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Abstract

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

Keywords: β-carotene; α-carotene; vitamin A deficiency; UV-C light; pulsed light; bioavailability.

Chemical compounds studied in this article:

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

1) Introduction

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

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

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

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

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

2) Materials and methods

Chemicals.

HPLC grade methanol, methyl tert-butyl ether (MTBE), dichloromethane, ethanol, n-hexane, as well as HPLC grade H_2O, were purchased from Carlo Erba reagents (Val de Reuil, France). HPLC standards including α-carotene, β-carotene and echinenone>95%, and digestive enzymes used in the *in vitro* digestion (i.e. pepsin 800-2.500 U/mg protein, pancreatin, and bile extract porcine), were obtained from Sigma Aldrich (France).

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

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

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

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

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

The full factorial design experiment was conducted on the *Daylance* variety of orange carrots. Carrots were sorted and divided into 45 homogeneous batches, i.e. homogeneous in size, diameter, and color. Pulsed light experiments were conducted with the same equipment as described above, but with different energy doses per flash. The first untreated batch represented the control, the second batch was treated with 2 kJ/m^2 per flash; the third batch with 5 kJ/m^2 per flash, the fourth batch with 10 kJ/m^2 per flash; and the last batch with 20 kJ/m^2 per flash. Due to the setting, only the central part in length of the carrots (approximately 10 cm) was irradiated. After treatment, carrot batches were stored on non-hermetic plastic trays (above absorbent paper saturated with 20 mL of water) in a dark chamber at different temperatures, i.e. +4°C, +20°C and +30°C, and for different durations, i.e. 3, 6 or 10 days. At the end of each 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^2. Carrots were treated with 1, 2, or 3 flashes
171 to obtain 20, 40, or 60 kJ/m^2, 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 $NaHCO_3$ 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 (α-carotene and β-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 (α- carotene and β-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: H2O) (Gleize, Steib, Andre, &
211 Reboul, 2012) at a flow rate of 1 $mL.min^{-1}$, 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 ± 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. α- and β-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.

3) Results

Effect of UV-C and pulsed light treatments on α-carotene concentrations.

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

Effect of UV-C and pulsed light treatments on β-carotene concentration.

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

Effect of storage time and temperature on proVA CAR concentration.

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

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

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

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

corner of the **figure 2C** SRC). Concerning β-carotene, the negative influence of storage time on the positive effect that the pulsed light treatment had was dramatic (**Figure 2D)**. The concentration of β-carotene measured 10-days after 20 kJ/m^2 treatment (back right of the SRC) was significantly lower than the β-carotene concentration measured 10-days after no pulsed light treatment (front right of the SRC).

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

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

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

Because results presented in **Figures 2B** and **2C** showed that the maximal effect of pulsed light was observed 3 days after treatment with the maximal dose used in this experiment, we decided to assess whether higher doses of pulsed light can further increase the concentrations in proVA CAR. Therefore, pulsed light doses of 20, 40, and 60 kJ/m^2 were applied and carrots were stored up to 7 days to assess whether we again observed an increase of proVA CAR after 3 days and a decrease between 3 and 7 days. The results of this experiment are shown in **Figure 3**. Note that the effects of the 60 kJ/m^2 dose are not shown because carrot epidermis appeared burnt 3 days after this treatment and thus we did not continue to make experiments with this condition. Conversely to what was observed in the previous experimental design (**Figures 2B** and **2C**), irradiating another variety of carrots with 20 kJ/m^2 pulsed light 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 kJ/m^2) on these concentrations. Similar results were
305 observed for α-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 °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 α-carotene (p=0.03) and 26.9% vs. 18.4% for β-carotene (p=0.059), respectively.

4) Discussion

In the first part of this study, we aimed to assess whether a short treatment with two artificial light sources, i.e. UV-C and pulsed light, can significantly increase proVA CAR concentrations in whole intact carrots stored in a temperate environment, i.e. 20 °C. Because data from the literature suggest that both UV-C and pulsed light irradiation can increase the concentration of several phytochemical species in various fruits and vegetables (Fgaier, de Almeida Lopes, de Oliveira Silva, Aarrouf, & Urban, 2019; Urban, Charles, de Miranda, & Aarrouf, 2016), we started by comparing the effect of these two irradiation methods. Because there are few studies on the effects of artificial light on proVA CAR content in whole carrots, and because data obtained in other crops have observed effects at very different energy levels, i.e. between 0.6 and 30 kJ/m² (Aguiló-Aguayo, Gangopadhyay, Lyng, Brunton, & Rai, 2017; Lopes, et al., 2016; Pataro, Sinik, Capitoli, Donsì, & Ferrari, 2015), we chose an intermediate energy dose, i.e. 5 kJ/m^2. The results obtained herein clearly show that UV-C treatment failed to modify the concentrations in our targeted micronutrients, i.e. α- and β-carotene. We acknowledge that we assessed the effect of only one dose of UV-C, i.e. 5 kJ/m^2, which was taken from previous studies showing that it led to an increase in CAR concentration in tomato (Bravo, et al., 2013; Liu, Zabaras, Bennett, Aguas, & Woonton, 2009), and it is possible that other doses would have given different results. Indeed, a lower dose of UV-C (0.78 kJ/m^2) increased proVA CAR content in carrots (Alegria, Pinheiro, Duthoit, Gonçalves, Moldão-Martins, & Abreu, 2012), but peeled carrots were treated, and the proVA CAR content was measured in shredded carrots that were first stored at 0 °C and then at 5 °C. In short, these conditions were very different from the conditions described herein that aimed to mimic what could happen in retail supermarkets. In agreement with a recent study (Aguiló-Aguayo, Gangopadhyay, Lyng, Brunton, & Rai, 2017), we observed that pulsed light treatment induced

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

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

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

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

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

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

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

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

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

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

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

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

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Credit author statement:

Faiza Hammaz: Conceptualization, Formal analysis, Investigation, Visualization. **Patrick Borel:** Conceptualization, Methodology, Formal analysis, Resources, Writing - Original Draft, Visualization, Supervision, Project administration, Funding acquisition. **Florence Charles:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing - Review & Editing. **Charlotte Halimi:** Validation, Resources. **Salah Fgaier:** Investigation. **Jawad Aarrouf:** Conceptualization, Resources, Writing - Review & Editing. **Laurent Urban:** Conceptualization, Methodology, Resources, Writing - Review & Editing. **Rachel E. Kopec**, Writing - Review & Editing.

Conflicts of interest:

None of the authors reported a potential conflict of interest.

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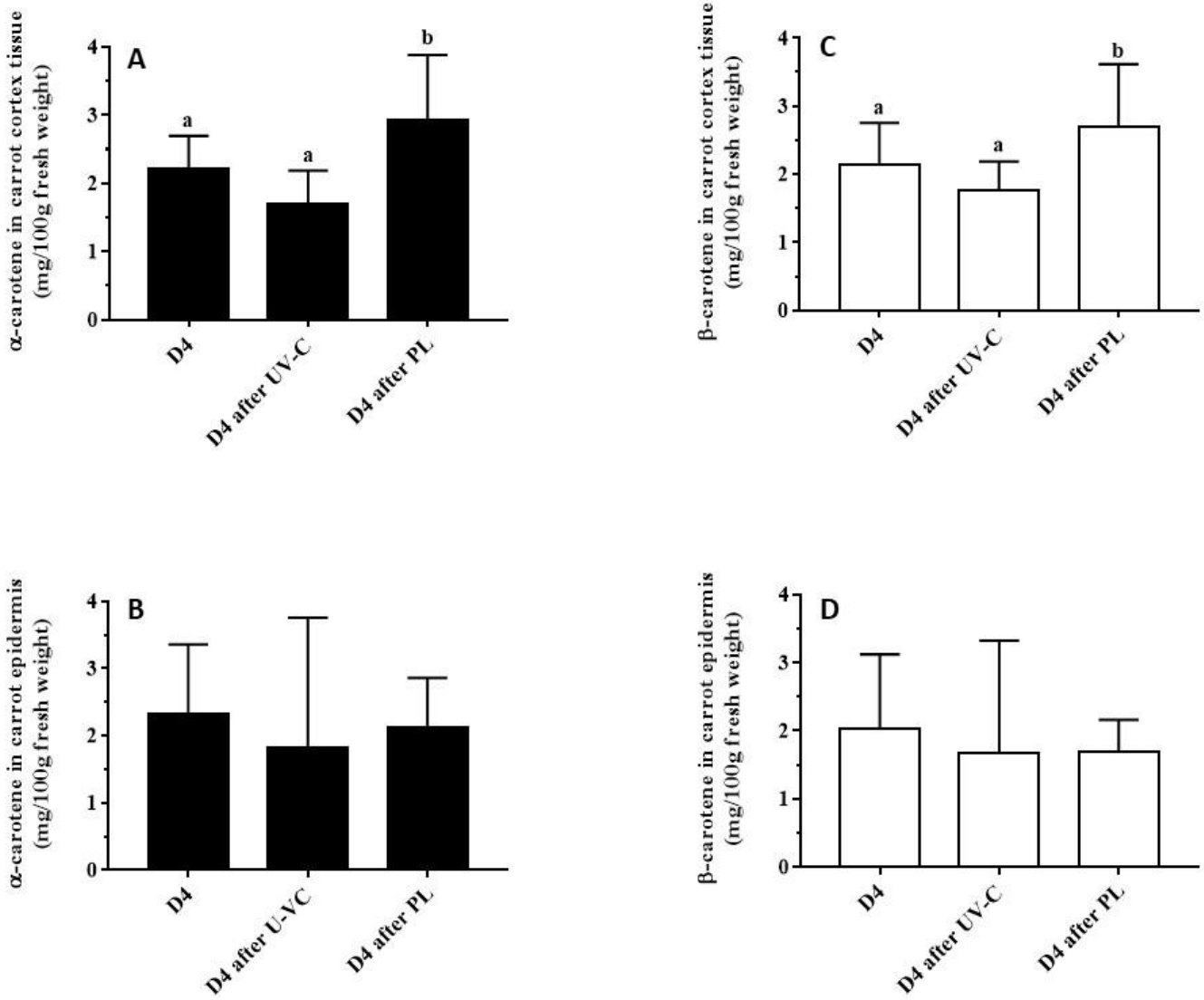
610

611 **Figures**

Figure 1



612

613 **Figure 1: Effect of UV-C and pulsed light treatments on α-carotene and β-carotene**
614 **concentrations in cortex and epidermis of carrots (cv. Daylance) stored at 20 °C.** A: cortex
615 tissue α-carotene concentration. B: epidermis α-carotene concentration. C: cortex tissue β-
616 carotene concentration. D: epidermis β-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 ± SEM of values

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

Figure 2

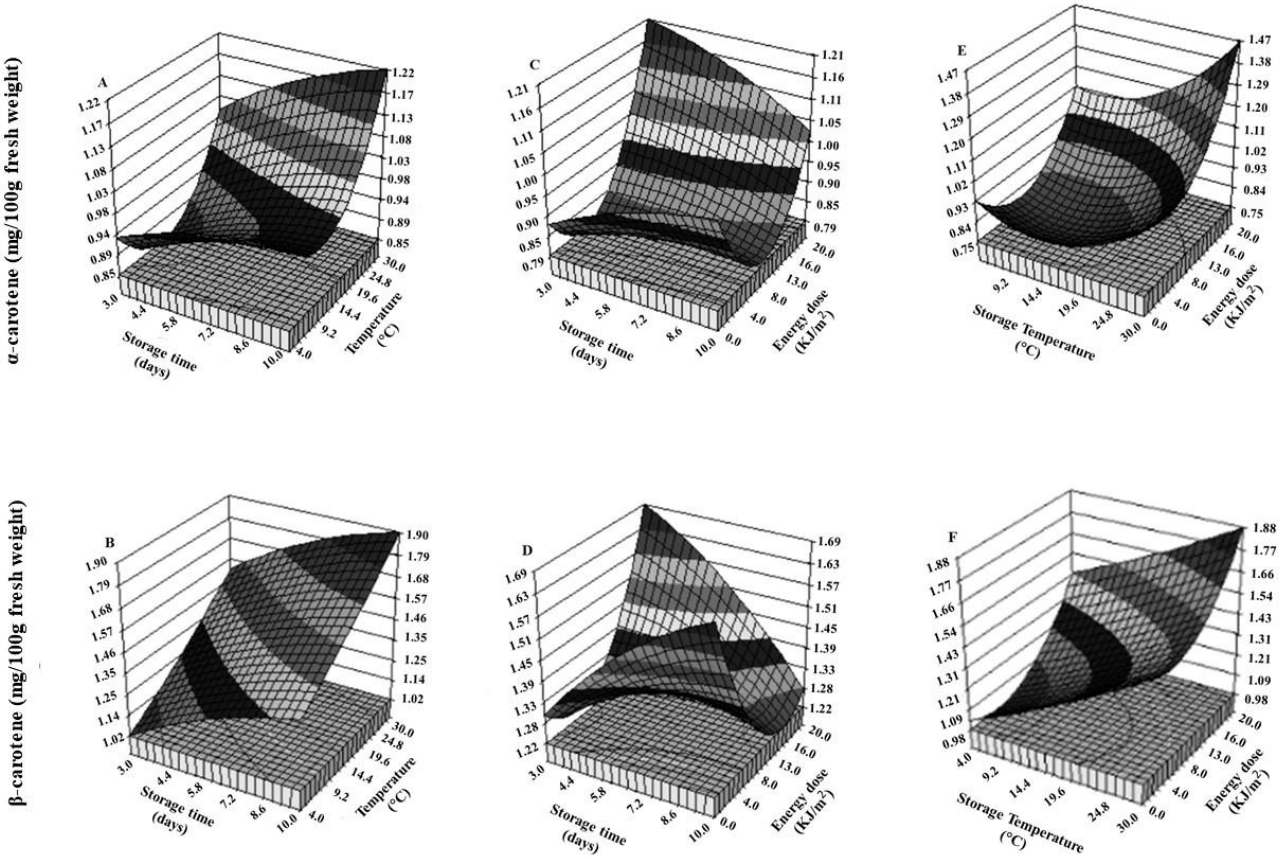
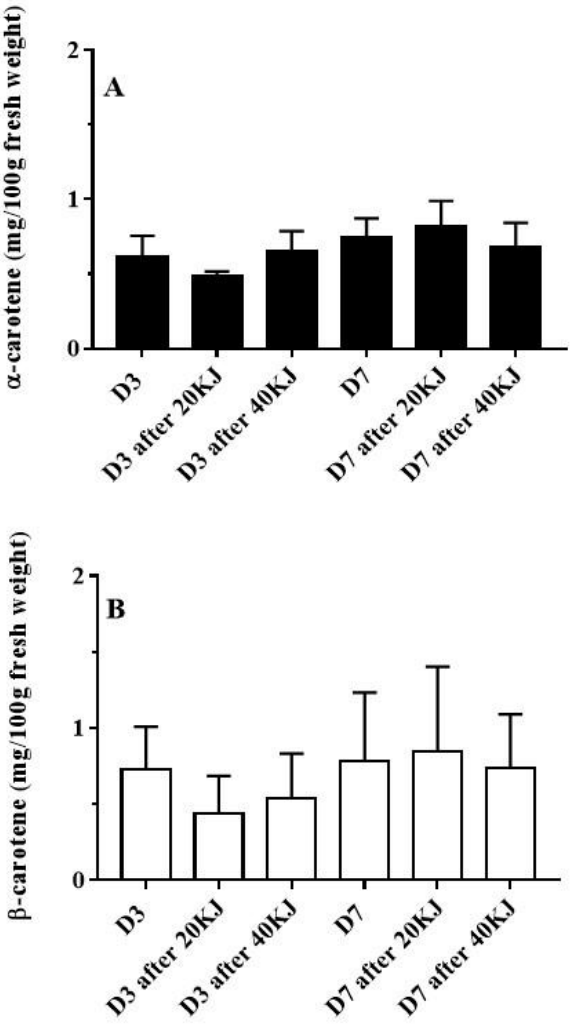


Figure 2: Pairwise surface response curves of α- and β-carotene concentrations in stored (cv. Daylance) carrots as a function of storage time, storage temperature and pulsed light dose. A, C and E: α-carotene concentrations. B, D and F: β-carotene concentrations. Results on the effect of storage time and energy dose of pulsed light (C and D) were obtained for a storage temperature of 20 °C. Results on the effect of storage temperature and energy dose of pulsed 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 α and β-carotene**
636 **concentrations in stored carrots (cv. Nantes).** D3: after 3-day storage. D3 after 20 kJ/m^2:
637 carrots irradiated with 20 kJ/m^2 pulsed light and then stored 3 days. D3 after 40 kJ/m^2: carrots
638 irradiated with 40 kJ/m^2 pulsed light and then stored 3 days. D7: after 7-day storage. D7 after

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

Figure 4

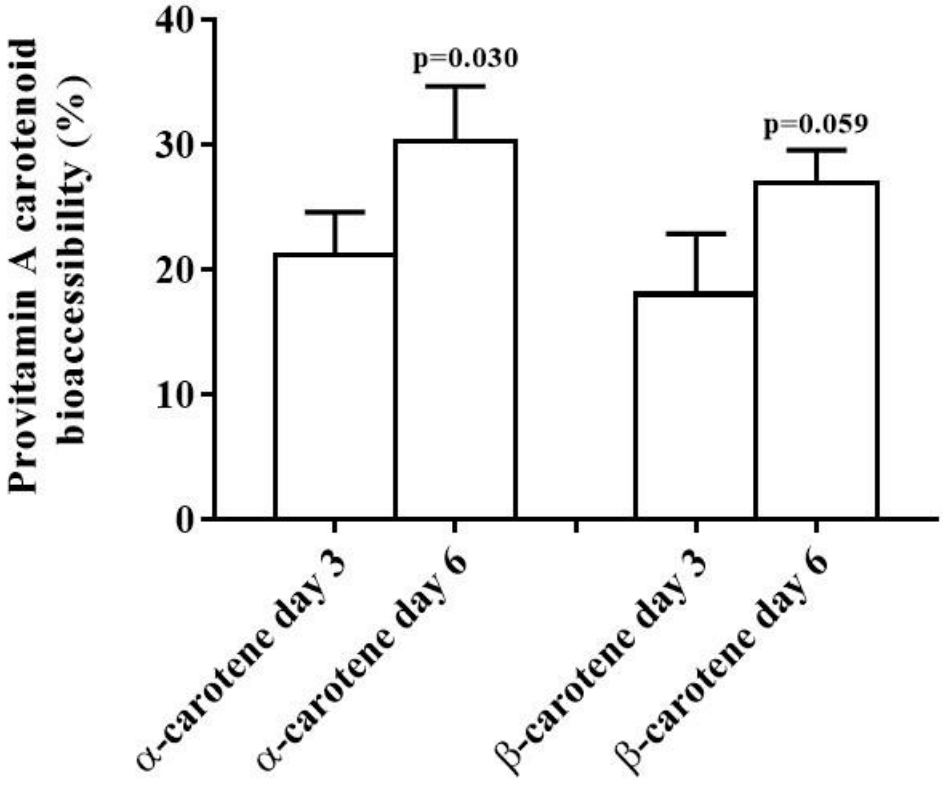


Figure 4: ProVA CAR bioaccessibility in carrots stored either 3 or 6 days at 20 °C. ProVA CAR bioaccessibility, i.e. the quantity of proVA CAR that is transferred to mixed micelles during digestion, was estimated by using an in vitro digestion model (see Materials & Methods). α- or β-carotene day 3: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

or β-carotene day 6: α- or β-carotene concentration in carrots stored for 6 days. Bars represent means ± SEM (n=5).

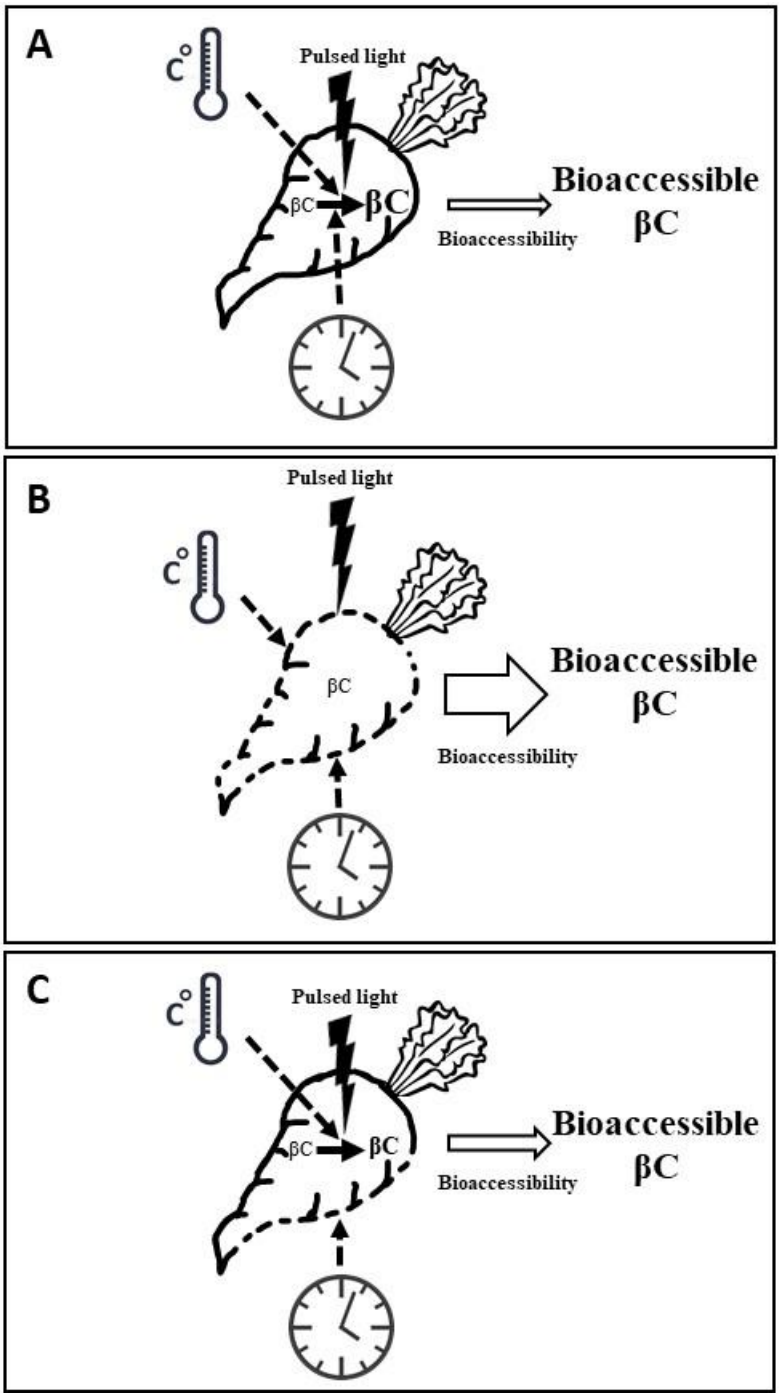


Figure 5: Mechanistic hypotheses to explain the apparent increase in the concentration of proVA CAR under the effect of the various factors. For reasons of clarity the proVA CAR,

i.e. α- and β-carotene, are represented by the abbreviation βC. A. In this scheme it is assumed that the different factors increase the biosynthesis of proVA CAR during storage. B. In this scheme it is assumed that the different factors accelerate the degradation of the plant matrix during storage, ultimately improving the bioaccessibility of the proVA CAR. C. In this scheme both mechanisms are involved. For example, an effect of the temperature on the plant matrix and time and temperature on biosynthesis is represented, but all possible combinations of 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.

Dependent variable: α-carotene					
Source	**Type I sum of squares**	**df**	**Mean square**	**F**	**p value**
Corrected model	9.531[a]	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			
Dependent variable: β-carotene					
Corrected model	16.738[b]	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			

[a]R squared = 0.424 (adjusted R squared = 0.273). [b]R squared = 0.525 (adjusted R squared = 0.395). Statistical analyses were based on the general linear model and used ANOVA. Independent variables were storage time (days), storage temperature (°C) and doses of pulsed light (kJ). Response variables were α- and β-carotene concentrations in carrots.