

1 **Melatonin content of pepper and tomato fruits: effects of cultivar**  
2 **and solar radiation**

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16 **Running title:** Melatonin content of pepper and tomato fruits

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18

19 **ABSTRACT**

20 We evaluated the effect of cultivar and solar radiation on the melatonin content of  
21 *Capsicum annuum* (pepper) and *Solanum lycopersicum* (tomato) fruits. The  
22 melatonin content of red pepper fruits ranged from 31 to 93 ng g<sup>-1</sup> (dry weight). The  
23 melatonin content of tomato ranged from 7.5 to 250 ng g<sup>-1</sup> (dry weight). We also  
24 studied the effect of ripeness on melatonin content and identified one group of  
25 pepper cultivars in which the melatonin content increased as the fruit ripened and  
26 another in which it decreased as the fruit ripened. Under shade conditions, the  
27 melatonin content in most of tomato cultivars tended to increase (up to 135%),  
28 whereas that of most pepper cultivars decreased (to 64%). Overall, the results also  
29 demonstrated that the melatonin content of the fruits was not related to carbon fluxes  
30 from leaves.

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32

33 *Keywords:* *N*-acetyl-5-methoxytryptamine, *Capsicum annuum*, *Solanum*  
34 *lycopersicum*, UHPLC-MS/MS.

35

36 **1. Introduction**

37

38 Plants can synthesize melatonin (*N*-acetyl-5-methoxytryptamine), a methoxylated  
39 indolamine (Murch, KrishnaRaj, and Saxena, 2000). Although the physiological  
40 functions of melatonin (MEL) in plants remain to be definitively established, some  
41 functional roles have recently been proposed (Tan, Hardeland, Manchester,  
42 Korkmaz, Ma, Rosales-Corral, and Reiter, 2012). Studies have documented that  
43 MEL is as a potent free-radical scavenger in plants (Paredes, Korkmaz, Manchester,  
44 Tan, and Reiter, 2009; Tan et al., 2012; Wang, Yin, Liang, Li, Ma, and Yue, 2012)  
45 and in animals (Gitto, Pellegrino, Gitto, Barberi, and Reiter, 2009; Melchiorri,  
46 Reiter, Sewerynek, Hara, Chen, and Nistico, 1996). In addition, MEL promotes  
47 growth of roots (Arnao and Hernández-Ruiz, 2007; Sarropoulou, Therios, and  
48 Dimassi-Theriou, 2012) and leaves (Okazaki, Higuchi, Aouini, and Ezura, 2010;  
49 Wang, Sun, Li, Wei, Liang, and Ma, 2013), and it may be involved in the regulation  
50 of circadian rhythms and photoperiodic reactions (Kolar and Machakova, 2005),  
51 among other plant functions.

52 The increasing interest in plant MEL expressed by the scientific community is  
53 partly due to beneficial effects of MEL on human health. When plants containing  
54 melatonin are eaten, the melatonin is absorbed and the antioxidant capacity of the  
55 blood is elevated (Iriti, Varoni, and Vitalini, 2010; Reiter, Manchester, and Tan,  
56 2005). In addition to its antioxidant properties, MEL can modulate the immune  
57 system (Carrillo-Vico, Lardone, Alvarez-Sanchez, Rodriguez-Rodriguez, and  
58 Guerrero, 2013; Mauriz, Collado, Veneroso, Reiter, and Gonzalez-Gallego, 2013),  
59 exert both direct and indirect anticancer effects (Alvarez-Garcia, Gonzalez, Alonso-  
60 Gonzalez, Martinez-Campa, and Cos, 2012; Uguz, Cig, Espino, Bejarano, Naziroglu,

61 Rodriguez, and Pariente, 2012) act as a neurotransmitter, thus alleviating  
62 neurodegenerative disorders such as Alzheimer's disease, and modulate sleep and  
63 circadian regulating systems in humans (Rosales-Corral, Acuña-Castroviejo, Coto-  
64 Montes, Boga, Manchester, Fuentes-Broto, Korkmaz, Ma, Tan, and Reiter, 2012;  
65 Slat, Claassen, Verbeek, and Overeem, 2013). Melatonin also plays other  
66 physiological roles in mammals and is involved in, e.g., regulation of body  
67 temperature, sexual maturation, mood and cardiovascular functions (Pandi-Perumal,  
68 Zisapel, Srinivasan, and Cardinali, 2005). Healthy subjects synthesize MEL not only  
69 in the pineal gland (Stehle, Saade, Rawashdeh, Ackermann, Jilg, Sebestény, and  
70 Maronde, 2011), but also in a wide range of other organs, i.e., gastrointestinal tract,  
71 airway epithelium, pancreas, adrenal glands, thyroid gland, thymus, urogenital tract  
72 and placenta (Kvetnoy, 1999). However, individuals who are deficient in MEL must  
73 ingest it from chemical or natural sources. In a recent human study, it has been  
74 demonstrated that concentrations of urinary aMT6 (a marker of circulating MEL in  
75 the body) increased significantly after consumption of some fruits (Johns, Johns,  
76 Porasuphatana, Plaimee, and Sae-Teaw, 2012). In addition, ingestion of a tart cherry  
77 juice concentrate increased the urinary MEL levels and was beneficial in improving  
78 sleep duration and quality in adults (Howatson, Bell, Tallent, Middleton, McHugh,  
79 and Ellis, 2012). The uptake of MEL from vegetables and fruits as an alternative to  
80 the intake of synthetic MEL is therefore of increasing interest (Korkmaz, 2011).

81 MEL occurs widely in higher plants, and it has been identified and quantified in  
82 several families of both monocotyledons and dicotyledons (Paredes et al., 2009).  
83 Nevertheless, available information on the natural effects of cultivar and solar  
84 radiation on the MEL contents of edible plants is rather scarce. Some data have  
85 shown intraspecific variations in the MEL content in tomatoes (Dubbels, Reiter,

86 Klenke, Goebel, Schnakenberg, Ehlers, Schiwara, and Schloot, 1995; Sturtz, Cerezo,  
87 Cantos-Villar, and Garcia-Parrilla, 2011), strawberries (Sturtz et al., 2011) and  
88 grapes (Iriti and Faoro, 2006). The environmental conditions under which plants  
89 grow also greatly influences their MEL content, e.g., its content increased from 22 to  
90 142 ng g f.w.<sup>-1</sup> in the leaves of tomato plants grown in a controlled growing chamber  
91 and in the open field respectively (Arnao and Hernández-Ruiz, 2013). Water  
92 hyacinth (*Eichhornia crassipes* (Mart) Solms) plants grown under sunlight contain  
93 more MEL than plants grown under artificial light (Arnao and Hernández-Ruiz,  
94 2013; Tan, Manchester, Di Mascio, Martinez, Prado, and Reiter, 2007).

95 The objectives of the present study were to explore the following: the MEL  
96 content of pepper fruits (*Capsicum annuum* L.), the effect of the ripeness of pepper  
97 fruits on the MEL content, the effect of cultivar and solar radiation on MEL content  
98 in pepper and tomato fruits, and the relationship between the MEL content and dry  
99 weight (d.w.) of the fruits, to elucidate whether the MEL content is associated with  
100 the carbon flux from leaves to fruits.

101

## 102 2. Materials and methods

103

### 104 2.1. Reagents

105

106 *N*-acetyl-5-methoxytryptamine (MEL) standard was purchased from Fluka (Neu-  
107 Ulm, Germany). All LC-MS grade solvents were obtained from J. T. Baker  
108 (Phillipsburg, NJ) and formic acid was purchased from Panreac Química S.A.  
109 (Barcelona, Spain).

110

### 111 2.2. Experimental conditions

112

113 Six varieties of pepper (*Capsicum annuum* L.) and seven varieties of tomato  
114 (*Solanum lycopersicum* Mill.) were chosen from commercial hybrids and local non-  
115 hybrid cultivars, all of which displayed different morphological types (Table 1).  
116 Seeds of the local cultivars were obtained from the germplasm stored by NEIKER.  
117 All cultivars were grown in soil, in the same unheated soft polyethylene-covered  
118 greenhouse in Derio (Basque Country, northern Spain) (latitude, 43° 17' N;  
119 longitude, 2° 52' W; altitude, 65 m above sea-level). The climate in the region is  
120 Atlantic temperate. Measured air temperature (T, °C) and total cumulated radiation  
121 (Rad, Wh m<sup>-2</sup>) in the greenhouse corresponding to the harvest periods were: min. T =  
122 7.9, max. T = 49, mean T = 23.8; min Rad = 390, max. Rad = 4546 and mean Rad =  
123 2779.

124 Tomato and pepper plants with four true leaves were planted at a density of 1.7  
125 plants per m<sup>2</sup>. Plants were drip irrigated with a nutrient solution (meq l<sup>-1</sup>) containing

126 1.0 Mg<sup>2+</sup>, 1.7 K<sup>+</sup>, 3.4 NO<sub>3</sub><sup>-</sup>, and 1.0 SO<sub>4</sub><sup>2-</sup>. The electrical conductivity of the nutrient  
127 solution was 0.5 dS m<sup>-1</sup> and the pH varied between 5.0 and 5.5.

128 When the fruits were 0.5-1 cm thick, some of them were shaded with aluminium foil.  
129 The foil was placed loosely around the fruit as a skirt, which was closed at the  
130 peduncle and open at the bottom to enable air circulation and thus minimize  
131 differences in air humidity and temperature between the shaded and non-shaded fruit.  
132 However, the temperature regime experienced by the non-shaded fruits obviously  
133 differed slightly because these fruits were sometimes directly exposed to solar  
134 radiation. It is very difficult to manipulate the amount of incident solar radiation that  
135 reaches fruit without inducing changes in the microclimate. Nevertheless, we  
136 considered that the main effect on shaded fruits was the large reduction in solar  
137 radiation.

138

### 139 *2.3. Sample preparation*

140

141 Batches of mature unshaded green, shaded and unshaded light-red pepper fruits  
142 and batches of shaded and unshaded light red tomato fruits were harvested from the  
143 same plants and washed with deionised water. Each batch of fruit weighed at least  
144 1.5 kg, except the batches of cherry tomatoes, which weighed 350 g. Four batches  
145 per treatment (unshaded green, unshaded red and shaded red) were processed. Non-  
146 edible parts (peduncle, calyx, placenta and seeds in pepper fruits and peduncle and  
147 columella in tomato fruits) were discarded. The edible parts were homogenized in a  
148 conventional food blender (Type 4184 Braun, Barcelona, Spain) at speed 5 for 1  
149 minute. Two subsamples of the puree thus obtained (from 40 to 50 g each) were  
150 dried at 65 °C for 7 days (to constant weight) to determine the d.w.

151 For each batch, 30 g of puree was mixed with 30 ml of absolute methanol (HPLC  
152 grade, Lab-Scan) and homogenized in a bullet blender 50 (Next Advance, USA) with  
153 1.4 mm stainless beads, at speed 8 for 12 min. The homogenate was filtered  
154 (Whatman paper No. 4) and centrifuged (Sorvall Legend XTR, Thermo Fischer  
155 Scientific, Madrid, Spain) at 7600 x g for 15 minutes. The pellet was discarded and  
156 the supernatant was evaporated to dryness under vacuum. The residue was  
157 resuspended in a mixture of methanol and water (1:1, v:v) before analysis of MEL.  
158 All steps were carried out under dim light.

159

#### 160 2.4. UHPLC-QqQ-MS/MS analysis

161

162 MEL determination and quantification was analyzed using a UHPLC-MS/MS  
163 (UHPLC-1290 Series and a 6460 QqQ-MS/MS; Agilent Technologies, Waldbronn  
164 Germany) with an ACQUITY BEH C18 column (2.1 x 150 mm; 1.7  $\mu$ m; Waters,  
165 Milford, MA). Chromatographic separation was achieved using a binary gradient  
166 consisting of (A) water and (B) methanol as LC grade solvents, both containing 0.1%  
167 formic acid (v/v). The flow rate was 0.30 ml min<sup>-1</sup> using a linear gradient (t; %B):  
168 (0.00; 40), (1.50; 40), (1.51; 90), (3.50; 90), (3.51; 40). The volume injection was 20  
169  $\mu$ l. Multiple reaction monitoring mode (MRM) in positive mode was carried out and  
170 MEL quantification was based on the 233/216 MRM transition (Mena, Gil-  
171 Izquierdo, Moreno, Martín, and García-Viguera, 2012; Rodriguez-Naranjo, Gil-  
172 Izquierdo, Troncoso, Cantos, and Garcia-Parrilla, 2011; Rodriguez-Naranjo, Gil-  
173 Izquierdo, Troncoso, Cantos-Villar, and Garcia-Parrilla, 2011). This MRM transition  
174 was selected because of its specificity and better signal-to-noise ratio. Nitrogen was  
175 used as the collision gas for the fragmentation by collision-induced dissociation of



176 the compounds at the collision cell of the triple quadrupole mass spectrometer. Mass  
177 spectrometer parameters were set as follows: drying-gas flow: 8 min<sup>-1</sup>; sheath gas  
178 flow: 12 min<sup>-1</sup>; sheath gas temperature: 350 °C; nebulizer pressure: 30 psi; capillary  
179 voltage: 4000 V and nozzle voltage: 1000 V. MassHunter Software version B 04.00  
180 was used for MS control and data gathering and MassHunter. Software version B  
181 03.01 was used for data processing, peak integration and linear regression.

182

183 *2.5. Statistical analysis*

184

185 We used an independent-samples t test to compare means, and Duncan's test to  
186 identify homogeneous subsets of means that are not different from each other (at a  
187 significant level of 0.05). We used SSPS 10 software for all analyses.

188

### 189 3. Results and discussion

190

#### 191 3.1. Effect of pepper and tomato cultivars on MEL contents

192

193 All six pepper cultivars analysed contained quantifiable amounts of MEL. The  
194 content of this compound in red fruits ranged from 4.48 ng g<sup>-1</sup> f.w. in the Barranca  
195 cultivar to 11.90 ng g<sup>-1</sup> f.w. in the F26 cultivar (Fig. 1A). One-way analysis of  
196 variance revealed a significant effect of cultivar on MEL content (p=0.000).  
197 Duncan's test identified three distinct groups of cultivars according to their contents:  
198 the group with the lowest MEL contents (4.48-6.23 ng g<sup>-1</sup> f.w.) included the  
199 Barranca, NC9, Derio and Velero cultivars; the group containing intermediate  
200 amounts of MEL (6.23-7.72 ng g<sup>-1</sup> f.w.) included Velero and Cristal and the group  
201 containing the highest amounts of MEL comprised the F26 cultivar. On a d.w. basis,  
202 its content ranged from 31.01 ng g<sup>-1</sup> d.w. to 93.40 ng g<sup>-1</sup> d.w. in Barranca and F26  
203 respectively (Fig. 1B).

204 MEL contents in samples of the seven cultivars of red tomato fruits ranged from  
205 0.64 ng g<sup>-1</sup> f.w. (Ciliegia) to 14.77 ng g<sup>-1</sup> f.w. (Optima) (Fig. 2A). The one-way  
206 analysis of variance revealed a significant effect of these cultivars on MEL content  
207 (p=0.000). On a d.w. basis, its contents ranged from 7.47 ng g<sup>-1</sup> d.w. (Ciliegia) to  
208 249.98 ng g<sup>-1</sup> d.w. (Optima) (Fig. 2B).

209 Although a lower MEL content has been reported for mature red tomato fruit (3-  
210 17 pg g<sup>-1</sup> f.w.) (Van Tassel, Roberts, Lewy, and O'Neill, 2001) and for pepper fruits,  
211 from 25.5 to 581 pg g<sup>-1</sup> f.w. (Huang and Mazza, 2011), the content in pepper and  
212 tomato fruits reported here is consistent with the values recently found for eleven  
213 varieties of tomato (4.1-114.5 ng g<sup>-1</sup> f.w.) (Sturtz et al., 2011). These authors

214 attributed the differences to the newly developed analytical method used. The latter  
215 data revealed intraspecific differences of MEL contents in fruit among members of *L.*  
216 *esculentum*, as previously suggested by Dubbels et al. (1995) and among members of  
217 *Prunus cerasus* (Burkhardt, Tan, Manchester, Hardeland, and Reiter, 2001).  
218 Nevertheless, our data confirm the important effect of cultivars on the MEL content  
219 per unit of d.w. of pepper and tomato fruits. Dry weight is a more reliable measure  
220 than the fresh weight (f.w.) because the water content of a plant or plant part will  
221 obviously depend on several different factors (variety, the amount of water available  
222 in soils, climatic conditions, time elapsed between harvest and analysis, and position  
223 of fruit within the plant). Choice of the unit can also considerably modify the results.  
224 For example, on a f.w. basis, the Optima cultivar contained 23 times more MEL than  
225 *Ciliegia*, but on a d.w. basis, the difference was 33.5-fold, due to the differences on  
226 their d.w. values. However, from a nutritional point of view, data are usually  
227 presented on a f.w. basis, as this enables calculation of the contribution of the edible  
228 part of a fruit or vegetable to the amount of this neurohormone in the human diet.

229 To explore the possible relationships between biomass allocation to the fruit and  
230 MEL content, the pepper and tomato cultivars were chosen to yield a wide range of  
231 fruit d.w. (%), from about 10 to 16% in pepper and from about 6 to 12% in tomato.  
232 There was no correlation between MEL content and fruit d.w. in either tomatoes or  
233 peppers (Fig. 3). Therefore, its allocation in fruit does not appear to be related to the  
234 carbon fluxes from leaves. Like other metabolites, the MEL content in fruit depends  
235 on its uptake from phloem vessels, the extent to which it is degraded and how it is  
236 metabolized. As far as we know, the possibility that MEL could be synthesized in  
237 fruit has not previously been considered. However, the following have led us to  
238 suggest that tomato fruit may biosynthesize MEL: the content in tomatoes harvested

239 at the mature green stage and allowed to ripen under controlled conditions increased  
240 (Van Tassel et al., 2001) and there is some evidence that mitochondria and  
241 chloroplasts (intracellular organelles present in green fruits) have the capacity to  
242 synthesize MEL *in situ* (Tan, Manchester, Liu, Rosales-Corral, Acuna-Castroviejo,  
243 and Reiter, 2013).

244

### 245 3.2. Effect of the stage of ripeness of pepper fruit on MEL contents

246

247 Except for the Velero pepper cultivar, the MEL content per fresh or d.w. units  
248 tended to increase from mature green to red fruits. However, these differences were  
249 only statistically significant in Barranca and F26 (Fig. 4 A and B), and there were no  
250 such significant differences in the Derio and NC9 cultivars. The opposite pattern was  
251 observed in Velero cultivar, in which the MEL contents in red fruits were lower than  
252 those of mature green fruits. Therefore, the stage of ripeness in *Capsicum annuum*  
253 fruit plays an important role in the final MEL content, which is consistent with  
254 previous data observed in tomatoes (Okazaki and Ezura, 2009; Van Tassel et al.,  
255 2001), i.e. that mature green fruits contained the lowest amounts and red tissue the  
256 highest amounts of MEL. In the current study, we identified two types of pepper  
257 cultivars: those in which the MEL content increased concomitantly with the  
258 maturation degree (Barranca, F26 and NC9 although not significant) and in others  
259 two cultivars where its content decreased (Velero and Derio although not  
260 significant). The present results therefore demonstrate that the effect of the stage of  
261 ripeness on MEL contents in pepper fruit is not straightforward and strongly depends  
262 on the genotype.

263

264 3.3. *Effect of the shading treatment on MEL contents in pepper and tomato fruits*

265

266 Shaded tomato fruits contained more MEL than non-shaded fruits, except in the  
267 Optima and Ciliegia cultivars. The content in the shaded fruits of Optima was lower  
268 than that of control fruits, whereas that of the shaded fruit of Ciliegia did not differ  
269 from the control fruit (Fig. 5A). In contrast, in most pepper cultivars, the MEL  
270 content of shaded fruit was lower than that shown for non-shaded fruit, whereas the  
271 shading treatment did not affect the content in Barranca fruits and increased it in  
272 Velero fruits (Fig. 5B). Therefore, it appears that the shading treatment had different  
273 effects in these plants, leading to an increase of the MEL content in the tomatoes  
274 cultivars and decrease in the pepper cultivars.

275 The differences in MEL contents between shaded and control fruits did not  
276 correlate with the differences in fruit d.w. (Table 2). For most of the pepper and  
277 tomato cultivars, the shading treatment induced a decrease in the d.w. of the fruits,  
278 which is consistent with previous findings that the d.w. tomato fruit tends to decrease  
279 linearly with decreasing incident solar radiation (Riga, Anza, and Garbisu, 2008).  
280 The present results demonstrate that the MEL contents of the fruits were not related  
281 to the carbon fluxes from leaves, as shown above from the analysis of the  
282 relationship between the MEL content in red fruit of each cultivar and respect to its  
283 d.w. (Fig. 3).

284 Regarding the tomato cultivars, Jack was the most sensitive to the shading  
285 treatment as the MEL content increased by 135%, whereas Ciliegia was not sensitive  
286 to the reduction in incident radiation (Table 2). Of the pepper cultivars, California  
287 and F26 were the most sensitive, but showed a different pattern of response; the

288 shading treatment led to an increase in the MEL content of 64% in the California  
289 cultivar and to a decrease of the same rate per cent in the F26 cultivar.

290 Light is known to affect MEL metabolism in plants, as Murch et al., (2000)  
291 demonstrated that light intensity regulates the incorporation of radio-labelled  
292 serotonin into MEL. Under low light conditions, plantlets of *Hypericum perforatum*  
293 cv. Anthos metabolized less MEL than under higher light intensities. Melatonin  
294 content of the leaves of *Eichhornia crassipes* (Mart.) Solms plants grown under  
295 sunlight are about 16 times higher than those of plants grown under artificial light  
296 (Tan et al., 2007). In a recent study, the MEL content measured in leaves of tomatoes  
297 grown in an open field was found to be about 6.5 fold higher than in plants grown in  
298 a controlled growing chamber and the difference between the plants grown in field  
299 and *in vitro* was about 9.4-fold (Arnao and Hernández-Ruiz, 2013). The phenomenon  
300 whereby light intensity induces the metabolism and accumulation of MEL appears to  
301 be related to the photoprotection against oxidative stresses induced by free radicals  
302 or reactive oxygen species, produced during the process of photosynthesis (Arnao  
303 and Hernández-Ruiz, 2009; Paredes et al., 2009; Tan et al., 2007). However, caution  
304 should be taken in extrapolating the role of MEL as a scavenger from photosynthetic  
305 tissues to non-photosynthetic organs such as a mature fruit.

306 Data on the effects of light on MEL content in fruit are rather scarce. Under field  
307 conditions, grapes (*Vitis vinifera*) from bunches naturally shaded by leaves retained  
308 higher MEL contents, about 10 fold higher than in grapes those exposed to solar  
309 radiation (Boccalandro, González, Wunderlin, and Silva, 2011). It has been  
310 suggested that under intense radiation, larger amounts of MEL are consumed  
311 (because of its role as a free radical scavenger) than its quantity produced or  
312 imported from leaves, thus leading to low MEL contents in the fruit. In contrast,

313 under low levels of radiation, less MEL is consumed and the content in fruits will be  
314 therefore higher. The response of most tomato cultivars and only one pepper cultivar  
315 to the shading treatment was similar to that of *V. vinifera*. However, the opposite  
316 response was observed in most pepper cultivars and two tomato cultivars, i.e. the  
317 shaded fruits contained less MEL and others were not sensitive to the treatment.  
318 These findings demonstrated at least two important aspects: there were intraspecific  
319 differences in the response of the plants to reduced incident light in both *C. annuum*  
320 and *S. lycopersicum*, and the MEL content in shaded fruit did not always increase as  
321 expected under the concept that the steady-state of this compound in fruit is directly  
322 affected by the light (Boccalandro et al., 2011).

323 It has been suggested that the decrease in MEL contents observed in fruit exposed  
324 to intense light (such as sunlight) may be a regulatory response of plants to maintain  
325 a high level of MEL in leaves exposed to light stress conditions and thereby to  
326 provide efficient protection against free radicals derived from photosynthetic  
327 processes, so that the allocation of MEL to a sink organ like a fruit will be reduced  
328 (Arnao and Hernández-Ruiz, 2013). However, in the present study, leaves were not  
329 shaded and all plants were grown under the same conditions, and therefore the  
330 differences in MEL contents between shaded and control fruit were not due to a  
331 higher or lower stress levels in leaves.

332 To conclude, the melatonin content of red pepper fruits ranged from 31.0 to 93.4  
333 ng g<sup>-1</sup> (d.w.) and tomato fruits from 7.47 to 249.98 ng g<sup>-1</sup> (d.w.). The effect of the  
334 stage of ripeness on MEL contents in pepper fruit is not straightforward and strongly  
335 depends on the genotype. Under shading treatment, the content of this hormone in  
336 most of tomato cultivars tended to increase (up to 135%), whereas that of most  
337 pepper cultivars decreased (to 64%). Overall, the results also demonstrated that the

338 melatonin content of the fruits was not related to carbon fluxes from leaves. Further  
339 studies should be performed in order to elucidate whether the MEL content of fruit is  
340 derived only from leaves or is also biosynthesized in the fruit, to identify the factors  
341 that affect MEL transport from leaves and/or roots, and to clarify the physiological  
342 functions of MEL in fruit.  
343

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344 **References**

345

346 Alvarez-Garcia, V., Gonzalez, A., Alonso-Gonzalez, C., Martinez-Campa, C., &  
347 Cos, S. (2012). Melatonin interferes in the desmoplastic reaction in breast  
348 cancer by regulating cytokine production. *Journal of Pineal Research*, 52,  
349 282-290.

350 Arnao, M. B. & Hernández-Ruiz, J. (2009). Protective effect of melatonin against  
351 chlorophyll degradation during the senescence of barley leaves. *Journal of*  
352 *Pineal Research*, 46, 58-63.

353 Arnao, M. B. & Hernández-Ruiz, J. (2007). Melatonin promotes adventitious- and  
354 lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L. *Journal*  
355 *of Pineal Research*, 42, 147-152.

356 Arnao, M. B. & Hernández-Ruiz, J. (2013). Growth conditions influence the  
357 melatonin content of tomato plants. *Food Chemistry*, 138, 1212-1214.

358 Bocalandro, H. E., González, C. V., Wunderlin, D. A., & Silva, M. F. (2011).  
359 Melatonin levels, determined by LC-ESI-MS/MS, fluctuate during the  
360 day/night cycle in *Vitis vinifera* cv Malbec: evidence of its antioxidant role in  
361 fruits. *Journal of Pineal Research*, 51, 226-232.

362 Burkhardt, S., Tan, D. X., Manchester, L. C., Hardeland, R., & Reiter, R. J. (2001).  
363 Detection and quantification of the antioxidant melatonin in montmorency  
364 and balaton tart cherries (*Prunus cerasus*). *Journal of Agricultural and Food*  
365 *Chemistry*, 49, 4898-4902.

- 366 Carrillo-Vico, A., Lardone, P., Alvarez-Sanchez, N., Rodriguez-Rodriguez, A., &  
367 Guerrero, J. (2013). Melatonin: buffering the immune system. *International*  
368 *Journal of Molecular Sciences*, 14, 8638-8683.
- 369 Dubbels, R., Reiter, R. J., Klenke, E., Goebel, A., Schnakenberg, E., Ehlers, C.,  
370 Schiwara, H. W., & Schloot, W. (1995). Melatonin in edible plants identified  
371 by radioimmunoassay and by high performance liquid chromatography-mass  
372 spectrometry. *Journal of Pineal Research*, 18, 28-31.
- 373 Gitto, E., Pellegrino, S., Gitto, P., Barberi, I., & Reiter, R. J. (2009). Oxidative stress  
374 of the newborn in the pre- and postnatal period and the clinical utility of  
375 melatonin. *Journal of Pineal Research*, 46, 128-139.
- 376 Howatson, G., Bell, P., Tallent, J., Middleton, B., McHugh, M., & Ellis, J. (2012).  
377 Effect of tart cherry juice (*Prunus cerasus*) on melatonin levels and enhanced  
378 sleep quality. *European Journal of Nutrition*, 51, 909-916.
- 379 Huang, X. & Mazza, G. (2011). Simultaneous analysis of serotonin, melatonin,  
380 piceid and resveratrol in fruits using liquid chromatography tandem mass  
381 spectrometry. *Journal of Chromatography A*, 1218, 3890-3899.
- 382 Iriti, M. & Faoro, F. (2006). Grape phytochemicals: A bouquet of old and new  
383 nutraceuticals for human health. *Medical Hypotheses*, 67, 833-838.
- 384 Iriti, M., Varoni, E. M., & Vitalini, S. (2010). Melatonin in traditional Mediterranean  
385 diets. *Journal of Pineal Research*, 49, 101-105.
- 386 Johns, N. P., Johns, J., Porasuphatana, S., Plaimmee, P., & Sae-Teaw, M. (2012).  
387 Dietary intake of melatonin from tropical fruit altered urinary excretion of 6-

- 388 sulfatoxymelatonin in healthy volunteers. *Journal of Agricultural and Food*  
389 *Chemistry*, 61, 913-919.
- 390 Kolar, J. & Machakova, I. (2005). Melatonin in higher plants: occurrence and  
391 possible functions. *Journal of Pineal Research*, 39, 333-341.
- 392 Korkmaz, A. (2011). Melatonin; from pineal gland to healthy foods. *Spatula DD -*  
393 *Peer Reviewed Journal on Complementary Medicine and Drug Discovery*, 1,  
394 33-36.
- 395 Kvetnoy, I. (1999). Extrapineal melatonin: location and role within diffuse  
396 neuroendocrine system. *Histochemical Journal*, 31, 1-12.
- 397 Mauriz, J. L., Collado, P. S., Veneroso, C., Reiter, R. J., & Gonzalez-Gallego, J.  
398 (2013). A review of the molecular aspects of melatonin's anti-inflammatory  
399 actions: recent insights and new perspectives. *Journal of Pineal Research*, 54,  
400 1-14.
- 401 Melchiorri, D., Reiter, R. J., Sewerynek, E., Hara, M., Chen, L., & Nistico, G.  
402 (1996). Paraquat toxicity and oxidative damage: Reduction by melatonin.  
403 *Biochemical Pharmacology*, 51, 1095-1099.
- 404 Mena, P., Gil-Izquierdo, Á., Moreno, D. A., Martín, N., & García-Viguera, C.  
405 (2012). Assessment of the melatonin production in pomegranate wines. *LWT*  
406 *- Food Science and Technology*, 47, 13-18.
- 407 Murch, S. J., KrishnaRaj, S., & Saxena, P. K. (2000). Tryptophan is a precursor for  
408 melatonin and serotonin biosynthesis in in vitro regenerated St. John's wort

- 409 (*Hypericum perforatum* L. cv. Anthos) plants. *Plant Cell Reports*, 19, 698-  
410 704.
- 411 Okazaki, M. & Ezura, H. (2009). Profiling of melatonin in the model tomato  
412 (*Solanum lycopersicum* L.) cultivar Micro-Tom. *Journal of Pineal Research*,  
413 46, 338-343.
- 414 Okazaki, M., Higuchi, K., Aouini, A., & Ezura, H. (2010). Lowering intercellular  
415 melatonin levels by transgenic analysis of indoleamine 2,3-dioxygenase from  
416 rice in tomato plants. *Journal of Pineal Research*, 49, 239-247.
- 417 Pandi-Perumal, S. R., Zisapel, N., Srinivasan, V., & Cardinali, D. P. (2005).  
418 Melatonin and sleep in aging population. *Experimental Gerontology*, 40, 911-  
419 925.
- 420 Paredes, S. D., Korkmaz, A., Manchester, L. C., Tan, D. X., & Reiter, R. J. (2009).  
421 Phytomelatonin: a review. *Journal of Experimental Botany*, 60, 57-69.
- 422 Reiter, R. J., Manchester, L. C., & Tan, D. X. (2005). Melatonin in walnuts:  
423 Influence on levels of melatonin and total antioxidant capacity of blood.  
424 *Nutrition (Burbank, Los Angeles County, Calif)*, 21, 920-924.
- 425 Riga, P., Anza, M., & Garbisu, C. (2008). Tomato quality is more dependent on  
426 temperature than on photosynthetically active radiation. *Journal of the*  
427 *Science of Food and Agriculture*, 88, 158-166.
- 428 Rodriguez-Naranjo, M. I., Gil-Izquierdo, A., Troncoso, A. M., Cantos, E., & Garcia-  
429 Parrilla, M. C. (2011). Melatonin: A new bioactive compound in wine.  
430 *Journal of Food Composition and Analysis*, 24, 603-608.

- 431 Rodriguez-Naranjo, M. I., Gil-Izquierdo, A., Troncoso, A. M., Cantos-Villar, E., &  
432 Garcia-Parrilla, M. C. (2011). Melatonin is synthesised by yeast during  
433 alcoholic fermentation in wines. *Food Chemistry*, 126, 1608-1613.
- 434 Rosales-Corral, S. A., Acuña-Castroviejo, D., Coto-Montes, A., Boga, J. A.,  
435 Manchester, L. C., Fuentes-Broto, L., Korkmaz, A., Ma, S., Tan, D., &  
436 Reiter, R. J. (2012). Alzheimer's disease: pathological mechanisms and the  
437 beneficial role of melatonin. *Journal of Pineal Research*, 52, 167-202.
- 438 Sarropoulou, V. N., Therios, I. N., & Dimassi-Theriou, K. N. (2012). Melatonin  
439 promotes adventitious root regeneration in in vitro shoot tip explants of the  
440 commercial sweet cherry rootstocks CAB-6P (*Prunus cerasus* L.), Gisela 6  
441 (*P. cerasus* × *P. canescens*), and MxM 60 (*P. avium* × *P. mahaleb*). *Journal*  
442 *of Pineal Research*, 52, 38-46.
- 443 Slats, D., Claassen, J. A. H. R., Verbeek, M. M., & Overeem, S. (2013). Reciprocal  
444 interactions between sleep, circadian rhythms and Alzheimer's disease: Focus  
445 on the role of hypocretin and melatonin. *Ageing Research Reviews*, 12, 188-  
446 200.
- 447 Stehle, J. H., Saade, A., Rawashdeh, O., Ackermann, K., Jilg, A., Sebestény, T., &  
448 Maronde, E. (2011). A survey of molecular details in the human pineal gland  
449 in the light of phylogeny, structure, function and chronobiological diseases.  
450 *Journal of Pineal Research*, 51, 17-43.
- 451 Sturtz, M., Cerezo, A. B., Cantos-Villar, E., & Garcia-Parrilla, M. C. (2011).  
452 Determination of the melatonin content of different varieties of tomatoes

- 453 (*Lycopersicon esculentum*) and strawberries (*Fragaria ananassa*). *Food*  
454 *Chemistry*, 127, 1329-1334.
- 455 Tan, D. X., Hardeland, R., Manchester, L. C., Korkmaz, A., Ma, S., Rosales-Corral,  
456 S., & Reiter, R. J. (2012). Functional roles of melatonin in plants, and  
457 perspectives in nutritional and agricultural science. *Journal of Experimental*  
458 *Botany*, 63, 577-597.
- 459 Tan, D. X., Manchester, L. C., Di Mascio, P., Martinez, G. R., Prado, F. M., &  
460 Reiter, R. J. (2007). Novel rhythms of *N*<sub>1</sub>-acetyl-*N*<sub>2</sub>-formyl-5-  
461 methoxykynuramine and its precursor melatonin in water hyacinth:  
462 importance for phytoremediation. *The FASEB Journal*, 21, 1724-1729.
- 463 Tan, D. X., Manchester, L. C., Liu, X., Rosales-Corral, S. A., Acuna-Castroviejo, D.,  
464 & Reiter, R. J. (2013). Mitochondria and chloroplasts as the original sites of  
465 melatonin synthesis: a hypothesis related to melatonin's primary function and  
466 evolution in eukaryotes. *Journal of Pineal Research*, 54, 127-138.
- 467 Uguz, A. C., Cig, B., Espino, J., Bejarano, I., Naziroglu, M., Rodriguez, A. B., &  
468 Pariente, J. A. (2012). Melatonin potentiates chemotherapy-induced  
469 cytotoxicity and apoptosis in rat pancreatic tumor cells. *Journal of Pineal*  
470 *Research*, 53, 91-98.
- 471 Van Tassel, D. L., Roberts, N., Lewy, A., & O'Neill, S. D. (2001). Melatonin in plant  
472 organs. *Journal of Pineal Research*, 31, 8-15.
- 473 Wang, P., Sun, X., Li, C., Wei, Z., Liang, D., & Ma, F. (2013). Long-term exogenous  
474 application of melatonin delays drought-induced leaf senescence in apple.  
475 *Journal of Pineal Research*, 54, 292-302.

476 Wang, P., Yin, L., Liang, D., Li, C., Ma, F., & Yue, Z. (2012). Delayed senescence  
477 of apple leaves by exogenous melatonin treatment: toward regulating the  
478 ascorbate–glutathione cycle. *Journal of Pineal Research*, 53, 11-20.

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Table 1. Origin and phenotypic characteristics of pepper and tomato cultivars. sd: standard deviation, n: number of samples.

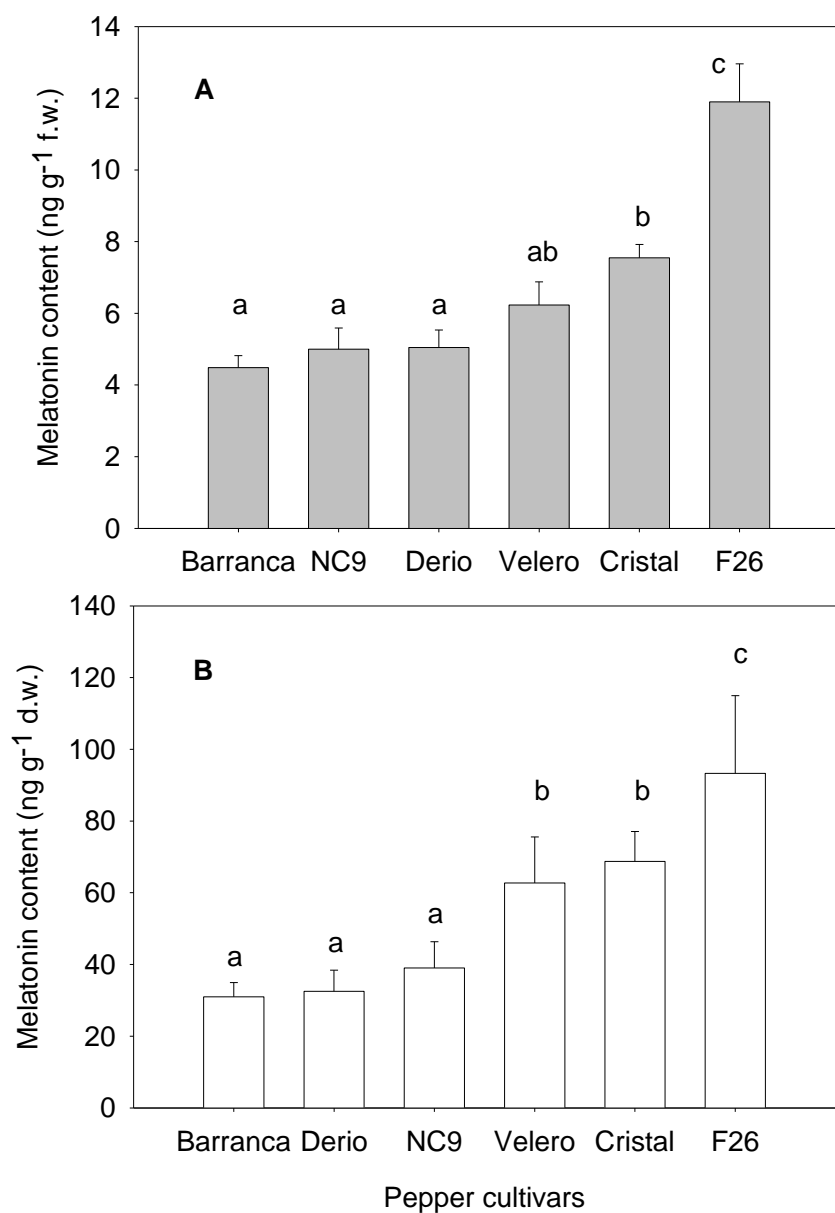
Cultivars	Origin	Type	Fruit weight (g)		Fruit width (mm)		Fruit length (mm)		length/width		n	
			mean	sd	mean	sd	mean	sd	mean	sd		
<u>Pepper</u>												
Barranca	Local cultivar	NEIKER	Triangular	59.1	10.3	51.5	13.9	110	20.4	2.30	0.67	16
Cristal	Comercial hybrid	Ramiro Arnedo	Elongate	67.6	11.5	44.5	4.52	115	14.0	2.61	0.36	26
Derio	Local cultivar	NEIKER	Elongate	35.3	7.51	33.4	5.90	125	12.2	3.88	0.93	28
F26	Local cultivar	NEIKER	Elongate	61.2	0.98	36.7	0.80	207	2.20	5.66	0.70	12
NC9	Local cultivar	NEIKER	Elongate	48.3	4.24	35.1	3.35	166	7.22	4.77	0.81	24
Velero	Comercial hybrid	Seminis	Blocky	175	24.1	86.8	6.01	78.6	6.20	0.91	0.11	24
<u>Tomato</u>												
Ciliegia	Comercial hybrid	Vilmorin	Cherry	7.78	2.38	24.2	1.73	23.8	1.84	0.98	0.04	16
Isis	Local cultivar	NEIKER	Cherry	16.4	3.37	30.6	2.25	28.8	2.58	0.94	0.04	25
Jack	Comercial hybrid	Seminis	Beef	280	78.4	84.7	9.64	68.9	5.57	0.82	0.05	12
Jesus	Local cultivar	NEIKER	Beef	462	167	110.7	13.2	79.5	10.8	0.72	0.07	16
NKT072	Local cultivar	NEIKER	Elongate	152	50.7	51.9	7.49	120	16.9	2.36	0.43	17
Optima	Comercial hybrid	Royal Sluis	Beef	221	35.0	77.4	7.22	65.6	3.95	0.86	0.13	12
Prico	Local cultivar	NEIKER	Beef	228	85.9	84.4	10.5	61.3	7.76	0.73	0.06	15



Table 2. Effect of the shading treatment on melatonin content pepper and tomato fruits of. Values were calculated using the following formula:  $Mel_{(\text{shaded fruit})} * 100 / Mel_{(\text{control fruit})} - 100$ , where Mel is the melatonin content per d.w.. ns: differences between shaded and control fruit are not significant.

Cultivar	Differences in melatonin content (%)	Differences in d.w. (%)
Peppers		
Velero	64.4	-20.1
Derio	-51.2	-5.16
F26	-64.0	ns
NC9	-41.4	-13.6
Barranca	ns	-9.41
Cristal	-40.8	ns
Tomatoes		
Optima	-36.6	-3.54
Jack	136	-7.00
Ciliegia	ns	10.7
Isis	73.5	-13.0
NKT072	95.9	-6.59
Prico	104	ns
Jesus	ns	-12.9

Figure 1.



*Fig. 1.* Effect of cultivar on the melatonin content of light-red pepper fruits. The melatonin contents are expressed on a fresh weight (A) and dry weight (B) basis. All cultivars were grown under the same greenhouse conditions. Each column represents the mean  $\pm$  standard deviation from 4 replicates. Means followed by the same letters are not significantly different at  $p < 0.05$  (Duncan test).

Figure 2.

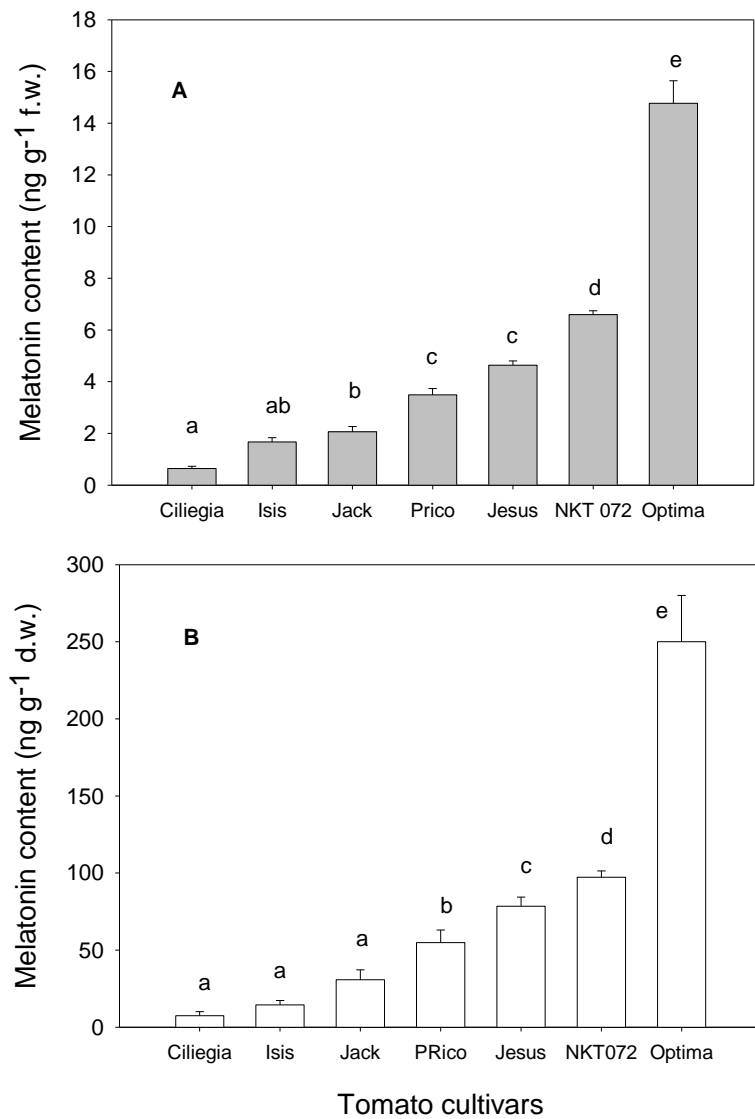
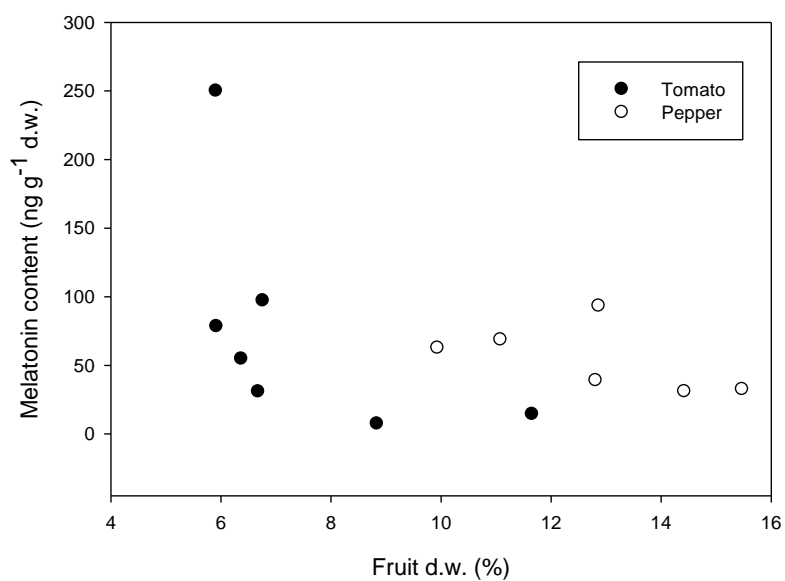


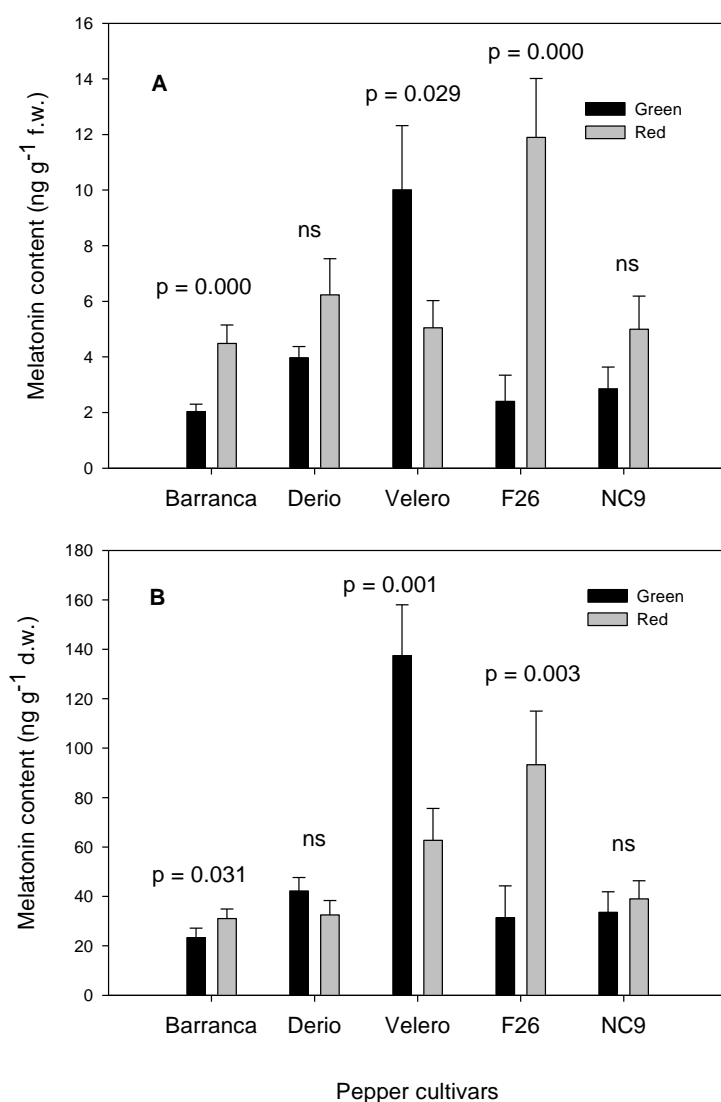
Fig. 2. Effect of cultivar on the melatonin content of light-red tomato fruits. The melatonin contents are expressed on a f.w. (A) and d.w. (B) basis. All cultivars were grown under the same greenhouse conditions. Each column represents the mean  $\pm$  standard deviation from 4 replicates. Means followed by the same letters are not significantly different at  $p < 0.05$  (Duncan test).

Figure 3.



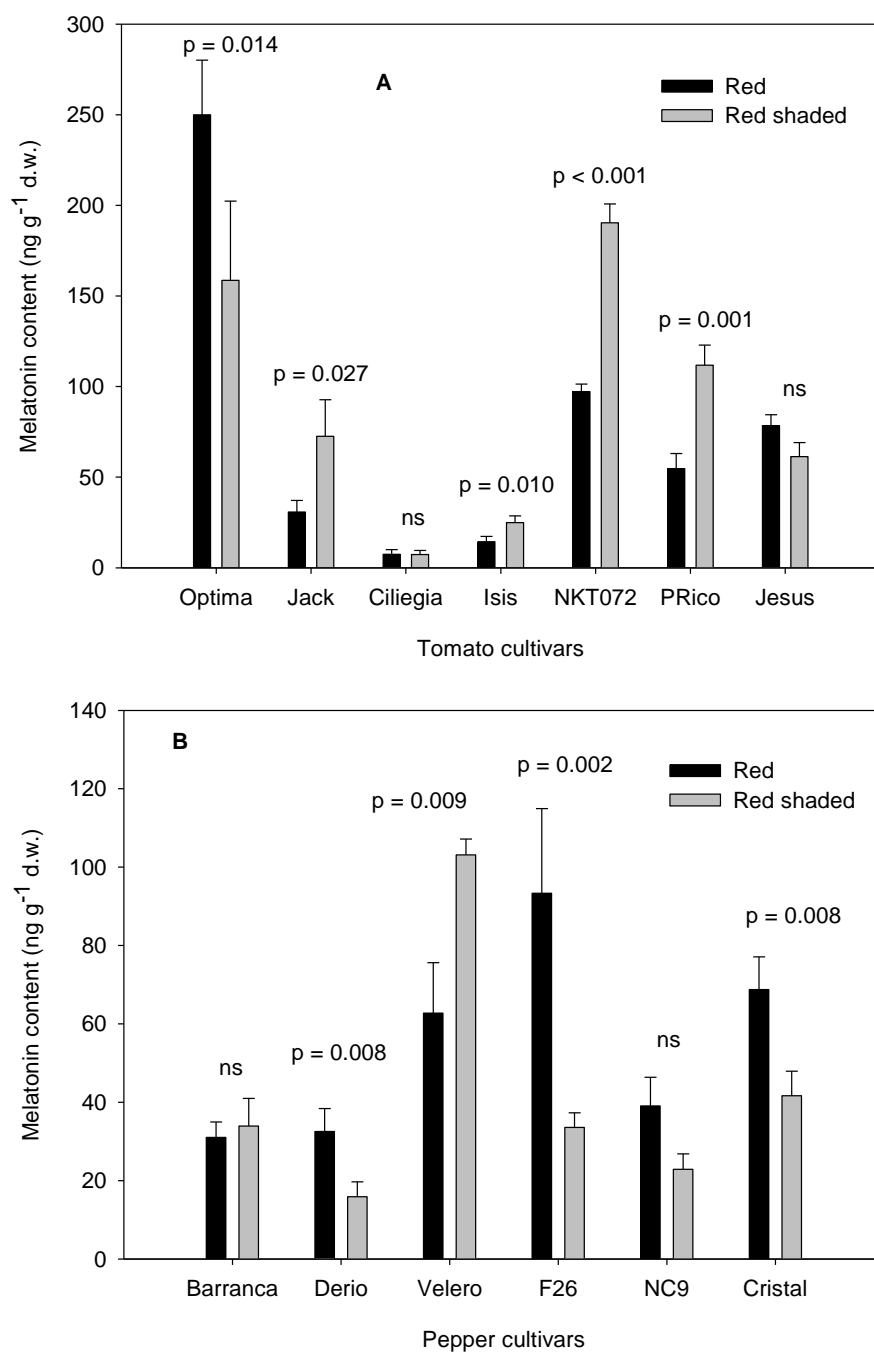
*Fig. 3.* Relationships between melatonin content and fruit d.w. for all tomato and pepper cultivars. Each point represents the mean value from 4 replicates for melatonin values and from 8 replicates for d.w. values.

Figure 4.



*Fig. 4.* Effect of stage of ripeness on melatonin content in mature green and light-red pepper fruits. Data are expressed on a f.w. (A) and a d.w. (B) basis. All cultivars were grown under the same greenhouse conditions. Each column represents the mean  $\pm$  standard deviation from 4 replicates. *p*-values from the independent-samples t test (Student's test) are shown. ns: not significant.

Figure 5.



*Fig. 5.* Effect of solar radiation on melatonin content in tomato (A) and pepper (B) cultivars. All cultivars were grown under the same greenhouse conditions and some fruits were shaded with an aluminium foil. Each column represents the mean  $\pm$  standard deviation from 4

replicates.  $p$ -values from the independent samples  $t$  test (Student's test) are shown. ns: not significant.

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**Highlights**

Melatonin in pepper and tomato fruits is strongly affected by incident solar radiation.

All cultivars did not show the same pattern of response to the shading treatment.

Melatonin accumulation in the fruits does not appear to depend on carbon flux from leaves.

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