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## Temperature and storage time increase provitamin A carotenoid concentrations and bioaccessibility in post-harvest carrots

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1 **Temperature and storage time increase provitamin A carotenoid**  
2 **concentrations and bioaccessibility in post-harvest carrots.**

3  
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23 **Abstract**

24           The aim was to enhance provitamin A carotenoid (proVA CAR) concentrations and  
25 bioaccessibility in carrots by manipulating post-harvest factors. To that end, we assessed the  
26 effects of Ultraviolet-C light, pulsed light, storage temperature, and storage duration. We also  
27 measured CAR bioaccessibility by using an *in vitro* model. Pulsed light, but not Ultraviolet-C,  
28 treatment increased proVA CAR concentrations in the cortex tissue ( $p<0.05$ ). Longer storage  
29 times and higher temperatures also increased concentrations ( $p<0.05$ ). The maximal increase  
30 induced by pulsed light was obtained after treatment with  $20 \text{ kJ/m}^2$  and 3-days of storage at  
31  $20^\circ\text{C}$ . However, the positive effect induced by pulsed light decreased considerably over the  
32 next seven days. ProVA CAR in carrots with the highest concentrations also proved to be more  
33 bioaccessible ( $p<0.05$ ). Thus, proVA CAR concentrations in stored carrots can be increased  
34 significantly through storage times and temperatures. Pulsed light can also significantly  
35 increase proVA CAR concentrations, but only temporarily.

36

37 **Keywords:**  $\beta$ -carotene;  $\alpha$ -carotene; vitamin A deficiency; UV-C light; pulsed light;  
38 bioavailability.

39

40 **Chemical compounds studied in this article:**

41 Beta-carotene (PubChem CID: 5280489); Alpha-carotene (PubChem CID: 6419725)

42

## 43 **1) Introduction**

44

45 Vitamin A deficiency is still a major health issue in many developing countries  
46 (Schmidhuber, et al., 2018). One strategy to fight this is to increase provitamin A carotenoid  
47 (proVA CAR) concentrations in fruits and vegetables commonly consumed in these countries.  
48 This can be achieved by providing fruits and vegetable cultivars that are naturally rich in  
49 proVA CAR or genetically modified foods, such as golden rice and the super banana (Tang,  
50 Qin, Dolnikowski, Russell, & Grusak, 2009; Waltz, 2014), or by acting on environmental  
51 conditions to modify the nutrient and phytochemical composition of crops. Indeed, it has been  
52 shown that several pre- and post-harvesting practices (e.g. drought, UV radiation) can increase  
53 apparent concentrations of bioactives (Atkinson, Nestby, Ford, & Dodds, 2005), including  
54 proVA CAR (Poiroux-Gonord, Bidel, Fanciullino, Gautier, Lauri-Lopez, & Urban, 2010; Saini  
55 & Keum, 2018). We specify “apparent” concentration because, in most cases, reports do not  
56 specify whether there was a true increase in concentration or if the plant structure modifications  
57 increased extraction of these compounds, leading to measurably higher concentrations during  
58 phytochemical quantification.

59 The orange carrot variety is one of the richest proVA CAR sources in the human diet  
60 and is widely consumed in several countries. Therefore, it is a very good candidate to identify  
61 environmental conditions that could increase its proVA CAR concentrations. In fact, it has  
62 been shown that both pre- and post-harvest factors can improve proVA CAR concentrations in  
63 carrots (Seljasen, et al., 2013). Acting on pre-harvest factors, e.g. watering, has the advantage  
64 of being able to simultaneously modify the proVA CAR content of very large quantities of  
65 carrots. However, this can have consequences in terms of yield, and this may lead to an  
66 increase in the cost of sale. Therefore, we assume it will be easier for inhabitants of countries  
67 where there is still vitamin A deficiency, and who are mostly people with low income and low

68 education, to modify some post-harvest factors of carrots stored by wholesalers, local retailers  
69 or even at home, than to advise/suggest to the cultivators to modify and control environmental  
70 conditions of their carrot culture. Therefore, we decided to focus on the effects of post-harvest  
71 factors on the concentration of proVA CAR in orange carrots.

72         There are several candidate post-harvest factors that could theoretically improve the  
73 proVA CAR concentration in carrots during storage (Saini & Keum, 2018). Those which are, a  
74 priori, the simplest to use are storage time and storage temperature. Concerning storage time, it  
75 has been observed in several studies that carrot proVA CAR concentrations increase during the  
76 first two to three weeks of storage, then decrease (Berger, Kuchler, Maassen, Busch-Stockfisch,  
77 & Steinhart, 2008; Brown, 1949; Howard, Wong, Perry, & Klein, 1999; Imsic, Winkler,  
78 Tomkins, & Jones, 2010; Lee, 1986). However, it is not known whether this increase is due to  
79 biosynthesis of proVA CAR during storage, or to better extractability of proVA CAR due to the  
80 slow degradation of the carrot matrix over time. Concerning the effect of storage temperature,  
81 there is surprisingly no clear conclusion. Although results presented in three studies suggest  
82 that it might modify proVA CAR concentration in carrots (Berger, Kuchler, Maassen, Busch-  
83 Stockfisch, & Steinhart, 2008; Imsic, Winkler, Tomkins, & Jones, 2010; Negi & Roy, 2000), it  
84 is not possible to conclude because these studies were not originally designed to answer this  
85 question. Ethylene and ambient oxygen concentrations are two additional candidate factors.  
86 However, we chose not to study their effects because both have a negative impact on carrot  
87 organoleptic properties (Carlton, Peterson, & Tolbert, 1961; Kato-Noguchi, 1998; Seljåsen,  
88 Hoftun, Selliseth, & Bengtsson, 2004). The last candidate factor we considered for improving  
89 the concentration of proVA CAR is treatment with artificial light. Indeed, it has been shown in  
90 several fruits and vegetables after harvest that different light treatments (i.e. UV-C, UV-B, high  
91 light, red light) with different time durations can significantly modify concentrations in several  
92 phytochemicals (Zhang, et al., 2015), including proVA CAR (Poiroux-Gonord, Bidel,

93 Fanciullino, Gautier, Lauri-Lopez, & Urban, 2010). For example, pulsed light successfully  
94 increased the concentration of proVA CAR in mango (de Almeida Lopes, Silva, Laurent,  
95 Charles, Urban, & de Miranda, 2017; Lopes, et al., 2016). While the mechanisms are not fully  
96 understood, it has been hypothesized that light irradiation can induce plants to produce  
97 antioxidant phytochemicals that participate in the defence against free radicals generated by  
98 these light radiations. However as previously suggested, this factor may also have an impact on  
99 the plant matrix, resulting in an increase in the extractability of antioxidants. Few studies are  
100 available concerning the effect of light treatments on proVA CAR concentrations specifically  
101 in carrots. Furthermore, in these studies the irradiation was performed on peeled or sliced  
102 carrots (Aguiló-Aguayo, Gangopadhyay, Lyng, Brunton, & Rai, 2017; Alegria, Pinheiro,  
103 Duthoit, Gonçalves, Moldão-Martins, & Abreu, 2012) and proVA CAR quantification was  
104 performed on shredded carrots (Aguiló-Aguayo, Gangopadhyay, Lyng, Brunton, & Rai, 2017).  
105 Following these observations and hypotheses, the first step of this study consisted of assessing  
106 whether short exposure to light, provided either by UV-C irradiation or by pulsed light, can  
107 significantly increase proVA CAR concentrations in stored carrots. Then we tried to optimize  
108 the proVA CAR concentrations by varying the storage duration, the storage temperature, and  
109 the dose of artificial light. Finally, we assessed whether carrots with higher concentrations of  
110 proVA CAR resulting from modification of a post-harvest factor during storage had greater  
111 quantities of bioaccessible proVA CAR, i.e. micellarized proVA CAR following simulated  
112 gastrointestinal digestion, since this step is assumed to be a pre-requisite for proVA CAR  
113 absorption (Borel, 2003; C Desmarchelier & Borel, 2017; Reboul, Richelle, Perrot,  
114 Desmoulins-Malezet, Pirisi, & Borel, 2006).

115 **2) Materials and methods**

116

117 *Chemicals.*

118 HPLC grade methanol, methyl tert-butyl ether (MTBE), dichloromethane, ethanol, n-  
119 hexane, as well as HPLC grade H<sub>2</sub>O, were purchased from Carlo Erba reagents (Val de Reuil,  
120 France). HPLC standards including  $\alpha$ -carotene,  $\beta$ -carotene and echinenone >95%, and digestive  
121 enzymes used in the *in vitro* digestion (i.e. pepsin 800-2.500 U/mg protein, pancreatin, and bile  
122 extract porcine), were obtained from Sigma Aldrich (France).

123

124 *Assessment of the effect of UV-C and pulsed light treatments on proVA CAR concentrations.*

125 In a first step, two irradiation devices, generating UV-C and pulsed light, were  
126 compared to evaluate their capacity to provoke an increase in proVA CAR concentrations in  
127 mature orange carrots (cultivar not specified) purchased from a local supermarket. UV-C  
128 treatments were performed in an enclosure system composed of 10 UV-C lamps (DSP UV-C  
129 tube, OSRAM HNL, 24 W) with an irradiation peak at 254 nm. Samples were placed on a  
130 quartz plate and irradiated with UV-C lamps located 20 cm above and below the samples. Each  
131 treatment lasted for 168 s to obtain a UV-C dose of 5 kJ/m<sup>2</sup>. Pulsed light was provided by  
132 Xenon lamps (Phoxène-Lumix S.R.L., Dardilly, France). The emitted spectrum ranged from  
133 UV-C to infrared. Whole carrots were placed onto the illumination area at 5 cm from the lamp  
134 and rotated manually (180°) in order to ensure both halves received even exposure to pulsed  
135 light. Each pulsed light flash lasted 500  $\mu$ s, i.e. 2000 Hz frequency, and provided a dose of 5  
136 kJ/m<sup>2</sup> on both carrot halves. After UV-C or pulsed light treatment, carrots were stored on non-  
137 hermetic plastic trays (above absorbent paper saturated with 20 mL of water) in a dark chamber  
138 at controlled temperature (i.e. 20 °C). The control batch was stored under the same conditions  
139 as the artificial light treated carrot batches. Sampling was performed after 4 days to evaluate the

140 effect of both types of irradiation on the proVA CAR concentrations in the epidermis and the  
141 cortex of the carrots. Each testing batch included 5 carrots, and proVA CAR measurements  
142 were performed on each carrot sample three times, i.e. 3 technical replicates. Therefore, 15  
143 measurements were performed per treatment condition.

144

145 *Factorial design to clarify the role of post-harvest factors on proVA CAR concentrations.*

146 To study the effect and the potential interactions between storage temperature, storage  
147 time, and dose of artificial light, a full factorial design was generated and tested using Trial Run  
148 1.0 software (SPSS, Chicago, IL, USA) (Tyssandier, Lyan, & Borel, 2001). The design  
149 comprised 3 to 5 levels for each factor: 3 levels for storage time, i.e. 3, 6 and 10 days; 3 levels  
150 for storage temperature, i.e. 4, 20 and 30 °C; and 5 levels for artificial light energy, i.e. 0 (no  
151 pulsed light, control samples), 2, 5, 10 and 20 kJ/m<sup>2</sup>. According to the design, 45 randomized  
152 experiments, i.e. 45 batches of 5 carrots, were carried out.

153 The full factorial design experiment was conducted on the *Daylance* variety of orange  
154 carrots. Carrots were sorted and divided into 45 homogeneous batches, i.e. homogeneous in  
155 size, diameter, and color. Pulsed light experiments were conducted with the same equipment as  
156 described above, but with different energy doses per flash. The first untreated batch represented  
157 the control, the second batch was treated with 2 kJ/m<sup>2</sup> per flash; the third batch with 5 kJ/m<sup>2</sup> per  
158 flash, the fourth batch with 10 kJ/m<sup>2</sup> per flash; and the last batch with 20 kJ/m<sup>2</sup> per flash. Due  
159 to the setting, only the central part in length of the carrots (approximately 10 cm) was  
160 irradiated. After treatment, carrot batches were stored on non-hermetic plastic trays (above  
161 absorbent paper saturated with 20 mL of water) in a dark chamber at different temperatures, i.e.  
162 +4°C, +20°C and +30°C, and for different durations, i.e. 3, 6 or 10 days. At the end of each  
163 experiment, carrots were cut into 3 parts and the central part (10 cm) was stored at -80°C until

164 further analysis. Note that we did not peel the carrots in this experiment and 3 extractions and  
165 proVA CAR measurements by HPLC were performed on each carrot (technical replicates).

166  
167 *Evaluation of the effect of high doses of pulsed light on the proVA CAR concentrations.*

168 In this experiment, we used the *Nantes* carrot variety because *Daylance*, a winter  
169 variety, was no longer available. The pulsed light equipment was the same as described in the  
170 previous protocol and we used flashes of 20 kJ/m<sup>2</sup>. Carrots were treated with 1, 2, or 3 flashes  
171 to obtain 20, 40, or 60 kJ/m<sup>2</sup>, respectively. Non-treated carrots represented the control group.  
172 After treatment, carrots were stored as previously described on non-hermetic plastic trays, and  
173 stored in the dark at 20°C for 3 or 7 days. As explained above, following each experiment non-  
174 peeled carrots were cut in 3 parts and stored at -80°C until extraction and proVA CAR analysis  
175 in triplicate (technical replicates).

176  
177 *Measurement of proVA CAR bioaccessibility.*

178 We compared two batches of carrots that had different proVA CAR concentrations  
179 because they were stored for different durations, i.e. 3 and 6 days. We used an *in vitro* digestion  
180 protocol adapted from Desmarchelier et al. (C. Desmarchelier, et al., 2013). In summary, 2 g of  
181 carrot cut into small pieces were mixed with a meal consisting of 6.7 g mashed potatoes, 1.2 g  
182 ground beef, 200 µL of olive oil (all purchased from a local supermarket) and 32 mL of 0.9%  
183 aq. NaCl. The mixture was homogenized for 10 min at 37°C using gentle stirring (190 shakes  
184 per minutes). Then, 2.5 mL an artificial saliva solution was added, and the mixture further  
185 incubated for 10 min at 37 °C under gentle stirring. The pH was then adjusted to 4±0.02 with 1  
186 M HCl. After the addition of 2 mL of pepsin solution, the mixture was incubated at 37°C for 30  
187 min under gentle stirring. The pH was then adjusted to 6±0.02 with 0.9 M NaHCO<sub>3</sub> buffer  
188 before adding 9 mL of a pancreatin solution and 4 mL of a 10% bile solution. The mixture was

189 further incubated for 30 min at 37°C using the same stirring. Aliquots of 4 mL of digesta were  
190 collected, and the remaining digesta centrifuged at 1200 x g for 1 h and 12 min at 10 °C. The  
191 recovered supernatant was filtered through a 0.8 µm and then a 0.22 µm syringe filter. The  
192 samples obtained were stored at -80 °C until proVA CAR extractions were made.

193

#### 194 *ProVA CAR extraction*

195 Carrot proVA CAR ( $\alpha$ -carotene and  $\beta$ -carotene) were extracted from 2 g of raw carrots  
196 first crushed with a knife mill (Grindomix GM 200, Retsh) under liquid nitrogen for 15 s and  
197 then homogenized in 50 mL of distilled water. A volume of 500 µL was taken to which 500 µL  
198 of echinenone (internal standard) diluted in ethanol was added. For micellar and digesta sample  
199 extractions, 2 mL sample was used. A double extraction with hexane was carried out (with two  
200 volumes of hexane per volume of the ethanol-sample mixture). After centrifugation at 1200 x g  
201 for 10 min at 4 °C, the hexane phases were pooled and evaporated under nitrogen gas until a  
202 dry film was obtained. Samples were redissolved in 200 µL of methanol/dichloromethane  
203 (65:35, v/v) for subsequent analysis by HPLC-DAD. Injection volumes of 50 µL (crushed  
204 carrots) and 100 µL (micelle samples and digestate samples) were used for HPLC analysis.

205

#### 206 *Quantification of ProVA CAR by HPLC-DAD*

207 The proVA CAR ( $\alpha$ - carotene and  $\beta$ -carotene) in the sample were identified due to  
208 consistent retention times and spectra of pure standards, and quantified at 450 nm. HPLC  
209 analyses were carried out using a gradient with the mobile phase consisting of (eluent A:  
210 methanol, eluent B: methyl tert-butyl ether and eluent C: H<sub>2</sub>O) (Gleize, Steib, Andre, &  
211 Reboul, 2012) at a flow rate of 1 mL.min<sup>-1</sup>, on a YMC C30 column (250 mm x 4.6 mm; 5µm  
212 particle size) with a pre-column (5 µm particle size, 10 mm x 4 mm) and a constant  
213 temperature (35 °C) (Gleize, Steib, Andre, & Reboul, 2012).

214

215 *Statistical analysis*

216 Means  $\pm$  SEM were obtained from 5 carrot samples. The value of each carrot sample  
217 was obtained from 3 technical replicates, i.e. extraction and HPLC measurement of 3 samples  
218 of the same carrot.

219 Statistical analyses of the factorial design study were based on the general linear model  
220 and used ANOVA. The relationships between the independent variables, i.e. storage time,  
221 storage temperature and doses of pulsed light, and the response variables, i.e.  $\alpha$ - and  $\beta$ -carotene  
222 concentrations in carrots, were shown by surface response curves (SRC). These statistical  
223 analysis as well as the drawing of the SRC were performed by the Trial Run software (SPSS,  
224 Chicago, IL, USA).

225 The other results were compared as follows: unpaired Student's t-test to compare means  
226 obtained in two carrot batches, and ANOVA followed by post-hoc Tukey/Kramer tests to  
227 compare means obtained in more than 3 carrot batches. These statistical analyses were  
228 performed using StatView software version 5.0 (SAS Institute, Cary, NC, USA). In all cases,  
229  $p < 0.05$  was considered statistically significant.

230 **3) Results**

231

232 *Effect of UV-C and pulsed light treatments on  $\alpha$ -carotene concentrations.*

233 **Figure 1** shows that UV-C treatment had no significant effect on  $\alpha$ -carotene  
234 concentration either in the cortex (A) or in the epidermis (B). Conversely, pulsed light  
235 treatment significantly increased  $\alpha$ -carotene concentration in the cortex tissue, +32% ( $p<0.05$ ),  
236 but not in the epidermis.

237

238 *Effect of UV-C and pulsed light treatments on  $\beta$ -carotene concentration.*

239 **Figure 1** shows that UV-C treatment had no significant effect on  $\beta$ -carotene  
240 concentration either in the cortex (C) or in the epidermis (D). Conversely, as observed for  $\alpha$ -  
241 carotene, pulsed light treatment significantly increased  $\beta$ -carotene concentration in the cortex  
242 tissue, +26% ( $p<0.05$ ), but not in the epidermis.

243

244 *Effect of storage time and temperature on proVA CAR concentration.*

245 **Figures 2A and 2B** show the effect of storage time and storage temperature on the  
246 concentrations of  $\alpha$ - and  $\beta$ -carotene in the central part of non-peeled whole carrots. Note that  
247 the test of between-sample effects showed that both factors significantly and independently  
248 modified both proVA CAR concentrations ( $p<0.03$ ), **Table 1**). Furthermore, there were  
249 interactions between these two factors, which almost reached significance for  $\alpha$ -carotene  
250 ( $p=0.052$ ) and significant for  $\beta$ -carotene ( $p=0.008$ , **Table 1**). The surface response curves  
251 (SRC) suggest that the effect of temperature was greater than that of storage time. They also  
252 suggest that the effect of the two parameters were greater for  $\beta$ -carotene than for  $\alpha$ -carotene.  
253 Finally, note that the values shown after 10-day storage at 30 °C are only theoretical. Indeed,  
254 they were calculated by the experimental design software which extrapolated them from the

255 experimental values. This is because most carrots started to rot at 30 °C after a few days, and  
256 thus we did not measure the proVA CAR concentrations in these samples.

257

258 *Effect of storage time and pulsed light dose on proVA CAR concentrations.*

259 **Figures 2C and 2D** show the effect of both storage time and pulsed light energy on the  
260 concentrations of  $\alpha$ - and  $\beta$ -carotene in the central part of non-peeled whole carrots stored at 20  
261 °C. Note that the test of between-sample effects showed that the dose of pulsed light  
262 significantly and independently modified both proVA CAR concentrations ( $p < 0.005$ , **Table 1**).  
263 Furthermore, there were interactions between the dose of pulsed light and the storage time,  
264 almost significant ( $p = 0.061$ ) for  $\alpha$ -carotene and significant ( $p = 0.005$ ) for  $\beta$ -carotene (**Table 1**).  
265 Concerning the SRC, the first observation is that, as observed in **Figures 2A and 2B**, when no  
266 pulsed light was applied (front parts of the **figures 2C and 2D SRC**) the storage time increased  
267 both proVA CAR concentrations and this effect was higher for  $\beta$ -carotene than for  $\alpha$ -carotene.  
268 The second observation is that there was a significant and independent effect of the energy dose  
269 of pulsed light on both proVA CAR concentrations ( $p = 0.001$  and  $p = 0.004$  for  $\alpha$  and  $\beta$ -carotene,  
270 respectively). Furthermore, this effect was not linear. Indeed, a distorted U-shaped curve was  
271 observed for  $\beta$ -carotene (**Figure 2D**) and a J-shaped curve for  $\alpha$ -carotene (**Figure 2C**), with  
272 minima at intermediate doses of pulsed light. Finally, it is noteworthy that the effect of the high  
273 dose pulsed light treatments decreased with increasing storage time. Three days after pulsed  
274 light treatment, the concentrations of both proVA CAR increased with the energy dose of  
275 pulsed light (left part of the C and D curves), but the positive effect of the energy dose on the  
276 proVA CAR concentrations decreased during storage time (from the left to the right of the  
277 **figures 2C and 2D SRC**). In fact, after 10 days of storage,  $\alpha$ -carotene concentrations were  
278 similar in carrots that had been treated with different doses of pulsed light (right end of the  
279 **figure 2C SRC**) as compared to carrots that had not been treated with pulsed light (front right

280 corner of the **figure 2C** SRC). Concerning  $\beta$ -carotene, the negative influence of storage time on  
281 the positive effect that the pulsed light treatment had was dramatic (**Figure 2D**). The  
282 concentration of  $\beta$ -carotene measured 10-days after 20 kJ/m<sup>2</sup> treatment (back right of the SRC)  
283 was significantly lower than the  $\beta$ -carotene concentration measured 10-days after no pulsed  
284 light treatment (front right of the SRC).

285 *Effect of storage temperature and pulsed light dose on proVA CAR concentrations.*

286 The effect of both storage temperature and pulsed light energy on the concentrations of  
287  $\alpha$ - and  $\beta$ -carotene in the central part of non-peeled whole carrots stored at 20 °C are shown in  
288 **Figures 2E** and **2F**. When no pulsed light was applied (front parts of the SRC), the storage  
289 time increased  $\beta$ -carotene concentration. This was not observed for  $\alpha$ -carotene. There was a  
290 significant and independent effect of the energy dose of pulsed light on both proVA CAR  
291 concentrations (p=0.001 and p=0.004 for  $\alpha$  and  $\beta$ -carotene, respectively).

292 *Effect of high doses of pulsed light on proVA CAR concentrations.*

293 Because results presented in **Figures 2B** and **2C** showed that the maximal effect of  
294 pulsed light was observed 3 days after treatment with the maximal dose used in this  
295 experiment, we decided to assess whether higher doses of pulsed light can further increase the  
296 concentrations in proVA CAR. Therefore, pulsed light doses of 20, 40, and 60 kJ/m<sup>2</sup> were  
297 applied and carrots were stored up to 7 days to assess whether we again observed an increase of  
298 proVA CAR after 3 days and a decrease between 3 and 7 days. The results of this experiment  
299 are shown in **Figure 3**. Note that the effects of the 60 kJ/m<sup>2</sup> dose are not shown because carrot  
300 epidermis appeared burnt 3 days after this treatment and thus we did not continue to make  
301 experiments with this condition. Conversely to what was observed in the previous experimental  
302 design (**Figures 2B** and **2C**), irradiating another variety of carrots with 20 kJ/m<sup>2</sup> pulsed light  
303 did not significantly modify proVA CAR concentrations after 3-day storage. There was also no

304 effect of the higher dose of pulsed light ( $40 \text{ kJ/m}^2$ ) on these concentrations. Similar results were  
305 observed for  $\alpha$ -carotene but with a lesser amplitude, as previously described in the  
306 experimental design.

307

308 *Bioaccessibility of proVA CAR in carrots stored under different environmental conditions.*

309 **Figure 4** shows that the bioaccessibility of proVA CAR from carrots stored for 6 days  
310 at  $20 \text{ }^\circ\text{C}$ , with greater concentrations in proVA CAR than carrots stored 3 days at the same  
311 temperature (**Figures 2A to 2D**), was higher than that in carrots stored for 3 days, i.e. 30.3% vs.  
312 21.2% for  $\alpha$ -carotene ( $p=0.03$ ) and 26.9% vs. 18.4% for  $\beta$ -carotene ( $p=0.059$ ), respectively.

#### 313 **4) Discussion**

314

315 In the first part of this study, we aimed to assess whether a short treatment with two  
316 artificial light sources, i.e. UV-C and pulsed light, can significantly increase proVA CAR  
317 concentrations in whole intact carrots stored in a temperate environment, i.e. 20 °C. Because  
318 data from the literature suggest that both UV-C and pulsed light irradiation can increase the  
319 concentration of several phytochemical species in various fruits and vegetables (Fgaier, de  
320 Almeida Lopes, de Oliveira Silva, Aarouf, & Urban, 2019; Urban, Charles, de Miranda, &  
321 Aarouf, 2016), we started by comparing the effect of these two irradiation methods. Because  
322 there are few studies on the effects of artificial light on proVA CAR content in whole carrots,  
323 and because data obtained in other crops have observed effects at very different energy levels,  
324 i.e. between 0.6 and 30 kJ/m<sup>2</sup> (Aguiló-Aguayo, Gangopadhyay, Lyng, Brunton, & Rai, 2017;  
325 Lopes, et al., 2016; Pataro, Sinik, Capitoli, Donsì, & Ferrari, 2015), we chose an intermediate  
326 energy dose, i.e. 5 kJ/m<sup>2</sup>. The results obtained herein clearly show that UV-C treatment failed  
327 to modify the concentrations in our targeted micronutrients, i.e.  $\alpha$ - and  $\beta$ -carotene. We  
328 acknowledge that we assessed the effect of only one dose of UV-C, i.e. 5 kJ/m<sup>2</sup>, which was  
329 taken from previous studies showing that it led to an increase in CAR concentration in tomato  
330 (Bravo, et al., 2013; Liu, Zabarar, Bennett, Aguas, & Woonton, 2009), and it is possible that  
331 other doses would have given different results. Indeed, a lower dose of UV-C (0.78 kJ/m<sup>2</sup>)  
332 increased proVA CAR content in carrots (Alegria, Pinheiro, Duthoit, Gonçalves, Moldão-  
333 Martins, & Abreu, 2012), but peeled carrots were treated, and the proVA CAR content was  
334 measured in shredded carrots that were first stored at 0 °C and then at 5 °C. In short, these  
335 conditions were very different from the conditions described herein that aimed to mimic what  
336 could happen in retail supermarkets. In agreement with a recent study (Aguiló-Aguayo,  
337 Gangopadhyay, Lyng, Brunton, & Rai, 2017), we observed that pulsed light treatment induced

338 a significant increase in apparent proVA CAR concentration, thus we decided to focus on this  
339 light treatment in the full factorial design. Note that we specified the apparent concentration  
340 because we could not tell the difference between a true increase of proVA CAR concentration  
341 and an increase of proVA CAR extractability due to an effect of pulsed light on the food matrix  
342 facilitating extraction (Lyan, et al., 2001; Serino, Gomez, Costagliola, & Gautier, 2009). The  
343 mechanisms that could explain these two potential phenomena are discussed later.  
344 Nevertheless, the fact that pulsed light led to an increase in proVA CAR concentration in the  
345 cortex tissue but not in the epidermis is noteworthy. Indeed, since carrots are generally peeled  
346 before consumption, prior pulsed light treatment would still be an effective way to increase  
347 their proVA CAR content.

348         Since previous studies have suggested that storage time (Brown, 1949; Howard, Wong,  
349 Perry, & Klein, 1999; Imsic, Winkler, Tomkins, & Jones, 2010; Lee, 1986) and storage  
350 temperature (Berger, Kuchler, Maassen, Busch-Stockfisch, & Steinhart, 2008; Imsic, Winkler,  
351 Tomkins, & Jones, 2010; Negi & Roy, 2000), can modulate proVA CAR concentrations in  
352 carrots, we also aimed to assess the interaction effects between pulsed light and these factors on  
353 proVA CAR concentrations. The results confirmed that pulsed light treatment can significantly  
354 increase the concentrations of the two proVA CAR in whole carrots. They also showed that the  
355 two other candidate factors can significantly and independently increase these concentrations.

356         Concerning the effect of the storage temperature, we observed that proVA CAR  
357 concentrations increased with temperature, i.e. between 4 °C and 30 °C for  $\beta$ -carotene and  
358 between around 9 °C and 30 °C for  $\alpha$ -carotene. Although several studies have been devoted to  
359 the evaluation of the effect of carrot storage duration on proVA CAR concentrations, only two  
360 studies tested different storage temperatures (Berger, Kuchler, Maassen, Busch-Stockfisch, &  
361 Steinhart, 2008; Imsic, Winkler, Tomkins, & Jones, 2010). Berger et al. (Berger, Kuchler,  
362 Maassen, Busch-Stockfisch, & Steinhart, 2008) found a decrease of proVA CAR

363 concentrations during storage at -18 °C and -25 °C and an increase at 4 °C and 20 °C.  
364 However, data was not provided to facilitate comparison of the increase at 4 °C and 20 °C for  
365 the same storage time. Imsic and colleagues (Imsic, Winkler, Tomkins, & Jones, 2010)  
366 observed a higher increase at 20 °C as compared to 4 °C, but the storage times were different,  
367 i.e. 14 and 3 days, respectively. Thus, to our knowledge, this is the first time that a significant  
368 positive relationship has been observed between the storage temperature and the concentration  
369 of proVA CAR in carrots. Note that we did not measure the effect of storage temperature at 30  
370 °C because carrots started to rot (see Results), thus the values shown in the SRC were  
371 calculated by the software. Nevertheless, it is likely that the optimal storage temperature to  
372 maximize the proVA CAR concentrations is higher than 20 °C, but it must be balanced with the  
373 risk of rotting and the decrease in organoleptic properties (Seljåsen, Hoftun, Selliseth, &  
374 Bengtsson, 2004), which increase with the storage temperature. Our observations suggest that  
375 the optimal temperature is between 20 °C and 30°C, depending on other post-harvest  
376 conditions, e.g. air humidity and storage duration.

377         The full factorial design experiment showed that proVA CAR concentrations increase  
378 with storage time, with 10 days as the longest duration tested, in agreement with several studies  
379 (Berger, Kuchler, Maassen, Busch-Stockfisch, & Steinhart, 2008; Brown, 1949; Imsic,  
380 Winkler, Tomkins, & Jones, 2010; Lee, 1986). Furthermore, between these publications, this  
381 effect has now been observed in at least 9 different carrot varieties, and thus we consider this to  
382 be a well demonstrated phenomenon. Nevertheless, it is important to state that this increase is  
383 transitory because, in most studies, it was followed by a continuous decrease in the  
384 concentration in proVA CAR when the storage was extended. The storage time at which this  
385 decrease starts varies among studies, from 7 days (Berger, Kuchler, Maassen, Busch-  
386 Stockfisch, & Steinhart, 2008) to 2 weeks (Howard, Wong, Perry, & Klein, 1999) and even 21  
387 days (Imsic, Winkler, Tomkins, & Jones, 2010). We hypothesize that these differences are due

388 to other post-harvest conditions, e.g. storage temperature or humidity, as well as to different  
389 carrot varieties.

390 The mechanisms that could explain the effect of storage time and storage temperature  
391 on proVA CAR concentrations have not yet been identified, but two hypotheses have been  
392 suggested (**Figure 5**). The first one is that the cell wall of the carrot matrix is gradually  
393 disaggregated by enzymes, e.g. cellulases and hemicellulases, during storage (Marx, Stuparic,  
394 Schieber, & Carle, 2003) and this disaggregation allows the CAR to be better extracted from  
395 the carrot matrix in the first stage of extraction, which most often consists of using organic  
396 solvents for proVA CAR removal from the vegetable matrix (Lyan, et al., 2001). It is further  
397 reasonable to hypothesize that this enzymatic disaggregation is enhanced by higher  
398 temperatures, which would also explain the positive effect of temperature on proVA CAR  
399 concentrations. However, the fact that proVA CAR concentration first increased during storage  
400 then decreased (Berger, Kuchler, Maassen, Busch-Stockfisch, & Steinhart, 2008; Howard,  
401 Wong, Perry, & Klein, 1999; Imsic, Winkler, Tomkins, & Jones, 2010) suggests that another  
402 mechanism is involved. Other molecules can be degraded during storage, especially  
403 antioxidants that are the more labile. Therefore, there is likely a time when the CAR  
404 degradation rate becomes higher than the extractability “boost” provided by matrix  
405 disaggregation that ultimately improves proVA CAR concentrations, explaining this biphasic  
406 curve. The second hypothesis to explain the increase of proVA CAR concentrations as a  
407 function of time and temperature is that proVA CAR are still being synthesized in the carrots  
408 during storage. Indeed, CAR biosynthesis after harvest has been suggested in many climacteric  
409 fruits, e.g. tomatoes or mangoes, as well as in tuberous vegetables, such as sweet potatoes. The  
410 hypothesis that carrots continue to biosynthesize proVA CAR (Rodriguez-Concepcion &  
411 Stange, 2013) after harvest is supported by two observations. First, there is a continuous  
412 increase of  $\beta$ -zeacarotene and  $\gamma$ -carotene, which are precursors of  $\beta$ -carotene in the biosynthetic

413 pathway, in carrots during storage (Lee, 1986). Secondly, treatment of carrots with 2-(4-  
414 chlorophenylthio)triethylamine HCl, which is a pesticide that inhibits CAR synthesis, results in  
415 reduction of proVA CAR concentration (Lee, 1986). Therefore, there are arguments supporting  
416 both hypotheses, and in fact both mechanisms may be at play concomitantly (**Figure 5**).  
417 Regardless of the mechanism(s) involved, the net result is equally of interest from a nutrition  
418 perspective. Indeed, greater extractability of proVA CAR would likely lead to a higher quantity  
419 of bioaccessible CAR (C Desmarchelier & Borel, 2017), and greater proVA CAR synthesis  
420 would also lead to more bioaccessible proVA CAR. This is the reason why we have compared  
421 the quantity of bioaccessible proVA CAR in carrots that exhibited different apparent proVA  
422 CAR concentrations in the last part of this study.

423         The interactions between the dose of pulsed light and the storage time (**Figures 2C** and  
424 **2D**) was intriguing. Although the highest dose of pulsed light led to a dramatic increase of  
425 proVA CAR concentrations after 3 days, these high concentrations significantly decreased  
426 upon the storage time. We hypothesize that pulsed light treatments induced the production of  
427 free radicals in the carrots and the carrots responded by synthesizing antioxidant  
428 phytochemicals to protect their tissues from free radical induced damage (Pataro, Sinik,  
429 Capitoli, Donsi, & Ferrari, 2015; Urban, Sari, Orsal, Lopes, Miranda, & Aarouf, 2018). Then,  
430 these newly synthesized antioxidants, including the proVA CAR, were eliminated by free  
431 radicals. Another hypothesis could be that pulsed light treatment modified the carrot matrix  
432 leading to a better extractability of the proVA CAR. Nevertheless, this hypothesis is less likely  
433 because it implies that the matrix modifications induced by the initial treatment with pulsed  
434 light were gradually repaired by the carrot during storage, which seems unlikely.

435         Results of the full factorial design experiment raised new questions about the effect of  
436 pulsed light treatment on proVA CAR concentrations. Indeed, they suggest that higher doses of  
437 pulsed light might further increase proVA CAR concentrations, especially during short storage

438 times. Therefore, we performed a third experiment where we compared the effect of higher  
439 doses of pulsed light, i.e. 40 and 60 kJ/m<sup>2</sup>, than the dose demonstrating maximal effect in the  
440 full factorial design, i.e. 20 kJ/m<sup>2</sup>. Unfortunately, this third experiment did not reproduce the  
441 effect observed at 20 kJ/m<sup>2</sup> on proVA CAR concentrations after 3-days storage. No effect was  
442 observed at 40 kJ/m<sup>2</sup> either. Finally, 60 kJ/m<sup>2</sup> pulsed light led to a burning of carrot epidermis.  
443 We hypothesize that our inability to reproduce the results obtained in both the first experiment  
444 and in the full factorial design was due to the difference in cultivars used. Cv. Daylance was  
445 used in the full factorial design, while cv. Nantes was used in the third experiment (as  
446 explained in the Material & Methods section). Furthermore, it has been shown that cv. Nantes  
447 carrots respond to light by decreasing proVA CAR concentration (Llorente, Martinez-Garcia,  
448 Stange, & Rodriguez-Concepcion, 2017) while there is no data for the cv. Daylance cultivar.  
449 Therefore, pulsed light treatment may not necessarily be effective, and/or its effect might only  
450 be transitory, as suggested by the results of the full factorial design. Taken together, we would  
451 not yet advise the use of this external stimulant to improve proVA CAR concentrations in  
452 stored carrots. Nevertheless, the data obtained suggest that this factor has a great potential to  
453 improve proVA CAR concentrations in stored carrots, and further studies are required to obtain  
454 a reproducible and lasting effect.

455         The last part of this study was dedicated to assess whether higher concentrations of  
456 proVA CAR induced by modifications of post-harvest factors during storage could lead to  
457 higher quantities of bioaccessible proVA CAR. In order to answer this question, we compared  
458 proVA CAR bioaccessibility in the carrots stored for 6 days at 20°C with the carrots stored for  
459 3 days at the same temperature. The results obtained, i.e. the observation that the carrots stored  
460 6 days had higher proVA CAR bioaccessibility than the carrots stored 3 days, confirm that  
461 higher apparent concentrations in proVA CAR translate in higher quantities of bioaccessible  
462 proVA CAR, although the mechanism(s) are not yet known.

463 In summary, pulsed light treatment, storage temperature and storage time can  
464 independently and additively improve apparent proVA CAR concentrations in carrots.  
465 Nevertheless, in our experiments the effect of pulsed light was only transitory and non-  
466 reproducible in different carrot cultivars, indicating that further studies are required to  
467 overcome inconsistencies in these results. Currently, we suggest that the easiest and most  
468 sustainable way to improve the quantity of bioaccessible proVA CAR in whole stored carrots is  
469 to manipulate storage temperature and storage time. More precisely, we suggest that it is better  
470 to store whole carrots at the highest possible temperature to best improve apparent proVA CAR  
471 concentration. Obviously, the temperature should not be too high to avoid decreasing  
472 organoleptic properties, and avoid carrot rotting. Therefore, we recommend room temperature,  
473 e.g. 20 °C to 25 °C, rather than cool temperatures, e.g. 4 °C to 8 °C. Concerning the storage  
474 duration, these results suggest it is best to store the whole carrot between 1 and 2-3 weeks after  
475 harvest. Indeed, it has been shown that carrots were marketable for 18 days when stored  
476 between 17 °C and 21°C (Negi & Roy, 2000). Obviously, the longer the storage duration, the  
477 lower the storage temperature should be to avoid negative organoleptic effects and carrot  
478 spoilage.

479 **Abbreviations:** carotenoid (CAR), provitamin A (proVA), surface response curve (SRC).

480

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489 **Credit author statement:**

490 **Faiza Hammaz:** Conceptualization, Formal analysis, Investigation, Visualization. **Patrick**

491 **Borel:** Conceptualization, Methodology, Formal analysis, Resources, Writing - Original Draft,

492 Visualization, Supervision, Project administration, Funding acquisition. **Florence Charles:**

493 Conceptualization, Methodology, Validation, Investigation, Resources, Writing - Review &

494 Editing. **Charlotte Halimi:** Validation, Resources. **Salah Fgaier:** Investigation. **Jawad**

495 **Aarrouf:** Conceptualization, Resources, Writing - Review & Editing. **Laurent Urban:**

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498

499 **Conflicts of interest:**

500 None of the authors reported a potential conflict of interest.

501

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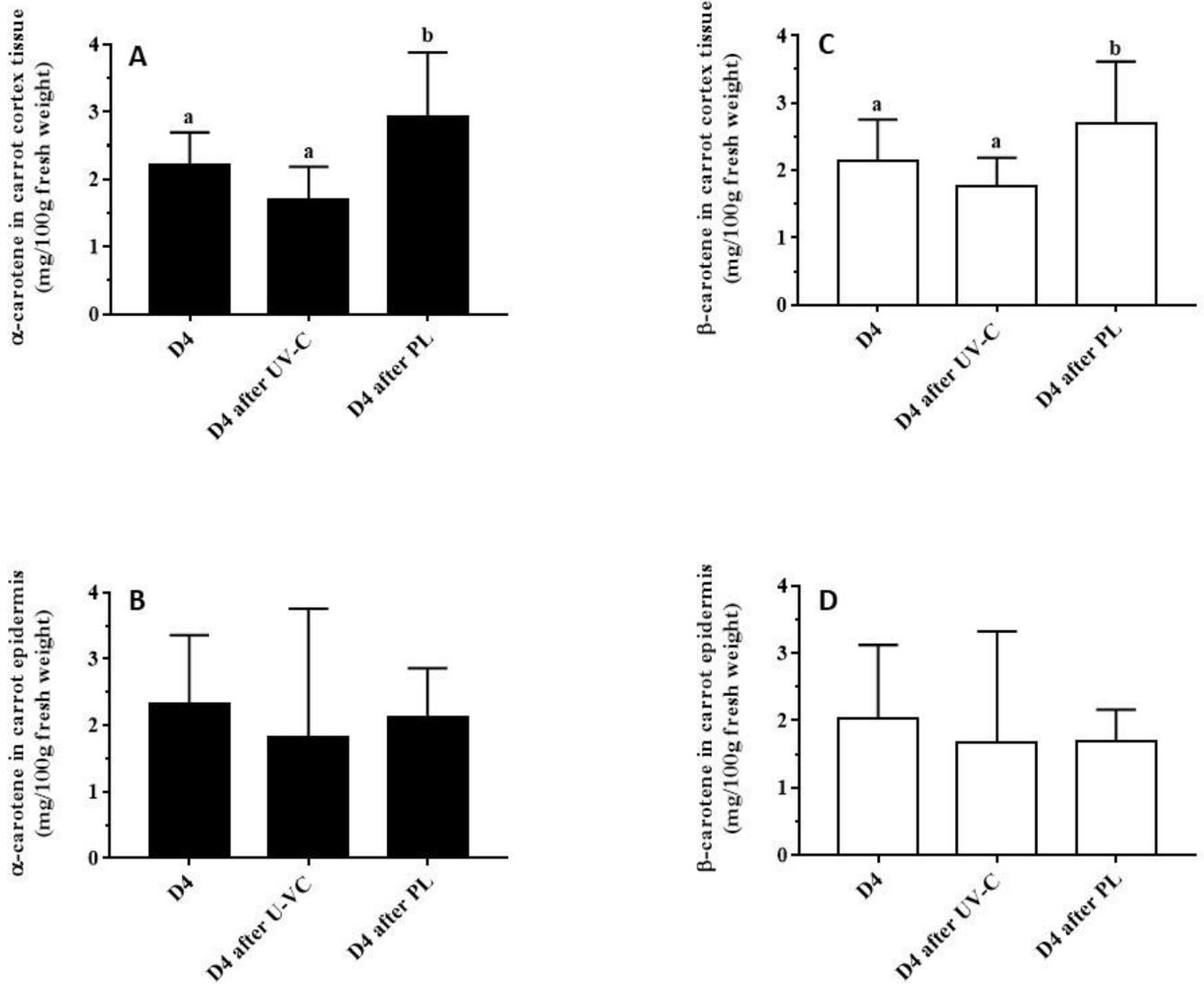
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610

**Figure 1**

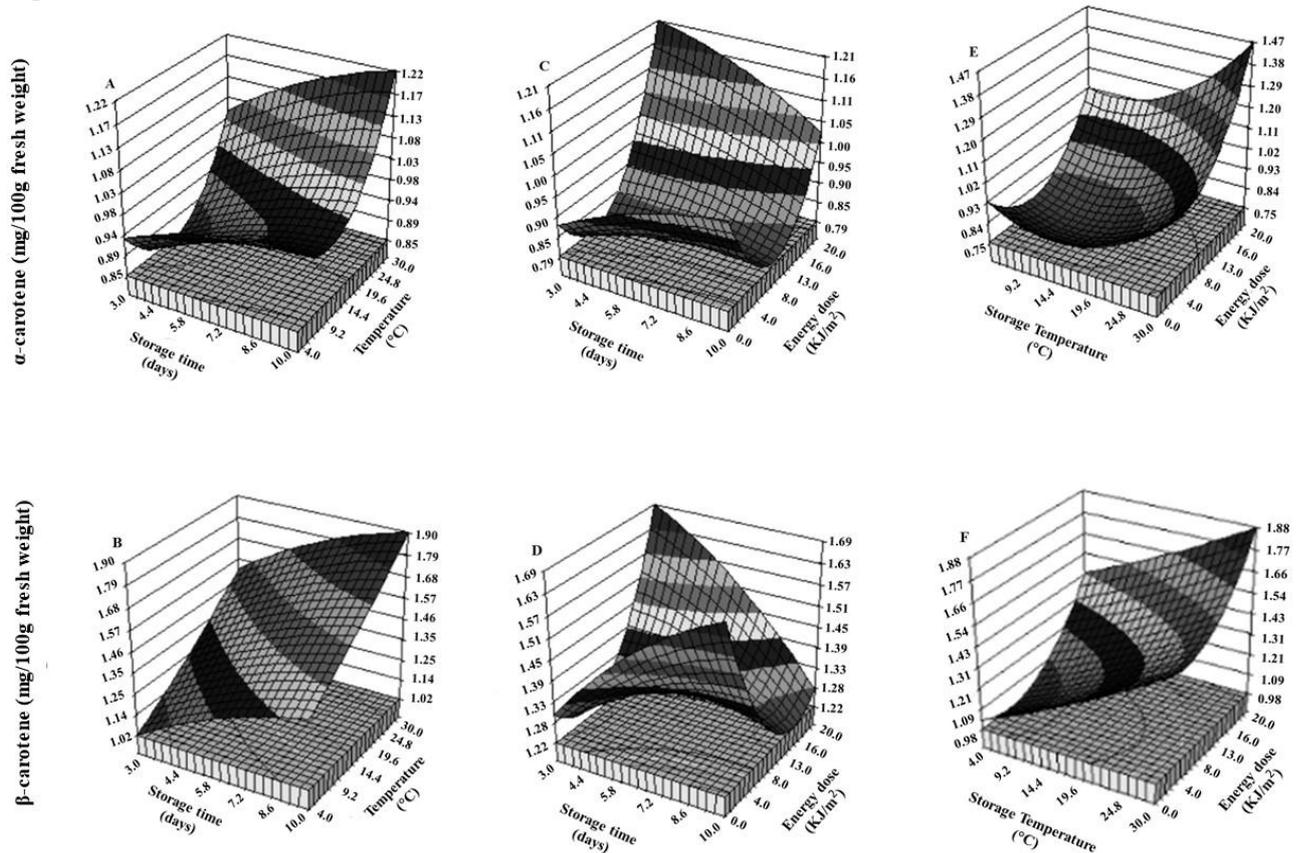


612

613 **Figure 1: Effect of UV-C and pulsed light treatments on  $\alpha$ -carotene and  $\beta$ -carotene**  
 614 **concentrations in cortex and epidermis of carrots (cv. Daylance) stored at 20 °C. A: cortex**  
 615 **tissue  $\alpha$ -carotene concentration. B: epidermis  $\alpha$ -carotene concentration. C: cortex tissue  $\beta$ -**  
 616 **carotene concentration. D: epidermis  $\beta$ -carotene concentration. D4: after 4 days of storage. D4**  
 617 **after UV-C: carrots irradiated with UV-C light and then stored 4 days. D4 after PL: carrots**  
 618 **irradiated with pulsed light and then stored 4 days. Bars represent mean  $\pm$  SEM of values**

619 measured in 5 carrots from which  $\alpha$ -carotene and  $\beta$ -carotene were extracted and measured 3  
 620 times by HPLC, i.e. 3 technical replicates. In each figure, means that bear different superscript  
 621 letters are significantly different from one another ( $p < 0.05$ ; ANOVA followed by Tukey-  
 622 Kramer post-hoc test).

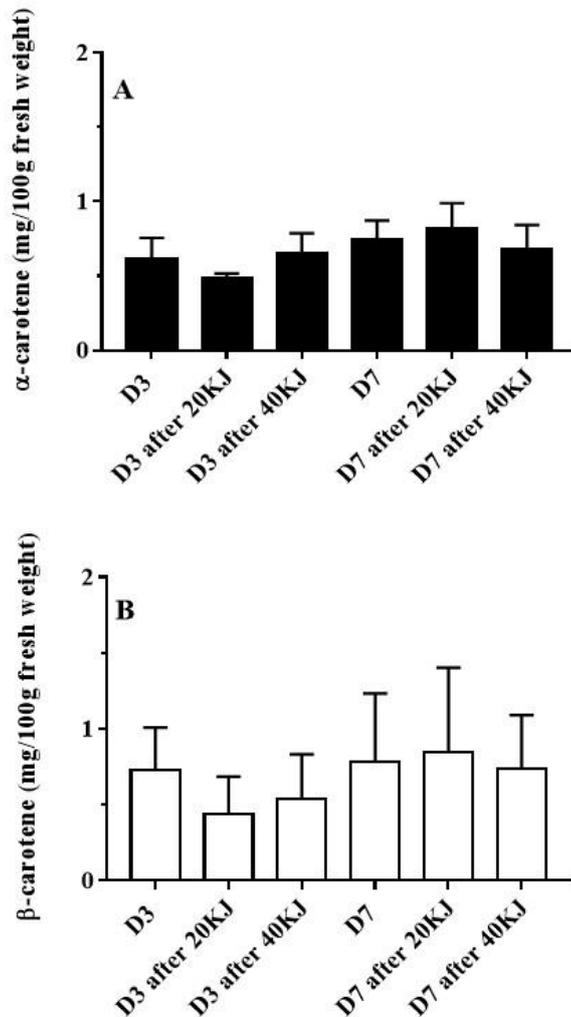
**Figure 2**



623  
 624  
 625 **Figure 2: Pairwise surface response curves of  $\alpha$ - and  $\beta$ -carotene concentrations in stored**  
 626 **(cv. Daylance) carrots as a function of storage time, storage temperature and pulsed light**  
 627 **dose. A, C and E:  $\alpha$ -carotene concentrations. B, D and F:  $\beta$ -carotene concentrations. Results on**  
 628 **the effect of storage time and energy dose of pulsed light (C and D) were obtained for a storage**  
 629 **temperature of 20 °C. Results on the effect of storage temperature and energy dose of pulsed**  
 630 **light (E and F) were obtained for a storage time of 3 days. The statistical analyses were**

631 performed on data obtained from a full factorial design experiment which consisted of 45

**Figure 3**



632 independent conditions where 5 carrots were used per condition (see Materials & Methods).

633 The surface response curves were generated by the statistical software.

634

635 **Figure 3: Effect of pulsed light dose and storage time at 20 °C on  $\alpha$  and  $\beta$ -carotene**

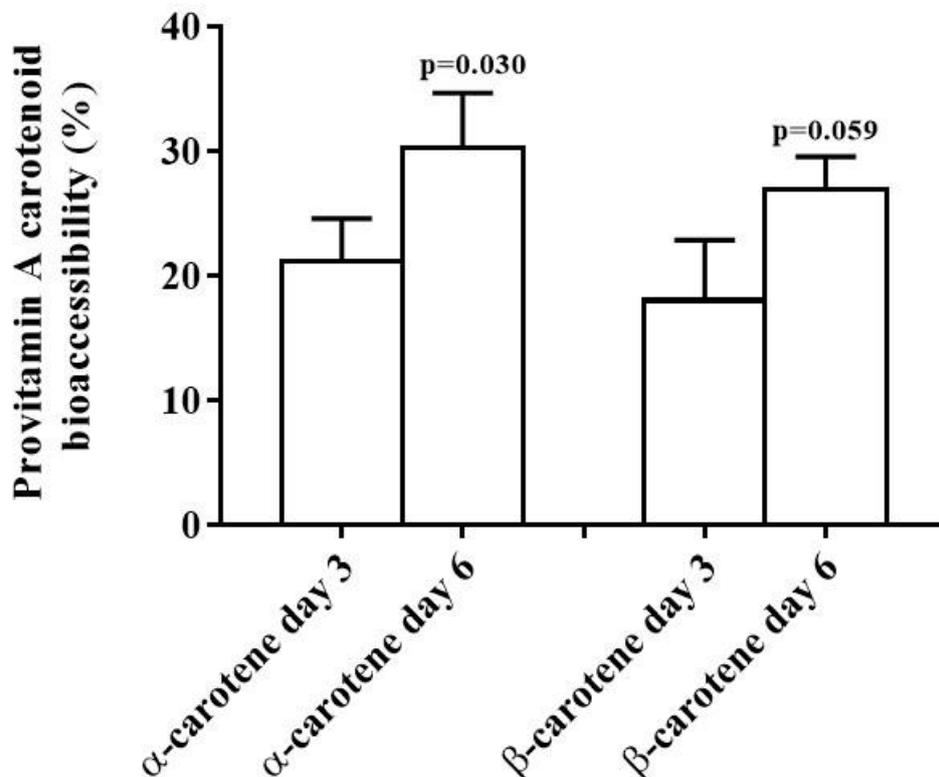
636 **concentrations in stored carrots (cv. Nantes).** D3: after 3-day storage. D3 after 20 kJ/m<sup>2</sup>:

637 carrots irradiated with 20 kJ/m<sup>2</sup> pulsed light and then stored 3 days. D3 after 40 kJ/m<sup>2</sup>: carrots

638 irradiated with 40 kJ/m<sup>2</sup> pulsed light and then stored 3 days. D7: after 7-day storage. D7 after

639 20 kJ/m<sup>2</sup>: carrots irradiated with 20 kJ/m<sup>2</sup> pulsed light and then stored 7 days. D7 after 40  
640 kJ/m<sup>2</sup>: carrots irradiated with 40 kJ/m<sup>2</sup> pulsed light and then stored 7 days. Bars represent  
641 means  $\pm$  SEM (n=5). For each experiment, the p-value of Fisher's test was no significant.

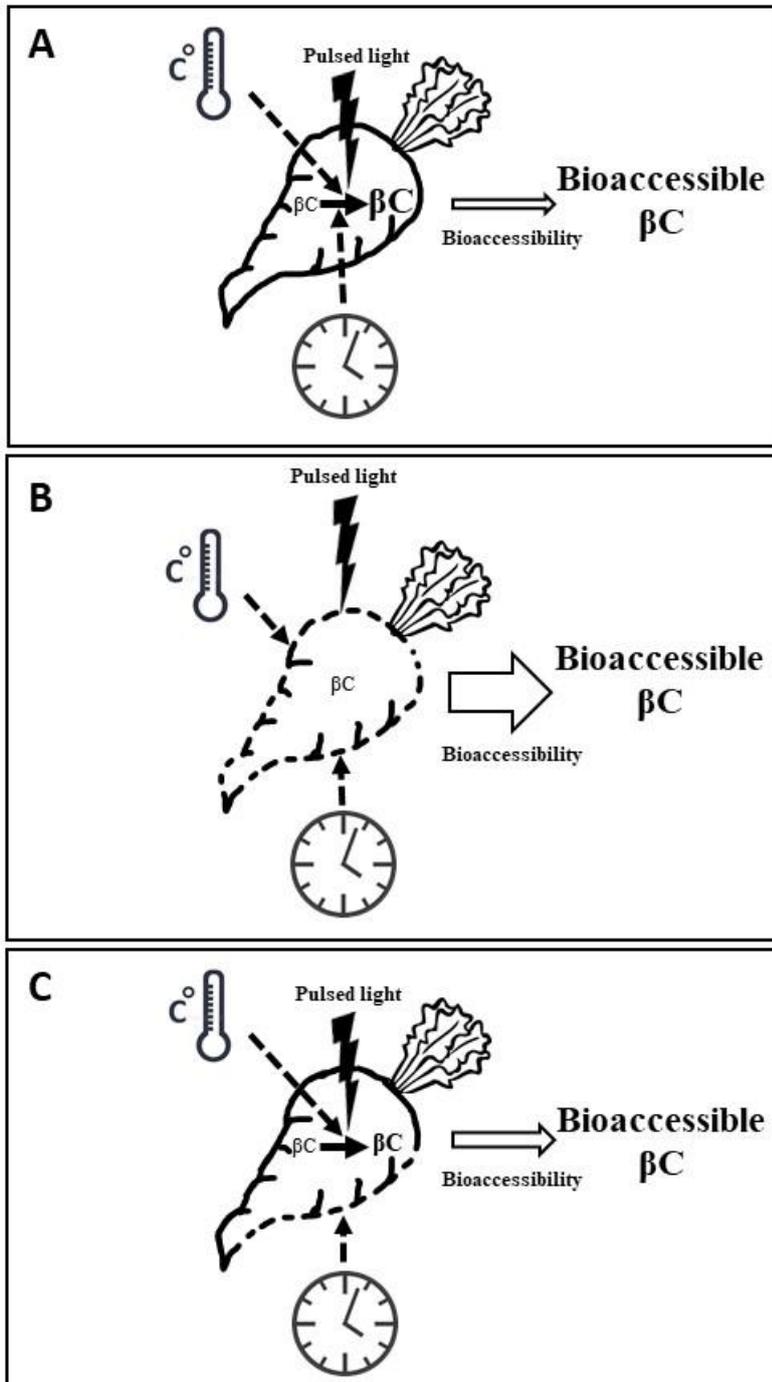
**Figure 4**



642  
643 **Figure 4: ProVA CAR bioaccessibility in carrots stored either 3 or 6 days at 20 °C.** ProVA  
644 CAR bioaccessibility, i.e. the quantity of proVA CAR that is transferred to mixed micelles  
645 during digestion, was estimated by using an in vitro digestion model (see Materials &  
646 Methods).  $\alpha$ - or  $\beta$ -carotene day 3:  $\alpha$ - or  $\beta$ -carotene concentration in carrots stored for 3 days.  $\alpha$ -

647 or  $\beta$ -carotene day 6:  $\alpha$ - or  $\beta$ -carotene concentration in carrots stored for 6 days. Bars represent  
648 means  $\pm$  SEM (n=5).

649



650

651 **Figure 5: Mechanistic hypotheses to explain the apparent increase in the concentration of**  
652 **proVA CAR under the effect of the various factors. For reasons of clarity the proVA CAR,**

653 i.e.  $\alpha$ - and  $\beta$ -carotene, are represented by the abbreviation  $\beta$ C. A. In this scheme it is assumed  
654 that the different factors increase the biosynthesis of proVA CAR during storage. B. In this  
655 scheme it is assumed that the different factors accelerate the degradation of the plant matrix  
656 during storage, ultimately improving the bioaccessibility of the proVA CAR. C. In this scheme  
657 both mechanisms are involved. For example, an effect of the temperature on the plant matrix  
658 and time and temperature on biosynthesis is represented, but all possible combinations of  
659 effects both on the biosynthesis and on the plant matrix are possible.

660 **Table 1:** Results (tests of between-subject effects) of the statistical analyses of the factorial  
 661 design study.

<b>Dependent variable: <math>\alpha</math>-carotene</b>					
<b>Source</b>	<b>Type I sum of squares</b>	<b>df</b>	<b>Mean square</b>	<b>F</b>	<b>p value</b>
Corrected model	9.531 <sup>a</sup>	39	0.244	2.812	0.000
Intercept	178.724	1	178.724	2056.519	0.000
Storage time (d)	0.626	2	0.313	3.603	0.030
Storage temperature (°C)	2.382	2	1.191	13.707	0.000
Pulsed light dose (kJ)	1.770	4	0.443	5.093	0.001
d x °C	0.688	3	0.229	2.640	0.052
d x kJ	1.336	8	0.167	1.922	0.061
°C x kJ	0.828	8	0.103	1.191	0.308
d x °C x kJ	1.899	12	0.158	1.821	0.050
Error	12.949	149	0.009		
Total	201.203	189			
Corrected total	22.479	188			
<b>Dependent variable: <math>\beta</math>-carotene</b>					
Corrected model	16.738 <sup>b</sup>	39	0.429	4.045	0.000
Intercept	326.855	1	326.855	3081.041	0.000
Storage time (d)	0.857	2	0.428	4.038	0.020
Storage temperature (°C)	5.064	2	2.532	23.865	0.000
Pulsed light dose (kJ)	1.713	4	0.428	4.037	0.004

d x °C	1.303	3	0.434	4.094	0.008
d x kJ	2.439	8	0.305	2.873	0.005
°C x kJ	1.990	8	0.249	2.345	0.021
d x °C x kJ	3.373	12	0.281	2.649	0.003
Error	15.170	143	0.106		
Total	358.763	183			
Corrected total	31.908	182			

662 <sup>a</sup>R squared = 0.424 (adjusted R squared = 0.273). <sup>b</sup>R squared = 0.525 (adjusted R squared =  
663 0.395). Statistical analyses were based on the general linear model and used ANOVA.  
664 Independent variables were storage time (days), storage temperature (°C) and doses of pulsed  
665 light (kJ). Response variables were  $\alpha$ - and  $\beta$ -carotene concentrations in carrots.