGF. Results are given from HPLC and spectrophotometry-MCR-ALS
 429 results in figure 7.



430



431

432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463

Figure 7: Relative proportion of lutein (LUT), lycopene (LYCO), and β -carotene (BC) from A) HPLC and B) Spectrophotometry + MCR-ALS

As can be seen, for each extract, the major expected form could be elucidated: lutein for KF, β -carotene for AN and lycopene for PGF and TP. Regarding the carotenoid species, lycopene and β -carotene were the best elucidated form as on every matrix, the error was about 10% vs 35% for lutein. This effective discrimination can be linked to the highest total carotenoid concentration with 200 mg.kg⁻¹ for tomato paste and 12 mg.kg⁻¹ for pink grapefruit juice respectively. Because of this concentration, even if the extract was diluted for spectral measurements, the noise due to other co-eluted compounds became minor. We can observe some aberrant concentrations of lutein in TP and lycopene in AN which were inferior to 5%. Conversely, the higher error for lutein could be due to a more complex extract and spectral proximity with other xanthophylls, pigments, or degradation compounds as can be seen in the chromatograms of Fig. 4. This fact may affect the efficiency of MCR-ALS-resolutions and create pure spectra that differ from the spectra of standards. This phenomenon is illustrated in Fig 6A, where S1 and S4, whose main contributors are lutein and β -carotene respectively, move away from the pure spectra of Fig 2A. Even if the MCR-ALS spectral resolution is better by taking a shorter range of wavelength from 400-600 nm (Fig 6B), this fact may explain the overestimation of lutein, contrary to β -carotene, in KPS as well as in all the mixtures that contain it: KPS-AN-PGJ, KPS-AN, KPS-PGF, and AN-PGJ.

Therefore, simple UV-Vis spectrophotometry associated to MCR-ALS treatment can enable good relative quantification in a mixture of pure carotenoid standards with a good sensitivity but also be used to get an estimation of the relative proportion of each specie in a real extract. Indeed, this technique was efficient to discriminate β -carotene and lycopene from other carotenoids with an error inferior to 10%. Therefore, with a simple UV-VIS spectrophotometer and a standard extraction protocol used to determine total carotenoids, addition of the MCR-ALS procedure can give more insights into the pigment composition of the food extract. In comparison to the usual and widespread "total carotenoid" experiments and with the same material, this methodology can give semi-quantitative information about the carotenoid

464 profile in a very reduced time (a few min) in comparison to 30-60 min with HPLC. Therefore,
465 this methodology could be applied for high throughput analysis such as for food process
466 controls where the carotenoid profile could be affected by thermal treatment, varietal
467 screening or ripening monitoring where hundreds of fruits/vegetables must be analyzed.
468 However, this paper also showed the limitations of such techniques that is mainly linked to
469 the types of carotenoids that need to be separated. MCR-ALS worked very differently as a
470 function of the spectra features of the compounds in mixture and its composition. The more
471 the spectra are different, the more the resolution can be possible and relevant. This fact is not
472 constant like the molecules we wanted to separate: spectra may be very different (like β -
473 carotene and lycopene) or the same (like β -carotene and β -cryptoxanthin). Therefore, the
474 performance of the method can be high for some plants but not at all for others. As a
475 consequence, this methodology worked very well for fruits that contained lycopene and β -
476 carotene like tomato and pink grapefruit and could be relevant for watermelon, guava, etc.
477 More generally, our observations showed that separation could be possible with a difference
478 of λ max of at least 10 nm and/or III/II ratio difference of 30%. Therefore, violaxanthin whose
479 maximal absorption wavelength is around 440 nm could be separated from β -carotene and
480 lycopene^{57,43}. Lastly, for different sectors of the agri-food industry, MCR-ALS could be useful
481 in estimating other pigments such as chlorophylls.

482 However, in the case of a mixture of pigments that have very similar features in the UV-Vis
483 such as β -carotene and lutein, use of MCR-ALS can lead to important ambiguities on the
484 composition and lead to a much lower accuracy. Therefore, this technique cannot replace
485 HPLC analysis especially when specific nutritional calculation has to be carried out. HPLC
486 remains much more robust and reliable for a fine and accurate carotenoid profile. Also,
487 substances to be analyzed that have a low absorption coefficient in the relevant spectral
488 region, or which are only minor components of the mixture cannot be determined with high
489 accuracy. Therefore, it is recommended that substances should have different spectral
490 features and be of similar concentrations to achieve a proper discrimination. Finally, the
491 removal of the UV region <350 nm can be useful to limit interferences, and the signal/noise
492 ratio must be high, so a good spectrophotometer, with a low spectral bandwidth is required.
493 Future improvements can be made, by adding a physical purification step, or mathematically
494 by adding spectra of other compounds in the mixture if they are known.

495

496

497 **Conflicts of interest statement**

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

500

501 **References**

502

- 503 (1) Azevedo-Meleiro, C. H.; Rodriguez-Amaya, D. B. Confirmation of the Identity of the Carotenoids
504 of Tropical Fruits by HPLC-DAD and HPLC-MS. *Journal of Food Composition and Analysis* **2004**,
505 *17* (3), 385–396. <https://doi.org/10.1016/j.jfca.2004.02.004>.
- 506 (2) De-Sa, M. C.; Rodriguez-Amaya, D. B. Carotenoid Composition of Cooked Green Vegetables
507 from Restaurants. *Food Chemistry* **2003**, *83*, 595–600.
- 508 (3) Amaya, D. B. *A Guide to Carotenoid Analysis in Foods*; OMNI Research: Washington D.C., 2001.
- 509 (4) Fanciullino, A. L.; Dhuique-Mayer, C.; Luro, F.; Casanova, J.; Morillon, R.; Ollitrault, P. Carotenoid
510 Diversity in Cultivated Citrus Is Highly Influenced by Genetic Factors. *Journal of agricultural and*
511 *food chemistry* **2006**, *54* (12), 4397–4406.
- 512 (5) Leiva-Brondo, M.; Valcarcel, M.; Marti-, R.; Rosello, S.; Cebolla-Cornejo, J. New Opportunities
513 for Developing Tomato Varieties with Enhanced Carotenoid Content. *Scientia Agricola* **2016**, *73*,
514 512–519.
- 515 (6) Zhou, W.; Niu, Y.; Ding, X.; Zhao, S.; Li, Y.; Fan, G.; Zhang, S.; Liao, K. Analysis of Carotenoid
516 Content and Diversity in Apricots (*Prunus Armeniaca* L.) Grown in China. *Food Chemistry* **2020**,
517 *330*, 127223. <https://doi.org/10.1016/j.foodchem.2020.127223>.
- 518 (7) Penicaud, C.; Achir, N.; Dhuique-Mayer, C.; Dornier, M.; Bohuon, P. Degradation of β -Carotene
519 during Fruits and Vegetables Processing or Storage: Reaction Mechanisms and Kinetics Aspects.
520 *FRUITS* **2011**, *66* (6), 417–440.
- 521 (8) Achir, N.; Hadjal, T.; Madani, K.; Dornier, M.; Dhuique-Mayer, C. Carotene Reactivity in Pink
522 Grapefruit Juice Elucidated from Model Systems and Multiresponse Modeling. *Journal of*
523 *Agricultural and Food Chemistry* **2015**, *63* (15), 3970–3979.
524 <https://doi.org/10.1021/acs.jafc.5b00509>.
- 525 (9) Haila, K. M.; Lievonen, S. M.; Heinonen, M. I. Effects of Lutein, Lycopene, Annatto, and γ -
526 Tocopherol on Autoxidation of Triglycerides. *Journal of Agricultural and Food Chemistry* **1996**,
527 *44*, 2096–2100.
- 528 (10) Varzakas, T.; Kiokias, S. HPLC Analysis and Determination of Carotenoid Pigments in
529 Commercially Available Plant Extracts. *Current Research in Nutrition and Food Science* **2016**, *4*
530 (01–14).
- 531 (11) Bohn, T.; Desmarchelier, C.; Dragsted, L. O.; Nielsen, C. S.; Stahl, W.; Rühl, R.; Keijer, J.; Borel, P.
532 Host-Related Factors Explaining Interindividual Variability of Carotenoid Bioavailability and
533 Tissue Concentrations in Humans. *Molecular Nutrition & Food Research* **2017**, *61* (6), 1600685.
534 <https://doi.org/10.1002/mnfr.201600685>.
- 535 (12) Rao, AV.; Rao, LG. Carotenoids and Human Health. *Pharmacol Res.* **2007**, *55* (3), 207–216.
536 <https://doi.org/10.1016/j.phrs.2007.01.012>.
- 537 (13) Haskell, M. J. The Challenge to Reach Nutritional Adequacy for Vitamin A: β -Carotene
538 Bioavailability and Conversion—Evidence in Humans. *The American Journal of Clinical Nutrition*
539 **2012**, *96* (5), 1193S-1203S. <https://doi.org/10.3945/ajcn.112.034850>.
- 540 (14) Koushan, K.; Rusovici, R.; Li, W.; Ferguson, L. R.; Chala, K. The Role of Lutein in Eye-Related
541 Disease Keyvan Koushan, Raluca Rusovici, Wenhua Li, Lee R. Ferguson and Kakarla V. Chala.
542 *Nutrients* **2013**, *5*, 1823–1839.

- 543 (15) Agarwal, S.; Rao, A. V. Tomato Lycopene and Its Role in Human Health and Chronic Diseases
544 Sanjiv Akkinappally Venketeshwer Rao. *JAMC* **2000**, *163*, 739–744.
- 545 (16) Zang, L.-Y.; Sommerburg, O.; van Kuijk, F. J. G. M. Absorbance Changes of Carotenoids in
546 Different Solvents. *Free Radical Biology and Medicine* **1997**, *23* (7), 1086–1089.
547 [https://doi.org/10.1016/S0891-5849\(97\)00138-X](https://doi.org/10.1016/S0891-5849(97)00138-X).
- 548 (17) Melendez-Martinez, A. J.; Stinco, C. M.; Liu, C.; Wang, X.-D. A Simple HPLC Method for the
549 Comprehensive Analysis of Cis/Trans (Z/E) Geometrical Isomers of Carotenoids for Nutritional
550 Studies. *Food Chemistry* **2013**, *138* (2), 1341–1350.
551 <https://doi.org/10.1016/j.foodchem.2012.10.067>.
- 552 (18) Rodriguez-Amaya, D. B.; Kimura, M.; Godoy, H. T.; Amaya-Farfan, J. Updated Brazilian Database
553 on Food Carotenoids: Factors Affecting Carotenoid Composition. *Journal of Food Composition*
554 *and Analysis* **2008**, *21* (6), 445–463. <https://doi.org/10.1016/j.jfca.2008.04.001>.
- 555 (19) Clemente, A. A.; Maciel, G. M.; Siquieroli, A. C. S.; Gallis, R. B. de A.; Pereira, L. M.; Duarte, J. G.
556 High-Throughput Phenotyping to Detect Anthocyanins, Chlorophylls, and Carotenoids in Red
557 Lettuce Germplasm. *International Journal of Applied Earth Observation and Geoinformation*
558 **2021**, *103*, 102533. <https://doi.org/10.1016/j.jag.2021.102533>.
- 559 (20) Qi, F.-F.; Tao, L.-M.; Dai, Y.-M.; Zhang, B.-M.; Wang, X.; Yu, Y. Optimization and Application of
560 High-Throughput Supported Liquid Extraction for Simultaneous Determination of Carotenoids
561 and Fat-Soluble Vitamins in Serum. *Journal of Chromatography B* **2021**, *1173*, 122672.
562 <https://doi.org/10.1016/j.jchromb.2021.122672>.
- 563 (21) Asker, D. High Throughput Screening and Profiling of High-Value Carotenoids from a Wide
564 Diversity of Bacteria in Surface Seawater. *Food Chemistry* **2018**, *261*, 103–111.
565 <https://doi.org/10.1016/j.foodchem.2018.03.109>.
- 566 (22) Biehler, E.; Mayer, F.; Hoffmann, L.; Krause, E.; Bohn, T. Comparison of 3 Spectrophotometric
567 Methods for Carotenoid Determination in Frequently Consumed Fruits and Vegetables. *J Food*
568 *Sci.* **2010**, *75* (1), 55–61. <https://doi.org/10.1111/j.1750-3841.2009.01417.x>.
- 569 (23) Biswas, A. K.; Sahoo, J.; Chatli, M. K. A Simple UV-Vis Spectrophotometric Method for
570 Determination of β -Carotene Content in Raw Carrot, Sweet Potato and Supplemented Chicken
571 Meat Nuggets. *LWT - Food Science and Technology* **2011**, *44* (8), 1809–1813.
572 <https://doi.org/10.1016/j.lwt.2011.03.017>.
- 573 (24) Hornero-Méndez, D.; Mínguez-Mosquera, M. I. Rapid Spectrophotometric Determination of
574 Red and Yellow Isochromic Carotenoid Fractions in Paprika and Red Pepper Oleoresins. *J. Agric.*
575 *Food Chem.* **2001**, *49* (8), 3584–3588. <https://doi.org/10.1021/jf010400l>.
- 576 (25) Jaramillo, A. M.; Londoño, L. F.; Orozco, J. C.; Patiño, G.; Belalcazar, J.; Davrieux, F.; Talsma, E.
577 F. A Comparison Study of Five Different Methods to Measure Carotenoids in Biofortified Yellow
578 Cassava (*Manihot Esculenta*). *PLOS ONE* **2018**, *13* (12), e0209702.
579 <https://doi.org/10.1371/journal.pone.0209702>.
- 580 (26) Britton, G.; Liaanen-Jensen, S.; Pfander, H. *Carotenoids. Handbook*; Birkhäuser, Series Ed.; Basel,
581 Switzerland, 2004.
- 582 (27) C. Rhee, K. *Current Protocols in Food Analytical Chemistry*; 2001.
583 <https://doi.org/10.1002/0471142913.fab0102s00>.
- 584 (28) Britton, G.; Liaanen-Jensen, S.; Pfander, H. *Carotenoids. Vol. 1B: Spectroscopy*; Birkhäuser,
585 Series Ed.; Basel, Switzerland, 1995.
- 586 (29) Bulda, O. V.; Rassadina, V. V.; Alekseichuk, H. N.; Laman, N. A. Spectrophotometric
587 Measurement of Carotenes, Xanthophylls, and Chlorophylls in Extracts from Plant Seeds.
588 *Russian Journal of Plant Physiology* **2008**, *55* (4), 544.
589 <https://doi.org/10.1134/S1021443708040171>.
- 590 (30) Scott, K. J. *Detection and Measurement of Carotenoids by UV/VIS Spectrophotometry*; Current
591 Protocols in Food Analytical Chemistry; 2001.
- 592 (31) Küpper, H.; Seibert, S.; Parameswaran, A. Fast, Sensitive, and Inexpensive Alternative to
593 Analytical Pigment HPLC: Quantification of Chlorophylls and Carotenoids in Crude Extracts by

- 594 Fitting with Gauss Peak Spectra. *Anal. Chem.* **2007**, *79* (20), 7611–7627.
595 <https://doi.org/10.1021/ac070236m>.
- 596 (32) Kimura, M.; Rodriguez-Amaya, D. B. A Scheme for Obtaining Standards and HPLC Quantification
597 of Leafy Vegetable Carotenoids. *Food Chemistry* **2002**, *78*, 389–398.
- 598 (33) Dhuique-Mayer, C.; Tbatou, M.; Carail, M.; Caris-Veyrat, C.; Dornier, M.; Amiot, M. J. Thermal
599 Degradation of Antioxidant Micronutrients in Citrus Juice: Kinetics and Newly Formed
600 Compounds. *J. Agric. Food Chem.* **2007**, *55* (10), 4209–4216.
- 601 (34) Lichtenthaler, H. K.; Buschmann, C. Chlorophylls and Carotenoids: Measurement and
602 Characterization by UV-VIS Spectroscopy. *Current Protocols in Food Analytical Chemistry* **2001**,
603 *1* (1), F4.3.1-F4.3.8. <https://doi.org/10.1002/0471142913.faf0403s01>.
- 604 (35) Azzouz, T.; Tauler, R. Application of Multivariate Curve Resolution Alternating Least Squares
605 (MCR-ALS) to the Quantitative Analysis of Pharmaceutical and Agricultural Samples. *Talanta*
606 **2008**, *74* (5), 1201–1210. <https://doi.org/10.1016/j.talanta.2007.08.024>.
- 607 (36) Garrido, M.; Rius, F. X.; Larrechi, M. S. Multivariate Curve Resolution–Alternating Least Squares
608 (MCR-ALS) Applied to Spectroscopic Data from Monitoring Chemical Reactions Processes.
609 *Analytical and Bioanalytical Chemistry* **2008**, *390* (8), 2059–2066.
610 <https://doi.org/10.1007/s00216-008-1955-6>.
- 611 (37) Ruckebusch, C.; Blanchet, L. Multivariate Curve Resolution: A Review of Advanced and Tailored
612 Applications and Challenges. *Analytica Chimica Acta* **2013**, *765*, 28–36.
613 <https://doi.org/10.1016/j.aca.2012.12.028>.
- 614 (38) Achir, N.; Sinela, A.; Mertz, C.; Fulcrand, H.; Dornier, M. Monitoring Anthocyanin Degradation
615 in Hibiscus Sabdariffa Extracts with Multi-Curve Resolution on Spectral Measurement during
616 Storage. *Food Chemistry* **2019**, *271*, 536–542.
617 <https://doi.org/10.1016/j.foodchem.2018.07.209>.
- 618 (39) Março, P. H.; Poppi, R. J.; Scarminio, I. S.; Tauler, R. Investigation of the PH Effect and UV
619 Radiation on Kinetic Degradation of Anthocyanin Mixtures Extracted from Hibiscus Acetosella.
620 *Food Chemistry* **2011**, *125* (3), 1020–1027. <https://doi.org/10.1016/j.foodchem.2010.10.005>.
- 621 (40) Silva, A. C.; Lourenço, A. S.; de Araujo, M. C. U. Simultaneous Voltammetric Determination of
622 Four Organic Acids in Fruit Juices Using Multiway Calibration. *Food Chemistry* **2018**, *266*, 232–
623 239. <https://doi.org/10.1016/j.foodchem.2018.06.005>.
- 624 (41) Vidot, K.; Achir, N.; Mertz, C.; Sinela, A.; Rawat, N.; Prades, A.; Dangles, O.; Fulcrand, H.; Dornier,
625 M. Effect of Temperature on Acidity and Hydration Equilibrium Constants of Delphinidin-3-O-
626 and Cyanidin-3-O-Sambubioside Calculated from Uni- and Multiwavelength Spectroscopic Data.
627 *Journal of Agricultural and Food Chemistry* **2016**, *64* (20), 4139–4145.
628 <https://doi.org/10.1021/acs.jafc.6b00701>.
- 629 (42) Eksi-Kocak, H.; Ilbasemis Tamer, S.; Yilmaz, S.; Eryilmaz, M.; Boyaci, I. H.; Tamer, U. Quantification
630 and Spatial Distribution of Salicylic Acid in Film Tablets Using FT-Raman Mapping with
631 Multivariate Curve Resolution. *Asian Journal of Pharmaceutical Sciences* **2018**, *13* (2), 155–162.
632 <https://doi.org/10.1016/j.ajps.2017.07.010>.
- 633 (43) Mendieta, J.; Díaz-Cruz, M. S.; Esteban, M.; Tauler, R. Multivariate Curve Resolution: A Possible
634 Tool in the Detection of Intermediate Structures in Protein Folding. *Biophysical Journal* **1998**,
635 *74* (6), 2876–2888. [https://doi.org/10.1016/S0006-3495\(98\)77994-9](https://doi.org/10.1016/S0006-3495(98)77994-9).
- 636 (44) Vdović, S.; Wang, Y.; Li, B.; Qiu, M.; Wang, X.; Guo, Q.; Xia, A. Excited State Dynamics of β -
637 Carotene Studied by Means of Transient Absorption Spectroscopy and Multivariate Curve
638 Resolution Alternating Least-Squares Analysis. *Phys. Chem. Chem. Phys.* **2013**, *15* (46), 20026–
639 20036. <https://doi.org/10.1039/C3CP53323A>.
- 640 (45) Wehrens, R.; Carvalho, E.; Masuero, D.; de Juan, A.; Martens, S. High-Throughput Carotenoid
641 Profiling Using Multivariate Curve Resolution. *Analytical and Bioanalytical Chemistry* **2013**, *405*
642 (15), 5075–5086. <https://doi.org/10.1007/s00216-012-6555-9>.
- 643 (46) de Juan, A.; Jaumot, J.; Tauler, R. Multivariate Curve Resolution (MCR). Solving the Mixture
644 Analysis Problem. *Analytical Methods* **2014**, *6* (14), 4964–4976.
645 <https://doi.org/10.1039/c4ay00571f>.

- 646 (47) Jaumot, J.; Gargallo, R.; de Juan, A.; Tauler, R. A Graphical User-Friendly Interface for MCR-ALS:
647 A New Tool for Multivariate Curve Resolution in MATLAB. *Chemometrics and Intelligent*
648 *Laboratory Systems* **2005**, *76* (1), 101–110. <https://doi.org/10.1016/j.chemolab.2004.12.007>.
- 649 (48) Soto, M.; Dhuique-Mayer, C.; Servent, A.; Jiménez, N.; Vaillant, F.; Achir, N. A Kinetic Study of
650 Carotenoid Degradation during Storage of Papaya Chips Obtained by Vacuum Frying with
651 Saturated and Unsaturated Oils. *Food Research International* **2020**, *128*, 108737.
652 <https://doi.org/10.1016/j.foodres.2019.108737>.
- 653 (49) Gies, M.; Descalzo, A. M.; Servent, A.; Dhuique-Mayer, C. Incorporation and Stability of
654 Carotenoids in a Functional Fermented Maize Yogurt-like Product Containing Phytosterols. *LWT*
655 **2019**, *111*, 105–110. <https://doi.org/10.1016/j.lwt.2019.04.103>.
- 656 (50) Jaumot, J.; Escaja, N.; Gargallo, R.; González, C.; Pedroso, E.; Tauler, R. Multivariate Curve
657 Resolution: A Powerful Tool for the Analysis of Conformational Transitions in Nucleic Acids.
658 *Nucleic Acids Research* **2002**, *30* (17), e92–e92.
- 659 (51) Chanforan, C.; Loonis, M.; Mora, N.; Caris-Veyrat, C.; Dufour, C. The Impact of Industrial
660 Processing on Health-Beneficial Tomato Microconstituents. *Food Chemistry* **2012**, *134* (4),
661 1786–1795. <https://doi.org/10.1016/j.foodchem.2012.03.077>.
- 662 (52) Schweiggert, RM.; Mezger, D.; Schimpf, F.; Steingass, CB.; Carle, R. Influence of Chromoplast
663 Morphology on Carotenoid Bioaccessibility of Carrot, Mango, Papaya, and Tomato. *Food*
664 *Chemistry* **2012**, *135* (4), 2736–2742.
- 665 (53) Achir, N.; Hadjal, T.; Madani, K.; Dornier, M.; Dhuique-Mayer, C. Carotene Reactivity in Pink
666 Grapefruit Juice Elucidated from Model Systems and Multi-Response Modelling. *J. Agric. Food*
667 *Chem.*, **2015**, *63* (15), 3970–3979. <https://doi.org/10.1021/acs.jafc.5b00509>.
- 668 (54) Colle, IJ.; Lemmens, L.; Tolesa, GN.; Van Buggenhout, S.; De Vleeschouwer, K.; Van Loey, AM.;
669 Hendrickx, ME. Lycopene Degradation and Isomerization Kinetics during Thermal Processing of
670 an Olive Oil/Tomato Emulsion. *J Agric Food Chem.* **2010**, *58* (24), 12784–12789.
- 671 (55) Leontowicz, H.; Leontowicz, M.; Latocha, P.; Jesion, I.; Park, Y.-S.; Katrich, E.; Barasch, D.;
672 Nemirovski, A.; Gorinstein, S. Bioactivity and Nutritional Properties of Hardy Kiwi Fruit *Actinidia*
673 *Arguta* in Comparison with *Actinidia Deliciosa* ‘Hayward’ and *Actinidia Eriantha* ‘Bidan.’ *Food*
674 *Chemistry* **2016**, *196*, 281–291. <https://doi.org/10.1016/j.foodchem.2015.08.127>.
- 675 (56) Dragovic-Uzelac, V.; Levaj, B.; Mrkic, V.; Bursac, D.; Boras, M. The Content of Polyphenols and
676 Carotenoids in Three Apricot Cultivars Depending on Stage of Maturity and Geographical
677 Region. *Food Chemistry* **2007**, *102* (3), 966–975.
678 <https://doi.org/10.1016/j.foodchem.2006.04.001>.
- 679 (57) Thrane, J.-E.; Kyle, M.; Striebel, M.; Haande, S.; Grung, M.; Rohrlack, T.; Andersen, T.
680 Spectrophotometric Analysis of Pigments: A Critical Assessment of a High-Throughput Method
681 for Analysis of Algal Pigment Mixtures by Spectral Deconvolution. *PLOS ONE* **2015**, *10* (9),
682 e0137645. <https://doi.org/10.1371/journal.pone.0137645>.
- 683