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## Impact of storage time and temperature of salad heads on the quality of fresh-cut *Cichorium endivia*

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1 **Impact of storage time and temperature of salad heads on the quality of fresh-cut**

2 *Cichorium endivia*

3

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18

19 **Highlights**

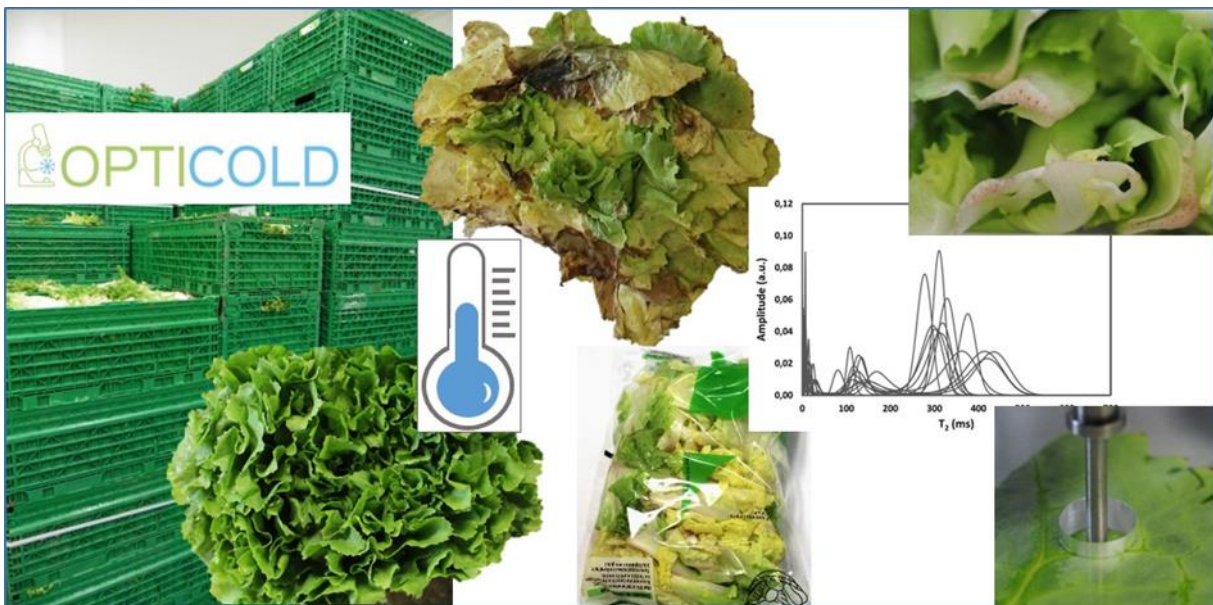
- 20 • Storage conditions of salad heads had an impact on the quality of fresh-cut salads.
- 21 • The visual quality of fresh-cut salads was the first criterion to be impacted.
- 22 • Salad-head storage for five days at 7 °C did not reduce the quality of fresh-cut salads.
- 23 • Increasing temperature and/or time of storage reduced the quality of fresh-cut salads.

24

25

26 **Graphical abstract**

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30

31 **ABSTRACT**

32 The impact of temperature and storage time of escarole salad heads on the quality of processed,  
33 fresh-cut salad was investigated. Based on temperatures recorded in a fresh-cut processing  
34 plant, salad heads were stored for four different storage times (0, 5, 9, and 12 d) at four  
35 temperatures (4, 7, 10, and 12 °C) before being processed. A range of quality criteria (technical  
36 yield, global visual aspect, pink cut surfaces, mechanical texture, aerobic microflora, respiration  
37 rate, atmosphere composition in fresh-cut salad pouches, nuclear magnetic resonance transverse

38 relaxation time) were measured on processing day, after 7 d storage at 4 °C of the fresh-cut  
39 salad packaged in pouches, and 1 d after pouch opening. The results are presented for salads  
40 grown in southeast France and early-season harvest, validated by experimental repetitions with  
41 a late-season harvest and salads from another geographical origin. Storage of salad heads caused  
42 deterioration of all quality attributes except for total aerobic bacteria. The global visual aspect  
43 was the most sensitive to changes in storage conditions of salad heads (significant reduction in  
44 quality for 5 d over 7 °C). In contrast, mechanical texture (maximum load for the shear test)  
45 was only significantly different for the fresh-cut salad prepared from salad heads stored for 9 d  
46 at 12 °C. For all quality criteria, fresh-cut salads processed from salad heads stored for 5 d at 4  
47 and 7 °C were not significantly different from those of non-stored salad heads. For storage time  
48 of 5 d or less, a temperature of 7 °C is likely as good as 4 °C for escarole salad-head storage  
49 intended for fresh-cut processing.

50

51 **Keywords:**

52 Fresh-cut, Modified Atmosphere Packaging, respiration rate, leaf texture, NMR relaxometry,  
53 visual aspect

54

55 **1. Introduction**

56 Fresh-cut vegetables are defined as pre-cut and pre-washed fresh vegetables packaged in a  
57 sealed polymeric film. These vegetables represented 7.6 % of the total purchase value (2.4 %  
58 by volume) of vegetables by French households in 2018 (FranceAgriMer, 2019), with 77.5 %  
59 of households purchasing fresh-cut vegetables. Green salads represented approximately 82 %  
60 of the sales of fresh-cut vegetables in France (FranceAgriMer, 2020), especially leafy green  
61 mixtures with escarole (broad-leaved endive, *Cichorium endivia* var. *latifolium*) as the major  
62 component. After processing, fresh-cut vegetables must be kept refrigerated (e.g., 4 °C in

63 France) during storage, transport, and retail; these vegetables have an average shelf life of  
64 approximately seven days. In processing plants, the guidelines for good manufacturing  
65 practices for fresh-cut vegetables (Anonymous 1988) do not recommend any particular storage  
66 temperature for entire salad heads. Therefore, while the product storage temperature is strictly  
67 regulated after processing ( $\leq 4\text{ }^{\circ}\text{C}$ ), the regulation offers some flexibility in the storage  
68 temperature of the salad heads. The storage time of salads in a processing plant is variable and  
69 depends on their origin and availability. Therefore, fresh-cut salads are processed from salad  
70 heads that may have been stored under different time and temperature conditions. Several  
71 studies have documented the effect of storage temperature of fresh-cut salads on their quality  
72 characteristics (Manzocco et al. 2017, Tsironi et al. 2017, Yahia et al. 2019). Furthermore, with  
73 respect to the storage conditions of the entire salad head, a few studies have reported the effect  
74 of storage time at one particular temperature (López-Gálvez et al. 1996; Rogers et al. 2006;  
75 Garrido et al. 2015; Koukounaras et al. 2018), However, to the best of our knowledge, the  
76 combined impact of the storage time and temperature of salad head has not yet been  
77 investigated.

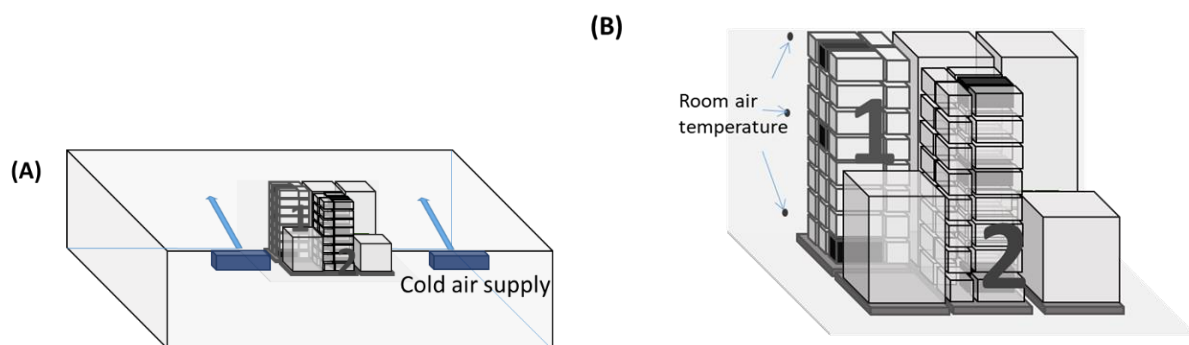
78 The objective of the present study was to determine whether temperature and time  
79 fluctuations prior to the processing of the salad heads influenced the physiology and quality of  
80 the fresh-cut salad. The storage conditions were chosen in accordance with the usual practices  
81 and temperature fluctuations observed in the partner factory. We used a range of methods to  
82 characterize fresh-cut salads: the percentage of raw salad used after trimming, global visual  
83 aspect, percentage of leaf pieces with pink cut surfaces, respiration rate, atmosphere  
84 composition in the fresh-cut salad pouches, fresh-cut leaf texture, total aerobic microflora, and  
85 nuclear magnetic resonance (NMR) relaxometry. It has been shown that NMR relaxometry can  
86 effectively evaluate the water status and distribution associated with cell and tissue structures.

87

88 **2. Materials and methods**

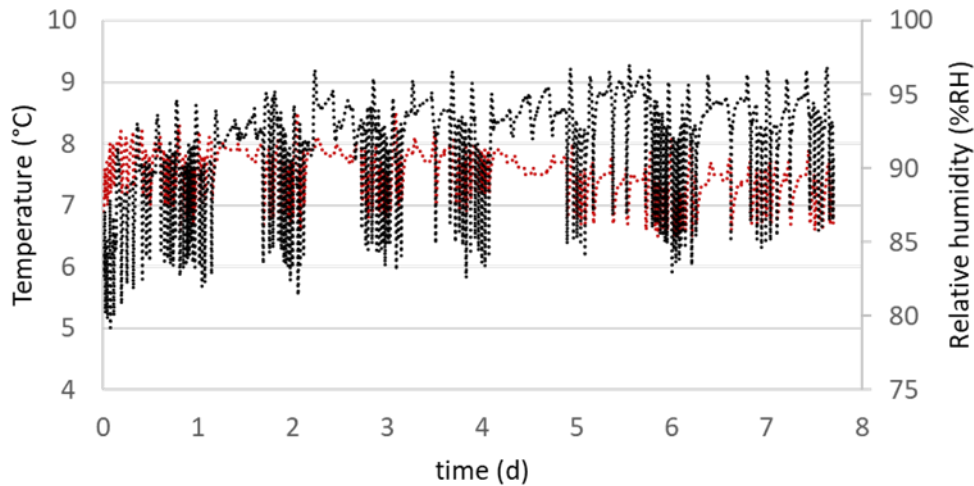
89 *2.1. Storage conditions in a fresh-cut salad processing plant*

90 The storage temperatures were determined based on the records collected at two distinct  
91 periods in the raw vegetable storage cold room of our partner factory (Small Medium  
92 Enterprise). The field study was conducted in autumn 2016 to measure the air temperature and  
93 relative humidity (RH) for eight days. Thereafter, in the late spring of 2017, another field  
94 experiment was conducted to record the air and product temperatures overnight. The sensors  
95 (Testo 171-4, +/-0.5 °C, range -35 °C to +60 °C, calibrated at -5, 0, 10, 20, and 30 °C) were  
96 positioned in accordance with the diagram in Fig. 1 at the top, middle, or bottom of two pallet  
97 stacks of salads.



98  
99 **Fig. 1.** Temperature monitoring in the factory cold room. (A) Disposition of the products in the cold  
100 room (real dimensions unknown). (B) Positions of the sensors for room air temperature and for product  
101 temperature measurements (black box) in the two pallets.

102 The evolution of air temperature and RH in the cold room is shown in Fig. 2. The air  
103 temperature varied slightly between 6.5 and 8 °C over time, with oscillations linked to defrost  
104 cycles and stable periods associated with reduced factory activity. The RH ranged from 85 to  
105 95 % RH.



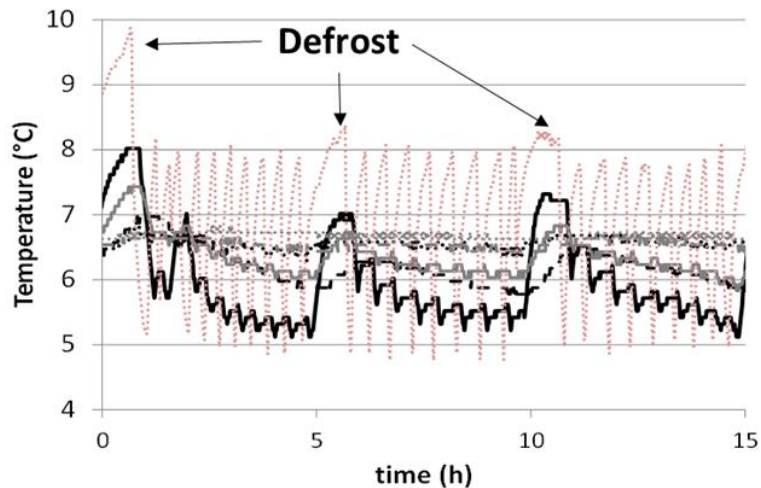
106

107 **Fig. 2.** Recording over eight days of cold room air temperature (red dotted line) and relative humidity  
 108 (black dotted) in a fresh-cut processing plant.

109

110 The temperature of the pallet was monitored (Fig. 3), and it was observed that the air  
 111 temperature remained in the same range (oscillations from 5 to 8 °C); furthermore, similar  
 112 profiles were observed between the two pallets. However, the profiles could be different due to  
 113 the location of the product in the pallets (top, middle or bottom, of Fig 1B). The product  
 114 temperatures at the top fluctuated with the ambient air temperature (on-off cycle, defrosting of  
 115 the refrigerating units). The impact of the refrigerating units on the product temperature at the  
 116 top is clearly observed. The fluctuations were attenuated for the products in the middle, and  
 117 almost no fluctuation was observed for the products at the bottom. This observation was  
 118 expected because the top of the pallet was directly subjected to the supply air of the refrigerating  
 119 units (Fig. 1A).

120



121

122 **Fig. 3.** Focus of a recording over one-day of air (red dotted line) and salad-head temperatures at different  
 123 locations in the pallets (pallet 1: black lines, pallet 2: gray lines, top: solid lines, middle: dashed lines,  
 124 bottom: dotted lines) in a fresh-cut processing plant.

125

126 According to the guidelines recommended by “Syndicat des Fabricants de Produits Végétaux  
 127 Frais Prêts à l’Emploi” (French association of ready-to-use fresh vegetable manufacturers), the  
 128 maximum temperature for raw material storage in fresh-cut salad processing plants should be  
 129 8 °C. For the experiments conducted in the laboratory, we considered the following  
 130 temperatures for the storage of salad heads:

131 • 4 °C was selected as the lowest temperature as it represents the temperature that should  
 132 be achieved by the fresh-cut product at the end of the processing;

133 • 7 and 10 °C were selected as intermediate temperatures because these temperatures  
 134 are close to the range of temperatures recorded in the partner factory;

135 • 12 °C was included as an extremely high temperature to ensure an impact on the  
 136 quality and physiology of the processed product.

137 In the fresh-cut processing plant, salad heads could be stored for 0 to 7 d, and occasionally  
 138 up to 12 d. Therefore, we considered as times of storage in our experiments the two extreme  
 139 situations, 0 d and 12 d, completed by two intermediate ones, 5 d and 9 d.



140

## 141 2.2. Plant material

142 Experiments were planned in 2018 with the open-field escarole salad *Cichorium endivia* var.  
143 *latifolium*. Salad var. *latifolium* Brillante (Syngenta, France) was grown in southeast France  
144 (Saint-Gilles, Gard; Mano Verde company), near INRAE Avignon, and purchased on April 30<sup>th</sup>  
145 (at the beginning of the season). Salads were harvested early in the morning and immediately  
146 transported to the laboratory in the INRAE Avignon. Salads to be characterized and processed  
147 in Avignon were first stored at 4 °C for 2 d; for NMR experiments, the salads were sent to  
148 INRAE Rennes by cold transport on the day of the harvest.

149 Two repetitions of the experiment were conducted:

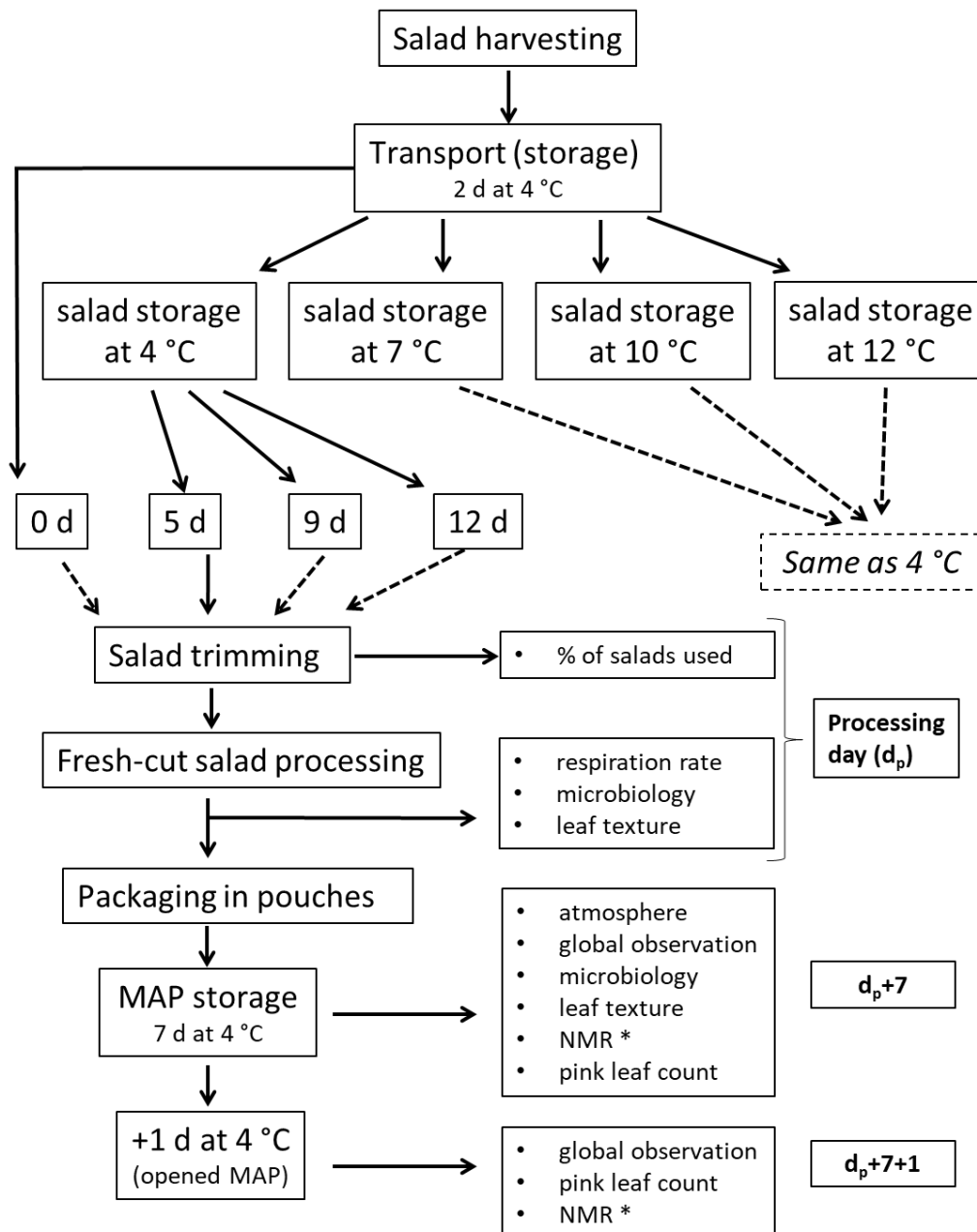
150 • One on escaroles harvested on June 4<sup>th</sup> 2018 (the end of the season) and purchased  
151 from the same producer.

152 • Another on open-field escaroles var. *latifolium* Leika (CLX 1001, Clause, France)  
153 purchased from a market gardener in northeast France (Balgau, Alsace) on June 25<sup>th</sup> (season  
154 beginning in this part of France) and sent to INRAE Avignon in refrigerated trucks.

155

## 156 2.3. Salad-head storage and fresh-cut processing

157 The global design of the experiment is shown in Fig. 4. The storage experiments began 2 d  
158 after harvesting because of the time taken during the refrigerated transport of the salads (from  
159 Avignon to Rennes or from Balgau to Avignon). Furthermore, when the salads were not  
160 transported (southern salads and experiment in Avignon), the plants were subjected to a similar  
161 pre-storage environment (stored for 2 d at 4 °C). Thereafter, temperatures were set at 4, 7, 10,  
162 or 12 °C (RH approximately 90 %) and the salad heads were stored for 5, 9, or 12 d in cold  
163 chambers before fresh-cut processing. The temperatures were monitored with a Wi-Fi sensor  
164 SPY T+ (JRI MySirius, France, ±0.4 °C in the range -20 °C to +30 °C).



165

166 **Fig. 4.** Global design of the storage experiment and analysis carried out at each stage.

167 \* Specific storage conditions: NMR was performed on fresh-cut leaves 5 d after processing from salads  
 168 stored for 0.5, 6 or 11 d at 4, 7 or 12 °C.

169 At each storage time (0, 5, 9, or 12 d), six escarole heads for each temperature (4, 7, 10, or  
 170 12 °C) were processed into fresh-cut salad pouches in the laboratory, according to the procedure  
 171 followed by the processing plant ( $d_p$ : processing day, Fig. 4). The salad heads were trimmed to  
 172 remove external, withered, and damaged leaves. Furthermore, the bases and tips of the salad  
 173 heads were removed; thereafter, the leaves were sliced into large pieces (approximately  $10 \times 6$

174 cm) and immediately rinsed in pre-wash bath to remove the soil residues, followed by a washing  
175 bath (4 °C, 80 mg L<sup>-1</sup> chlorine, 2 min shaking). Leaves were drained and dipped in a rinsing  
176 bath (4 °C, 1 min shaking), and then drained and spun using a manual salad spinner (10 turns;  
177 Dynamic 10 L, France). The washing and rinsing baths were changed for each temperature.

178 Leaf pieces of fresh-cut escarole were packed into four pouches for each temperature  
179 condition, using a polypropylene packaging film provided by the industrial project partner.  
180 Each pouch contained a mixture of green and yellow leaves (300 g per pouch). Thereafter, the  
181 pouches were sealed under air (passive modified atmosphere packaging, MAP). Regardless of  
182 the initial storage conditions of the raw salads, the fresh-cut salad pouches were stored for 7 d  
183 at 4 °C (d<sub>p</sub>+7, Fig. 4) before characterization. Moreover, fresh-cut salads were observed and  
184 analyzed 1 d after opening the pouches (d<sub>p</sub>+7+1). Fresh-cut salads prepared from salad heads  
185 stored for 12 d were characterized at d<sub>p</sub>; however, no pouches were prepared because of the  
186 extremely poor quality of the salad leaves.

187 For the NMR experiments, the same raw salads were used; however, the storage conditions  
188 were slightly different. Salad heads were stored for 0.5, 6, or 11 d at 4, 7, and 12 °C before  
189 fresh-cut processing (d<sub>p</sub>); NMR measurements were performed on leaves at d<sub>p</sub>+5 and d<sub>p</sub>+5+1.

190 The same global design was used for the repetitions with the late-season salads and those  
191 from the northeast of France; however, only three temperatures were studied (4, 7, and 10 °C),  
192 and neither bacterial determination nor NMR measurements were performed.

193

## 194 *2.4. Characterization of fresh-cut salads*

### 195 *2.4.1. Raw material used after trimming*

196 The salads were weighed before and after trimming to determine the percentage of salad heads  
197 used to process fresh-cut salads.

198

199 2.4.2. *Microbiology*

200 The total aerobic bacteria in fresh-cut salads at  $d_p$  and  $d_p+7$  was counted in accordance with  
201 the methodology proposed by Tsironi et al. (2017). For microbial analysis, salad leaf pieces (10  
202 g) were homogenized with 90 mL peptone buffered water using a stomacher (Blender 400®)  
203 for 60 s. Ten-fold serial dilutions for each homogenized sample were prepared using peptone  
204 buffered water. Aliquots (0.1 mL) of each dilution were spread on Plate Count Agar (PCA,  
205 Biokar Diagnostics, France). This rich and non-selective medium enabled the counting of total  
206 aerobic bacteria after incubation at 25 °C for 48 h (Jacques et al. 1995). For each condition of  
207 salad-head storage, analyses were performed on the four-replicate fresh-cut salad pouches  
208 ( $d_p+7$ ) and on four randomly selected samples of fresh-cut leaves at  $d_p$ . Microbial counts were  
209 expressed as  $\log_{10}$  CFU  $g^{-1}$ .

210

211 2.4.3. *Respiration rate and atmosphere composition*

212 The respiration rate (RR) of fresh-cut salads was measured at all  $d_p$  using the jar technique  
213 (Varoquaux et al. 2002). Leaves (30 to 45 g per jar) were placed in a tight gas jar (500 or 750  
214 mL, three jars for each storage/temperature condition), and oxygen consumption and carbon  
215 dioxide production were measured at 20 °C (after temperature equilibrium). The results were  
216 expressed in mmol gas produced or consumed per h and per kg of leaves. The measurement of  
217 RR for both the gases allowed for the calculation of the respiratory quotient ( $RR_{CO_2}/RR_{O_2}$ ) for  
218 all samples. Moreover, for escarole characterization, we calculated the  $Q_{10}$  (the multiplication  
219 factor for the RR for a temperature increase of 10 °C) in accordance with Gore's law  
220 (Varoquaux et al. 2002) by measuring the RR of fresh-cut leaves on 0 d of storage at 4, 7, 12,  
221 and 20 °C at  $d_p$  and  $d_p+1$ .

222 The atmosphere composition within all fresh-cut salad pouches (four pouches for each  
223 storage condition of the salad heads) was measured at  $d_p+7$  and expressed in %  $O_2$  and %  $CO_2$ .

224 The atmosphere composition in the sealed jars and fresh-cut salad pouches was analyzed by gas  
225 chromatography using a  $\mu$ GC (Agilent 3000A). O<sub>2</sub> and CO<sub>2</sub> were separated on two capillary  
226 columns (MS-5A and Poraplot) under argon and helium, respectively, and quantified using a  
227 catharometric detector (TCD).

228

#### 229 *2.4.4. Global observations of stored fresh-cut salad pouches*

230 The global visual quality of the four pouches was evaluated by two experts for each salad-  
231 head storage condition. Global visual quality was scored on a scale from 5 to 0, where 5 = good,  
232 4 = quite good, 3 = medium, 2 = poor, 1 = bad, and 0 = very bad. In addition, the percentage of  
233 leaf pieces with pink discoloration on the cut surfaces and veins (a visible sign of alteration of  
234 the salad leaves) was calculated. Both global visual quality and pink discoloration were  
235 determined just at the opening of pouches ( $d_p+7$ ) and after 1 d ( $d_p+7+1$ ) to mimic consumer  
236 behavior.

237

#### 238 *2.4.5. Leaf texture*

239 To analyze leaf texture, two tests were performed using a multi-purpose texture analyzer  
240 (TaPlus, Lloyd Instruments, UK). The Kramer shear test was performed on approximately 4 g  
241 of green leaves (without central ribs and laid flat) with a 1000 N load cell at a rate of 20 cm  
242 min<sup>-1</sup>, with 10 blades. For each time x temperature storage condition, the results were expressed  
243 as the maximum load (N) standardized for 4 g of sample for three replicates of fresh-cut salads  
244 at  $d_p$  or height replicates at  $d_p+7$  (two measurements per pouch).

245 A puncture test was performed with a flat probe (3 mm in diameter) at a rate of 1 mm s<sup>-1</sup>  
246 (50 N load cell) until the green leaf piece held in place with a pierced plate ruptured. The results  
247 were expressed as the maximum load (N), and the corresponding deflection (mm) for six  
248 replicate leaves of fresh-cut salads at  $d_p$  or for sixteen replicate leaf pieces at  $d_p+7$  (four leaf

249 pieces per pouch × four pouches per salad-head storage condition). To overcome the  
250 fluctuations at the start of the test (leaf tension), the zero value for the deflection was set at a  
251 load of 0.025 N. In addition, to better take into account the differences in the shape of the curve  
252 load=f(deflection) between the different treatments, curves were fitted to a binomial equation  
253  $y=ax^2+bx+c$ , where y is the load, x is the deflection, and a, b, and c are the estimated parameters  
254 used to characterize each curve.

255

#### 256 2.4.6. Nuclear magnetic resonance (NMR) relaxometry

257 Transverse relaxation measurements were performed on a 20 MHz spectrometer (Minispec  
258 PC 120, Bruker, Karlsruhe, Germany). For each NMR analysis, discs (8 mm in diameter) were  
259 sampled from several pieces of green leaves collected from two salad pouches. Discs were  
260 sampled to avoid the presence of major leaf veins. Measurements were performed in 12  
261 replicates unless the leaves were severely damaged and no green piece was available. The  
262 temperature was set at 4 °C for all samples.  $T_2$  was measured using a Carr-Purcell-Meiboom-  
263 Gill (CPMG) sequence with a 90-180° pulse spacing of 0.2 ms and 32 averages. The number  
264 of successive echoes recorded was adjusted for each sample to establish the baseline of the  
265 relaxation curve. The recycle delay for each sample was adjusted to  $5 \times T_1$  after measuring  $T_1$   
266 using a fast saturation recovery sequence. The CPMG signal was fitted using the Scilab  
267 software in accordance with the maximum entropy method (MEM) (Mariette et al. 1996), which  
268 provides continuous distribution of relaxation time components without any assumption  
269 concerning their number. In this representation, the peak areas corresponded to the intensities  
270 of the  $T_2$  components.

271 The specific leaf water weight per NMR component ( $LWW_i$ ) was calculated using the  
272 following equation:

273 (Eq. 1):  $LWW_i = \frac{I_{R,i} \times m_w}{A}$

274 where  $m_w$  corresponds to the water mass (g) of the leaf samples,  $A$  is the area of the discs ( $m^2$ ),  
275 and  $I_{R,i}$  is the relative intensity of the  $i^{\text{th}}$  NMR signal component (%). The water mass of the  
276 leaf samples was calculated as the difference between their fresh and dry weights. The dry  
277 weight was estimated at the end of the NMR experiments by oven drying the discs at  $70\text{ }^\circ\text{C}$  for  
278 36 h.  $LWW_{\text{tot}}$  corresponded to the total amount of water per area of the leaf.

279

## 280 *2.5. Statistical analysis*

281 Data were subjected to Analysis of variance (ANOVA) using XLstat (Addinsoft). For the  
282 main experiment (sections 3.1 to 3.6), for each variable (quality criteria), two factors, storage  
283 time and temperature of salad head were considered. ANOVA was performed in two ways, with  
284 and without non-stored raw material as a reference. Mean values were compared to each other  
285 by Tukey's HSD test (ANOVA performed without the reference), and mean values obtained  
286 from stored salad heads were compared to those of non-stored salads using Dunnett's test  
287 (ANOVA performed with the reference). Unless stated otherwise, a probability of 5 % was used  
288 to determine significant differences between treatments.

289 Results from the replications (section 3.7) were also analyzed through ANOVA by taking  
290 into consideration three factors, i.e., experiment, storage time, and temperature of salad head;  
291 moreover, two other factors, time and temperature (combining the results from the experimental  
292 replicates), were also considered.

293

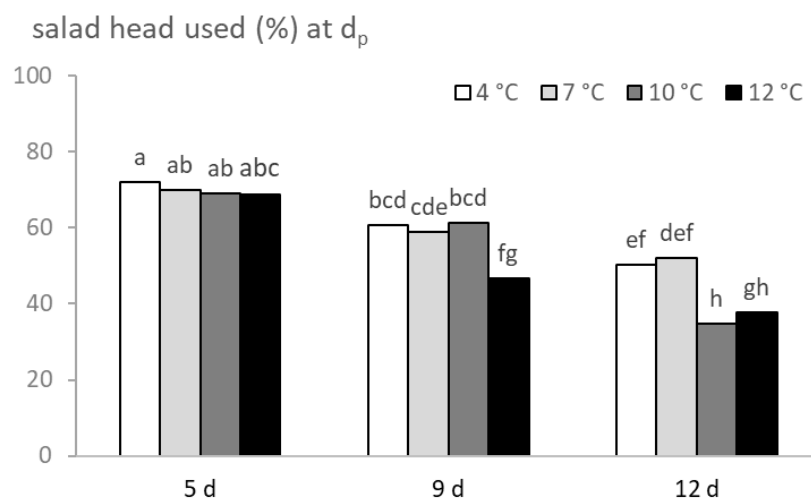
## 294 **3. Results**

### 295 *3.1. Impact of salad-head storage conditions on technical yield*

296 ANOVA indicated that both the storage time and temperature of the salad head, as well as  
297 the interaction between these two factors, had a significant effect on the technical yield (i.e., %  
298 of the salad head not discarded at trimming and used to process fresh-cut salads) ( $p < 0.0001$ ).

299 For the three storage times, the mean technical yields (considering all temperatures) decreased  
 300 significantly with increasing storage time (70, 57, and 44 % for 5, 9, and 12 d, respectively).  
 301 The mean technical yields for the four temperatures, 4, 7, 10, and 12 °C, were 61, 60, 55 and  
 302 51 %, respectively. No significant differences were observed between 4 and 7 °C and 10 and  
 303 12 °C

304 The technical yield for each time × temperature combination is shown in Fig. 5. For each  
 305 storage time, the technical yields decreased with the storage temperature. However, the values  
 306 were only significantly different for 9 d at 12 °C and 12 d at 10 or 12 °C. Under these three  
 307 storage conditions, almost all the green leaves were discarded by trimming, thereby leaving  
 308 only the youngest, pale green and yellow leaves.



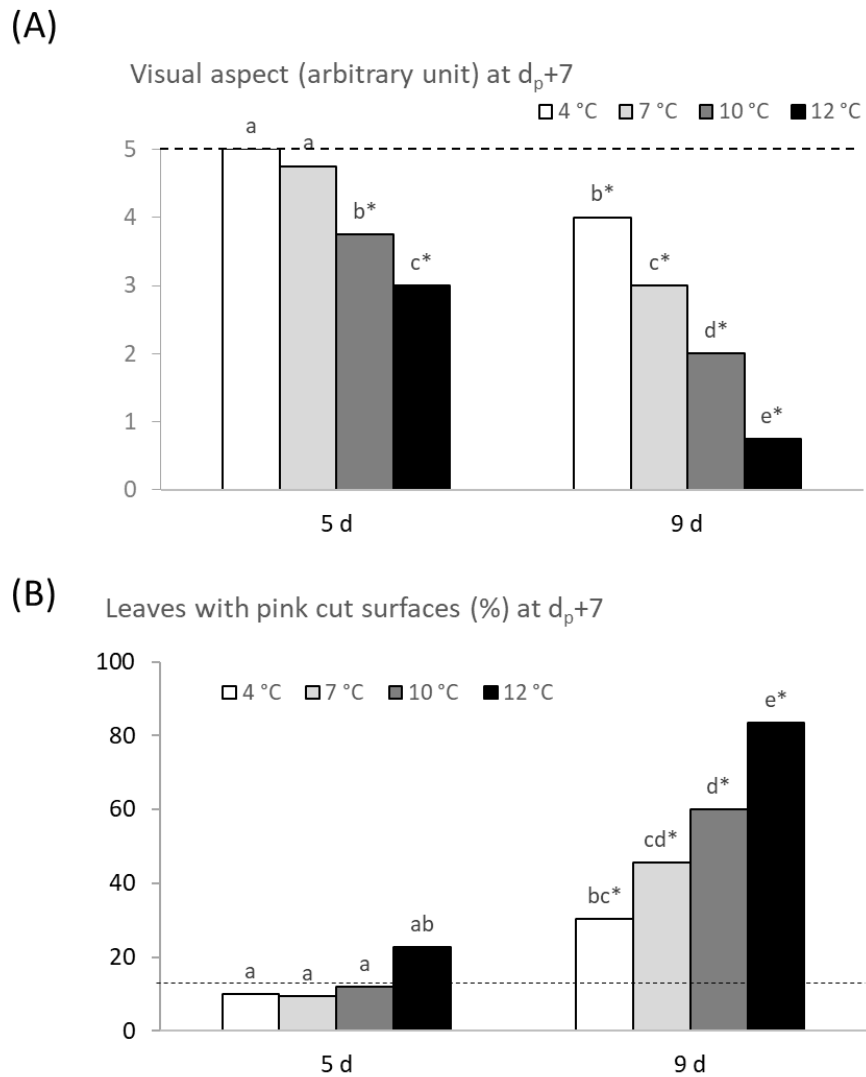
309  
 310 **Fig. 5.** Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on the percentage of  
 311 salad used after trimming to process fresh-cut salads ( $d_p$ : processing day). Bars represent the average of  
 312 the trimming carried out on six salad heads. ANOVA was performed on the twelve storage conditions,  
 313 and bars with different letters are significantly different ( $p < 0.05$ ) according to the Tukey HSD test.

314  
 315 *3.2. Impact of salad-head storage conditions on the visual quality of fresh-cut salads*

316 The salad heads stored at different times and temperatures were processed into fresh-cut  
 317 salads; thereafter, all fresh-cut bags were stored for 7 d at 4 °C ( $d_p+7$ ) and examined at the



318 opening. Moreover, the fresh-cut bags were evaluated after 1 d of further storage at 4° C after  
 319 opening ( $d_p+7+1$ ). The global visual aspect was evaluated on an arbitrary scale of 0 ( poorest )  
 320 to 5 ( best ). The results are presented in Fig. 6A.



321  
 322 **Fig. 6.** Impact of salad-head storage conditions (5 or 9 d at 4, 7, 10 or 12 °C) on visual aspect (A)  
 323 and the percentage of leaf pieces with pink discoloration of the cut surfaces (B) of the fresh-cut salad  
 324 pouches stored for 7 d at 4 °C ( $d_p+7$ ). With respect to visual aspect, 5 represented the best and 0 the  
 325 worst. Each bar represents the mean of four-replicate fresh-cut salad pouches. ANOVA was performed  
 326 on the eight different storage conditions, and bars with different letters are significantly different  
 327 ( $p<0.05$ ) according to the Tukey HSD test. Dotted lines represent fresh-cut salads processed from non-  
 328 stored (0 d) salads. (\*) indicates results significantly different from those of the non-stored raw salad  
 329 according to the Dunnett test ( $p<0.05$ ).

330

331 At opening ( $d_p+7$ ), the visual aspect was good (5 or close to 5); similar results were observed  
332 for fresh-cut salads processed from non-stored salad heads and salads stored for 5 d at 4 °C and  
333 7 °C (Fig. 6A). Under other storage conditions, the visual aspect of the processed salad  
334 gradually deteriorated with increasing storage time and temperature.

335 The pink discoloration of cut surfaces and veins is responsible for an unpleasant aspect of  
336 fresh-cut salads (Couture et al. 1993, Rico et al. 2007, Charles and Varoquaux 2016). The  
337 proportions of leaf pieces with pink cut surfaces immediately after opening of the pouches were  
338 approximately 9-13 % for the fresh-cut processed from salads stored for 5 d at 4, 7, and 10 °C  
339 (Fig. 6B). Furthermore, these values were comparable with those of fresh-cut salads processed  
340 from non-stored salad heads. Salad heads that were stored for 5 d at 12 °C resulted in a 23 %  
341 rate in pink cut surfaces; this increase was insignificant. After 9 d of storage, the pink  
342 discoloration increased to a significantly higher percentage than that observed in non-stored  
343 salads (dotted line), regardless of the storage temperature.

344

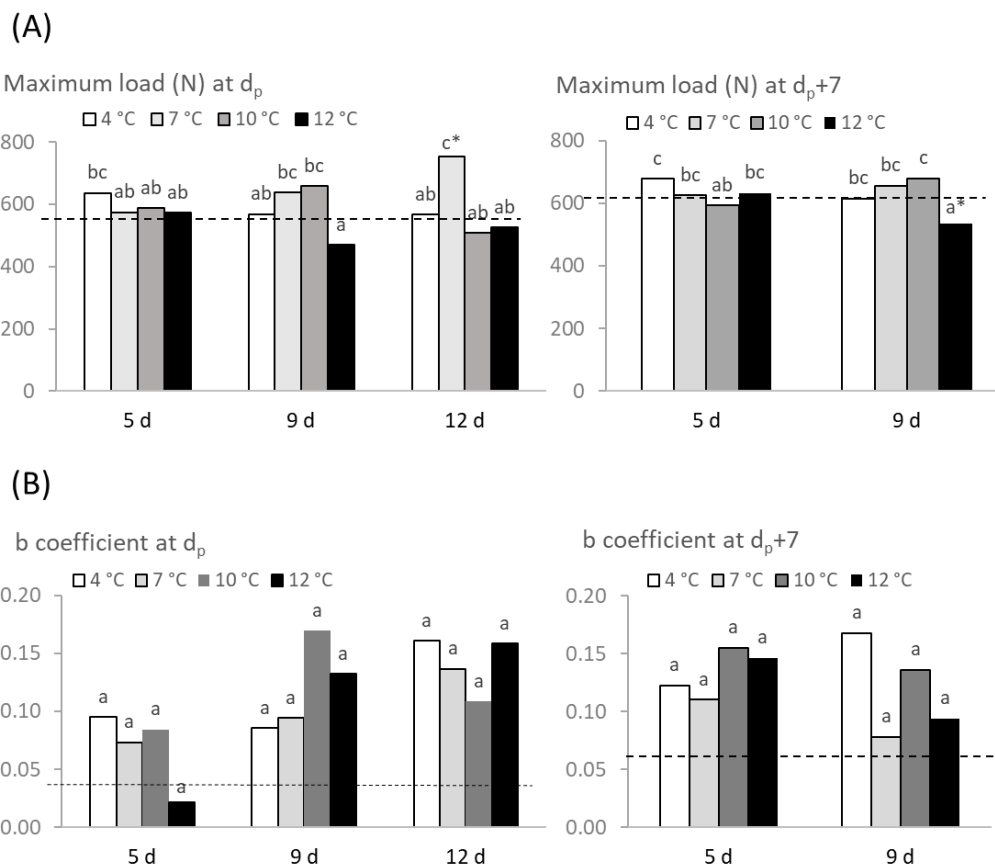
345 The global visual quality was a more stringent quality criterion than the percentage of pink  
346 discoloration of cut surfaces; the storage conditions giving similar results to non-stored salad  
347 (taken as reference) were 5 d at 4 °C and 7 °C for global visual quality in contrast to 5 d at all  
348 storage temperatures (4 °C to 12 °C) for pink discolorations.

349 The visual aspect of fresh-cut salads observed at  $d_p+7+1$  was lower than that observed at the  
350 opening. The highest scores for the global visual aspect were of 4 or close to 4 for non-stored  
351 salads and salads stored 5 d at 4 and 7 °C (non-significantly different), with the proportion of  
352 leaves with pink cut surfaces ranging between 53-63 %; this value was significantly lower than  
353 that observed for all other conditions (82-100 %).

354

355 *3.3. Impact of salad-head storage conditions on leaf texture*

356 The maximum loads for each time × temperature combination are shown in Fig. 7A. The  
 357 Kramer shear test performed at  $d_p$  showed mean maximum loads from 470 to 754 N, with  
 358 relatively low values for the longest storage time and highest temperature. At  $d_p+7$ , the mean  
 359 maximum load ranged from 534 to 680 N, depending on the storage conditions of the salad  
 360 head.



361  
 362 **Fig. 7.** Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on the leaf texture of  
 363 fresh-cut salad just after processing ( $d_p$ ) and after 7 d storage at 4 °C ( $d_p+7$ ). (A) Maximum load (N) for  
 364 the Kramer shear test. (B) Polynomial b factor obtained from puncture test spectra. Bars represent means  
 365 of three to eight replicates for the Kramer test and six to sixteen replicates for the puncture test. ANOVA  
 366 was performed on the twelve storage conditions, and bars with different letters are significantly different  
 367 ( $p < 0.05$ ) according to the Tukey HSD test. The dotted line corresponds to fresh-cut salads processed  
 368 from non-stored (0 d) salads. (\*) indicates results significantly different from those of the non-stored  
 369 salads according to the Dunnett test ( $p < 0.05$ ).

370 For each time  $\times$ temperature storage, load values were higher for leaves at  $d_p+7$  than at  $d_p$ .  
371 At  $d_p+7$ , the maximum load of the fresh-cut salads processed from the salads stored for the  
372 longest time (9 d) and the highest temperature (12 °C) was significantly lower than that for  
373 fresh-cut salads processed from non-stored (0 d) salads; these non-stored salads were  
374 considered as reference.

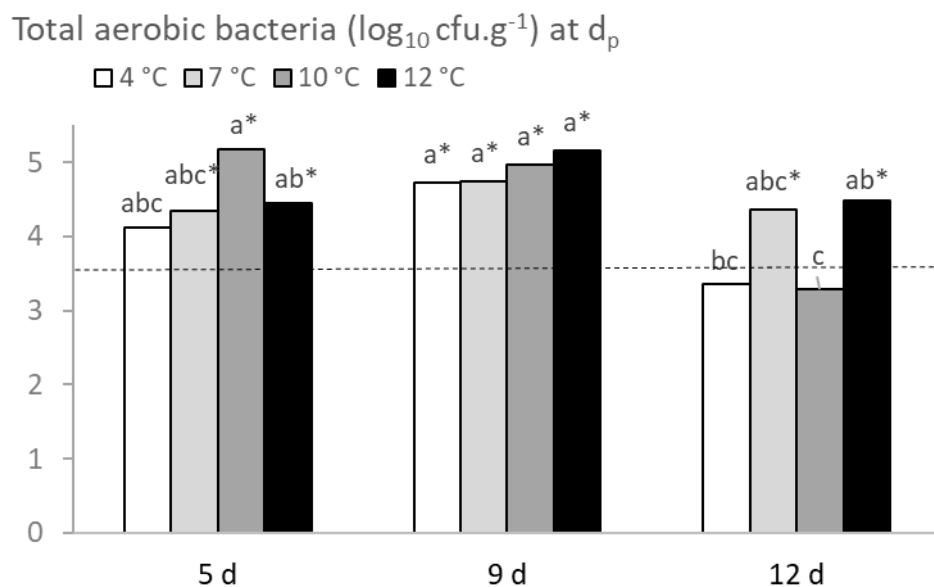
375 ANOVA performed on the maximum loads for the puncture test showed no significant effect  
376 on any salad-head storage conditions at  $d_p$  (means from 1.41 to 1.75 N) (supplementary Fig.  
377 S1). Furthermore, at  $d_p+7$ , only leaves prepared from salads stored for 5 d at 4 °C or 10 °C  
378 presented maximum loads, i.e., highest and lowest, respectively. During the measurements, we  
379 observed that the texture of fresh-cut leaves changed based on the increase in salad-head storage  
380 time and temperature. The leaves became more elastic but with maximum loads equivalent to  
381 those of fresh and crunchy leaves. A polynomial adjustment with synchronization of the spectra  
382 from a force of 0.025 N was performed to integrate all leaf deformations until the breakthrough  
383 (Fig. S2). Despite contrasting mean values for the b coefficient (from 0.022 to 0.17 at  $d_p$  and  
384 from 0.094 to 0.168 at  $d_p+7$ ), ANOVA did not distinguish any storage conditions (Fig. 7B) in  
385 accordance with the results of the Tukey HSD test and Dunnett test (with non-stored salads as  
386 reference). At  $d_p+7$ , b coefficients decreased with the storage time, except at 4 °C; furthermore,  
387 it varied from 0.094 (7 °C) to 0.146 (10 °C) for storage temperature.

388

#### 389 *3.4. Impact of salad-head storage conditions on total aerobic bacteria*

390 Total aerobic bacteria in fresh-cut salad leaves were counted at  $d_p$  and  $d_p+7$ . At  $d_p$  (washed-  
391 cut leaves ready to be packaged), the microbial counts of samples prepared from salad heads  
392 stored between 0 d and 12 d, at temperatures from 4 to 12 °C, ranged from 3.3 log<sub>10</sub> CFU g<sup>-1</sup> to  
393 5.2 log<sub>10</sub> CFU g<sup>-1</sup>. ANOVA indicated that both storage time and temperature, as well as the  
394 interaction between the two factors, had a significant impact ( $p<0.0001$ ) on total aerobic

395 bacteria. Furthermore, it was observed that fresh-cut salads prepared from salads stored for 5 d  
 396 at 4 °C and 12 d at 4 and 10 °C were not significantly different from those prepared using non-  
 397 stored salads (Fig. 8). In accordance with the results of the Tukey HSD test, only fresh-cut  
 398 salads prepared from salad heads stored for 12 d at 4 or 10 °C were significantly different from  
 399 those prepared in other conditions (Fig. 8). Storage of salad heads tended to increase the counts  
 400 of aerobic bacteria on the fresh-cut product at  $d_p$  (compared to non-stored salads), with some  
 401 exceptions for the longest storage time (12 d). This phenomenon may be explained by the  
 402 increased elimination of external dark green leaves during trimming after 12 d of storage.



403  
 404 **Fig. 8.** Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on total aerobic  
 405 bacteria of fresh-cut salad after processing, before pouches storage ( $d_p$ ). Bars represent the means of  
 406 four replicates. ANOVA was performed on the twelve storage conditions, and bars with different letters  
 407 are significantly different ( $p < 0.05$ ) according to the Tukey HSD test. The dotted line corresponds to  
 408 fresh-cut salads processed from non-stored (0 d) salad heads. (\*) indicates results significantly different  
 409 from those of the non-stored salads according to the Dunnett test ( $p < 0.05$ ).

410

411 No significant effect of storage time and temperature of the salad heads was observed on the  
412 microbial counts of the fresh-cut salads that were analyzed at  $d_p+7$ ; the microbial count ranged  
413 from  $5.5 \log_{10} \text{CFU g}^{-1}$  to  $6.2 \log_{10} \text{CFU g}^{-1}$  (results not shown).

414

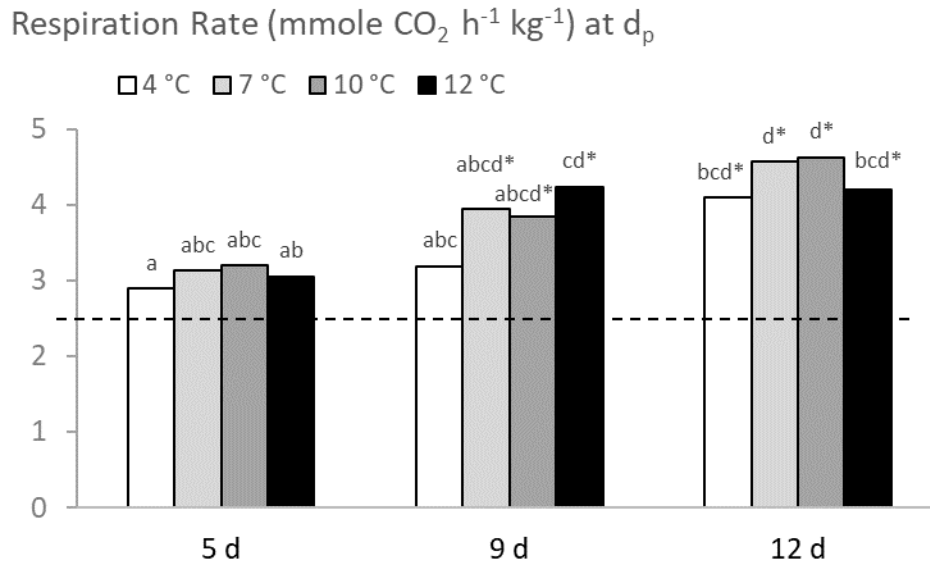
### 415 *3.5. RR of fresh-cut salads and modified atmosphere in the fresh-cut salad pouches*

416 The  $Q_{10}$  (multiplying factor of the RR caused by a  $10^\circ\text{C}$  increase) was calculated based on  
417 RRs measured at 4, 7, 12, and  $20^\circ\text{C}$ . For fresh-cut salads made from non-stored salads (0 d),  
418 the  $Q_{10}$  value was 2.44 for  $\text{RR}_{\text{O}_2}$  and 2.52 for  $\text{RR}_{\text{CO}_2}$ . After 24 h, the RR of fresh-cut leaves  
419 decreased at the four temperatures. Moreover, a decrease in  $Q_{10}$  was observed for both  $\text{RR}_{\text{O}_2}$   
420 (1.95) and  $\text{RR}_{\text{CO}_2}$  (2.16) (results not shown).

421 RR at  $20^\circ\text{C}$  indicates the physiological activity of the salads. For all the samples, the  
422 respiratory quotient ( $\text{RR}_{\text{CO}_2}/\text{RR}_{\text{O}_2}$ ) was  $0.95 \pm 0.06$ , and no modification was observed in this  
423 value throughout the experiment. Therefore, only the  $\text{RR}_{\text{CO}_2}$  results ( $\text{mmol h}^{-1} \text{kg}^{-1}$ ) are  
424 presented. Storage of the salad heads increased the RR (measured at  $20^\circ\text{C}$ ) of fresh-cut salad  
425 leaves at  $d_p$ . The values for RR ranged from  $2.4 \text{ mmol h}^{-1} \text{kg}^{-1}$  for non-stored salads to  
426  $4.6 \text{ mmol h}^{-1} \text{kg}^{-1}$  for salad heads stored for 12 d (Fig. 9).

427 ANOVA revealed that storage time had a significant impact on the RRs (at  $20^\circ\text{C}$ ) of fresh-  
428 cut leaves ( $p < 0.0001$ ). The mean RRs (all salad-head storage temperatures included) were 2.4,  
429 3.1, 3.8, and  $4.4 \text{ mmol h}^{-1} \text{kg}^{-1}$  for 0, 5, 9 and 12 d storage, respectively. In contrast, salad head  
430 storage temperature (all storage times included) had no significant impact on the mean  
431 respiration rates. According to Tukey's HSD test (Fig. 9), the RR of fresh-cut samples prepared  
432 from salads stored for the same time was not significantly affected by the storage temperature.  
433 With respect to the storage times, fresh-cut samples from salad heads stored for 5 d at 4, 7, and  
434  $10^\circ\text{C}$  had significantly lower RR than those stored for 12 d at the same temperatures.

435



436

437 **Fig. 9.** Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on the respiration rate  
 438 (RR<sub>CO<sub>2</sub></sub>) measured at 20 °C of fresh-cut leaves just after processing (d<sub>p</sub>). Bars represent the means of  
 439 three replicates. ANOVA was performed on the twelve storage conditions, and bars with different letters  
 440 are significantly different (p<0.05) according to the Tukey HSD test. The dotted line corresponds to  
 441 fresh-cut salads processed from non-stored salad heads. (\*) indicates results significantly different from  
 442 those of the non-stored salads according to the Dunnett test (p<0.05).

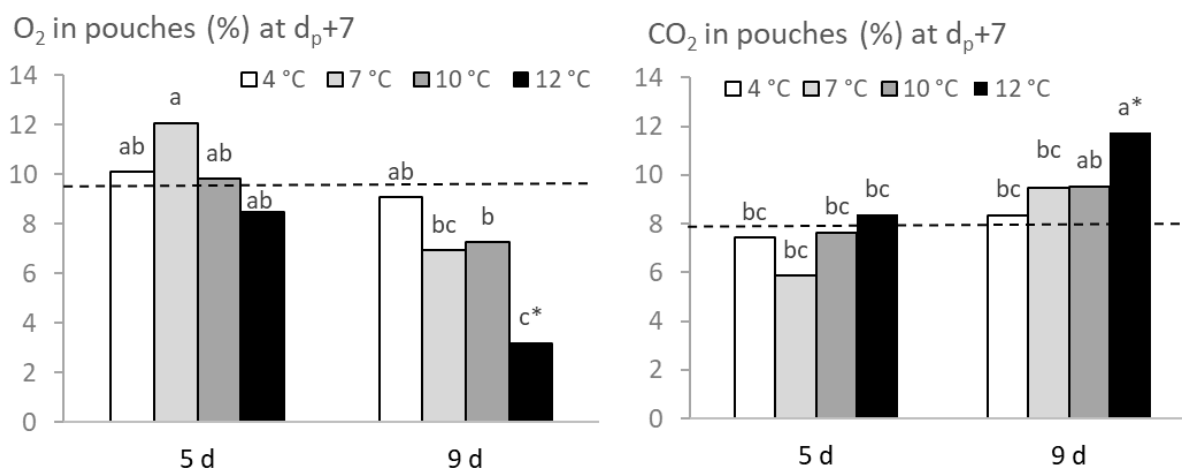
443

444 However, the fresh-cut samples prepared from salads stored for 9 d were not significantly  
 445 different from those stored at other conditions (5 and 12 d). RR was significantly higher for  
 446 fresh-cut leaves from salad heads stored for 9 d at 12 °C than for 5 d at the same temperature.  
 447 Dunnett's test revealed that the RR of fresh-cut leaves prepared from salad heads stored for 5  
 448 d, for all temperatures, and 9 d at 4 °C was not significantly different from that of leaves  
 449 prepared from non-stored salads (dotted line).

450

451 The atmospheres in fresh-cut salad pouches at d<sub>p</sub>+7 for different storage conditions of salad  
 452 heads ranged from 12.1 % O<sub>2</sub>/5.9 % CO<sub>2</sub> to 3.2 % O<sub>2</sub>/11.7 % CO<sub>2</sub>. ANOVA revealed that the  
 453 storage conditions of the salad heads had a significant impact on atmosphere composition  
 (p<0.0001). Furthermore, while time and temperature had a significant effect on the atmosphere

454 composition, their interaction did not have any significant impact. Considering all the time×  
 455 temperature combinations of salad-head storage (Fig. 10), no significant difference was  
 456 observed among pouches prepared from salads stored for 5 d at all temperatures (4, 7, 10, and  
 457 12 °C). For salads stored for 9 d, the O<sub>2</sub> concentration was significantly lower in pouches with  
 458 leaves from salads stored at 12 °C than those stored at 4 and 10 °C. The atmosphere  
 459 compositions of pouches for salads stored for 9 d at 7 and 12 °C were significantly different  
 460 (lower O<sub>2</sub> and higher CO<sub>2</sub>) than those stored for 5 d at the same temperature. Compared to  
 461 pouches prepared from non-stored salads (0 d, dotted line), only those from salad heads stored  
 462 for the longest time and highest temperature (9 d at 12 °C) had a significantly different  
 463 atmosphere composition and were the most modified (Fig. 10).  
 464



465  
 466 **Fig. 10.** Impact of salad-head storage conditions (5 or 9 d at 4, 7, 10 or 12 °C) on atmosphere  
 467 composition (% O<sub>2</sub> and % CO<sub>2</sub>) in the fresh-cut salad pouches after 7 d at 4 °C. Bars represent the mean  
 468 of four replicates. ANOVA was performed on the eight storage conditions, and bars with different letters  
 469 are significantly different (p<0.05) according to the Tukey HSD test. The dotted line corresponds to  
 470 fresh-cut salads processed from non-stored salads (0 d). (\*) indicates results significantly different from  
 471 those of the non-stored salad according to the Dunnett test (p<0.05).

472

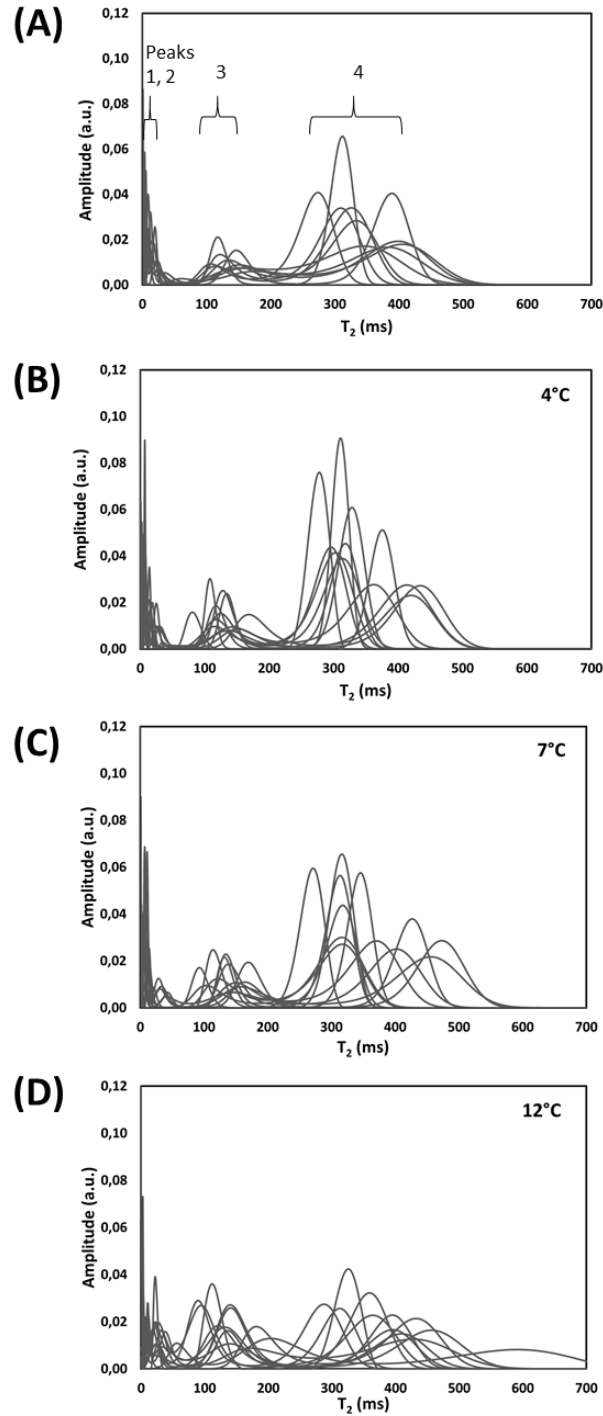


473 *3.6. Impact of salad-head storage conditions on the transverse NMR relaxation time of fresh-*  
474 *cut leaves*

475 Transverse relaxation time ( $T_2$ ) spectra of non-stored salad leaves showed four distinct peaks  
476 (Fig. 11A), each corresponding to particular water fractions. According to Sorin et al. (2019),  
477 the two fast relaxing peaks correspond to 1) water inside starch granules and cell walls and 2)  
478 chloroplast water. The third and fourth peaks were characterized by relaxation times of  
479 approximately 130 and 350 ms and relative signal intensities of 19 and 72 %, respectively.  
480 These peaks are associated with the vacuolar water of cells with distinct volume distributions  
481 (peaks 3 and 4 for small and large vacuoles, respectively, Sorin et al. 2019). In the following  
482 section, only the relaxation peaks associated with the vacuole water pools are analyzed. The  
483 third and fourth peaks of the  $T_2$  spectra ( $T_{2-3}$  and  $T_{2-4}$ ) obtained from the non-stored salad leaves  
484 were relatively homogeneous (Fig. 11A), to the extent that can be expected for vegetable  
485 materials grown under natural conditions.

486 Fig. 11B, C, and D present spectra of fresh-cut salads stored for 5 d at 4 °C ( $d_p+5$ ), (prepared  
487 from salad heads stored for 11 d at 4, 7, and 12 °C).  $T_2$  spectra of the fresh-cut leaves prepared  
488 from salads stored at 4 °C (Fig. 11B) were similar to those of non-stored salad leaves (Fig.  
489 11A). For the processed salads prepared from the salad heads stored at 7 °C, dispersion of the  
490 fourth peak increased with salad storage time and fresh-cut leaves from salads stored for 11 d  
491 exhibited relatively large variability in spectra (Fig. 11C), thereby demonstrating the  
492 heterogeneity of the samples. This phenomenon was prominent in the case where the salad  
493 heads were stored at 12 °C (Fig. 11D).

494



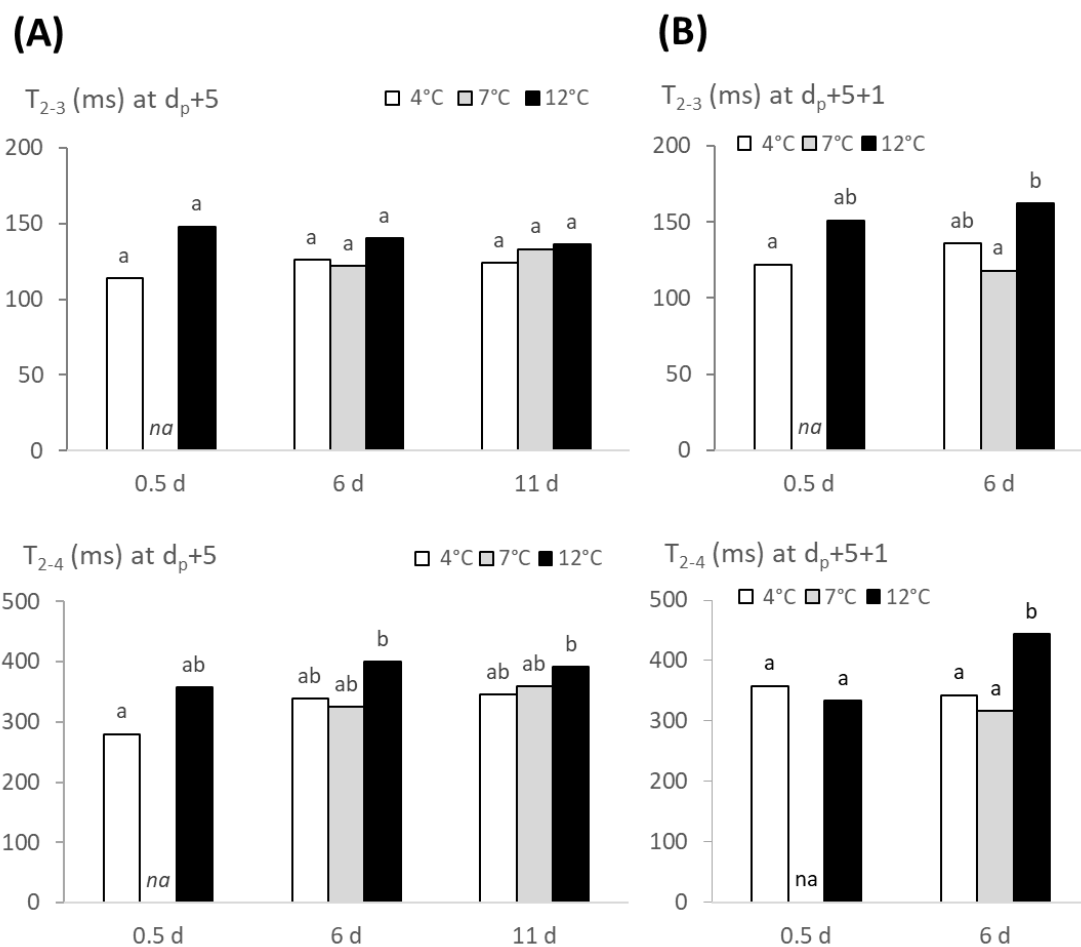
495

496 **Fig. 11.**  $T_2$  (transverse relaxation time) spectra of non-stored salad leaves (A) and of fresh-cut leaves  
 497 after 5 d at 4 °C ( $d_p+5$ ), prepared from salad heads stored for 11 d at 4 °C (B), 7 °C (C) and 12 °C (D).

498

499 Fig. 12 depicts the mean  $T_2$  of the two vacuolar peaks (3 and 4) in fresh-cut salad leaves at  
 500  $d_p+5$ ,  $d_p+5+1$ , and for different storage conditions of the salad head (storage times and  
 501 temperatures). For fresh-cut salads at  $d_p+5$ ,  $T_{2-3}$  remained constant over time for all conditions,

502 with no significant differences among the various salad-head storage conditions (Fig. 12A). For  
 503 salads stored at 4 and 7 °C,  $T_{2-4}$  did not significantly differ with the storage time (0.5, 6, or 11  
 504 d). Considering all salad-head storage temperatures and times, only the salads stored for 6 and  
 505 11 d at 12 °C showed a significant increase in  $T_{2-4}$  (compared to salads stored for 0.5 d at 4 °C).  
 506 None of the other conditions were significantly different from each other.  
 507

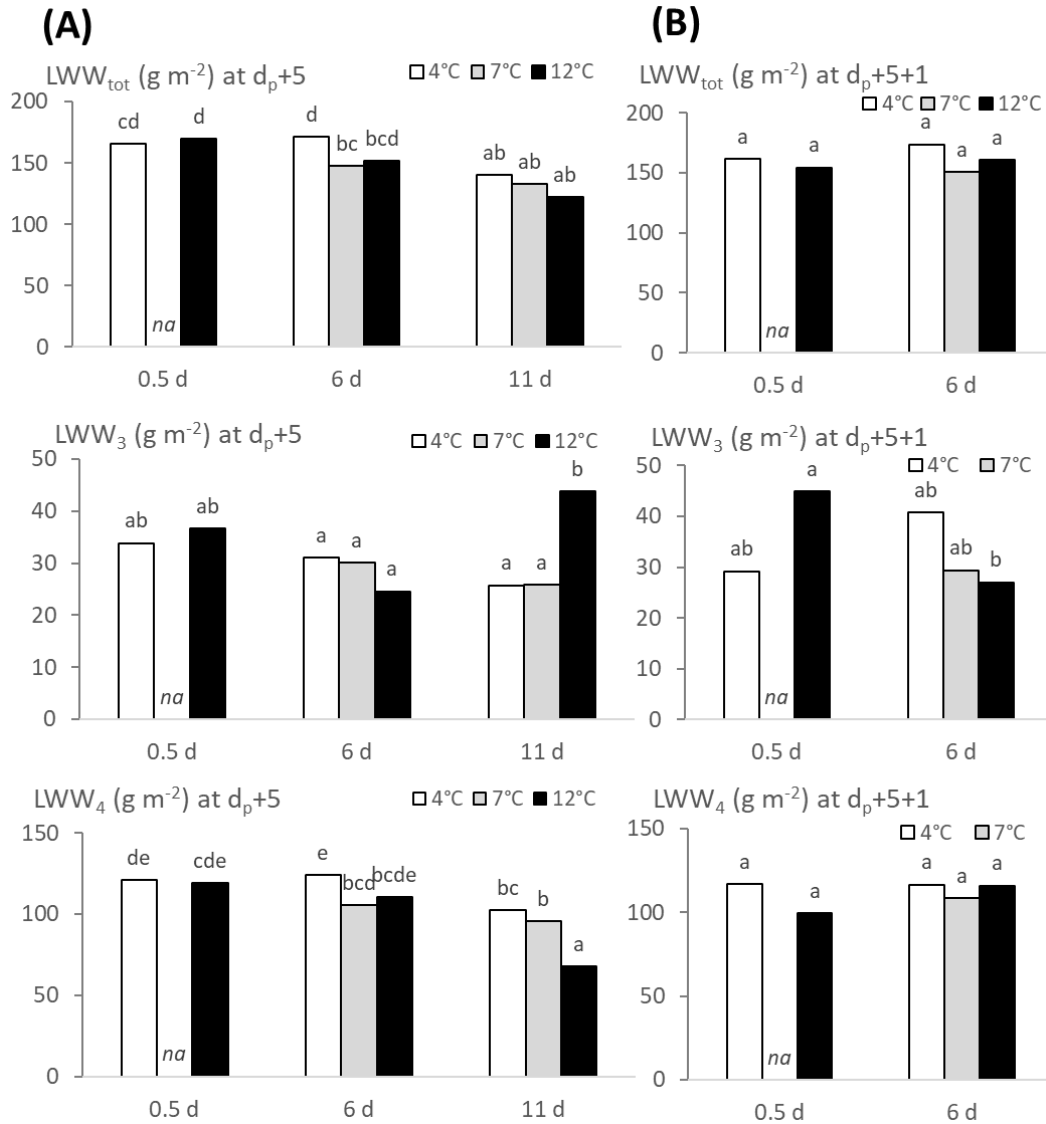


508  
 509 **Fig. 12.**  $T_2$  (transverse relaxation time) of the two vacuolar peaks (3 and 4) for fresh-cut packaged leaves  
 510 after 5 d at 4 °C ( $d_p+5$ ) (A) and 1 d after pouch opening ( $d_p+5+1$ ) (B), processed from salad heads stored  
 511 0.5, 6, and 11 d at 4, 7, and 12 °C. The results represent the mean of twelve replicates unless too many  
 512 leaves were damaged. ANOVA was performed for the nine storage conditions, and bars with different  
 513 letters are significantly different ( $p<0.05$ ) according to the Tukey HSD test. *na*: not available because  
 514 of technical difficulties.

515 One day at 4 °C in the open pouches increased the  $T_{2-4}$  of fresh-cut salads; however, this was  
516 not observed for all salad-head storage conditions (comparing panels A and B in Fig. 12). Fresh-  
517 cut salads from salads stored at the highest temperature and the longest time (12 °C, 6 d) had a  
518 significantly higher  $T_{2-4}$  than all other conditions; these values were not significantly different  
519 from each other (Fig. 12B). Similarly, the results recorded at the opening of the pouches were  
520 not significantly different (Fig. 12A). Notably, at  $d_p+5+1$ , fresh-cut leaves prepared from salads  
521 stored for 11 d (regardless of the temperature) were too damaged to be analyzed.

522

523  $LWW_{tot}$  decreased with increase salad-head storage time, thereby indicating loss of water  
524 from the leaves (Fig. 13A). The amount of water in the specific vacuolar compartments was  
525 followed by that in  $LWW_3$  and  $LWW_4$ . For salad heads stored at 4 °C and 7 °C,  $LWW_3$  and  
526  $LWW_4$  almost remained constant, while at 12 °C,  $LWW_3$  and  $LWW_4$  remained stable until the  
527 last day of measurement; this indicates that water redistribution between vacuoles of two cell  
528 types occurred at 11 d. At  $d_p+5+1$  (Fig. 13B),  $LWW$  remained stable between 0.5 and 6 d,  
529 except for  $LWW_3$ , which decreased for salad-heads stored at for 12 °C.



530

531 **Fig. 13.** LWW<sub>tot</sub> (total leaf water weight) and specific LWW of the two vacuolar peaks (3 and 4) for  
 532 fresh-cut packaged leaves after 5 d at 4 °C (d<sub>p</sub>+5) (A) and 1 d after pouch opening (d<sub>p</sub>+5+1) (B),  
 533 processed from salad heads stored for 0.5, 6 and 11 d at 4, 7 and 12 °C. The results represent the mean  
 534 of twelve replicates unless too many leaves were damaged. ANOVA was performed on the nine storage  
 535 conditions, and bars with different letters are significantly different (p<0.05) according to the Tukey  
 536 HSD test. *na*: not available due to technical difficulties.

537

### 538 3.7. Experiments with salads from the different seasons and geographical origin

539 The main experiment (presented in sections 3.1 to 3.6) was conducted on salads grown in  
 540 southeast France and harvested in spring. The results were completed by two experiments,

541 performed on salads grown in the same area but later in the season (end of June) and on salads  
542 grown in a different area (northeast of France, season beginning). ANOVA was performed on  
543 the results of these three experiments, considering the following factors: “experiment,” “storage  
544 time of salad head (d),” and “storage temperature of salad head (°C)”. The conditions selected  
545 for time and temperature were those common for all three experiments: 5, 9 and 12 d, and 4, 7  
546 and 10 °C (12 °C was not repeated as it highly deteriorated the quality of the fresh-cut product).  
547 Variables included were the percentage of salad heads used to process the fresh-cut salads after  
548 trimming (at  $d_p$ ), the visual aspects of the fresh-cut salads and the percentage of pink cut  
549 surfaces at the opening of the pouches ( $d_p+7$ ), and the atmosphere composition (% CO<sub>2</sub>) in the  
550 fresh-cut salad pouches at  $d_p+7$ . No significant difference was observed between salad-head  
551 storage conditions (see section 3.4); thus, the determination of total aerobic bacteria of the fresh-  
552 cut salads were not repeated.

553 ANOVA revealed a significant impact of the three factors on all tested variables. Table 1  
554 presents the results of the Tukey HSD test for the three factors and four variables. Fresh-cut  
555 salads prepared from south-late season and east-early season salads had a significantly poorer  
556 visual aspect than those from the south-early season salads (main experiment previously  
557 presented), with an average visual aspect of 2.1 and 2.2 instead of 3.7, respectively. Based on  
558 the pink cut surfaces, fresh-cut leaves from northeast salads were of lower quality than those  
559 from the late season in southeast France; furthermore, both these fresh-cut salads showed more  
560 pink cut surfaces than those observed in south/early season salads (Table 1). The differences in  
561 quality were not associated with the atmosphere composition of the pouches. The mean values  
562 of the four variables were significantly different among the salad storage times (5, 9, and 12 d  
563 for the percentage of salads used; 5 and 9 d for the visual aspect, pink cut surfaces, and  
564 atmosphere composition). With respect to storage temperatures of salad head (4, 7, and 10 °C),  
565 the mean values were significantly different at 10 °C (high trimming, low visual aspect, high

566 CO<sub>2</sub>); however, the values were similar at 4 and 7 °C, except for pink cut surfaces, which were  
 567 significantly less important for fresh-cut leaves from salads stored at 4 than at 7 and 10 °C.

568

569 **Table 1:** Impact of experiments, times and temperatures of salad-head storages on the percentage of  
 570 salads used after trimming to process fresh-cut salads (processing day, d<sub>p</sub>) and on the visual aspect,  
 571 percentage of pink cut surfaces and atmosphere composition (% CO<sub>2</sub>) of the fresh-cut salad pouches  
 572 after 7 d at 4 °C (d<sub>p</sub>+7). Results of Tukey HSD test from an ANOVA with three factors (Experiment,  
 573 Time, and Temperature), three conditions each and four variables.

Factors	Conditions	Variables							
		% used after trimming <sup>1</sup>		Visual aspects <sup>2</sup>		Pink cut surface <sup>2</sup>		%CO <sub>2</sub> <sup>2</sup>	
		Mean value <sup>3</sup>	Tukey HSD groups <sup>4</sup>	Mean value <sup>3</sup>	Tukey HSD groups <sup>4</sup>	Mean value <sup>3</sup>	Tukey HSD groups <sup>4</sup>	Mean value <sup>3</sup>	Tukey HSD groups <sup>4</sup>
<b>Experiment<sup>5</sup></b>	<b>south/early</b>	58.7	a	3.7	a	27.9	a	8.1	b
	<b>south/late</b>	62.4	b	2.1	b	76.2	b	7.9	b
	<b>east/early</b>	65.8	b	2.2	b	84.0	c	8.8	a
<b>Time (d)<sup>6</sup></b>	<b>5</b>	71.9	a	3.1	a	56.6	a	7.6	b
	<b>9</b>	62.3	b	2.2	b	68.7	b	8.9	a
	<b>12</b>	52.6	c	nd	nd	nd	nd	nd	nd
<b>Temperature (°C)<sup>7</sup></b>	<b>4</b>	63.6	a	3.0	a	58.0	a	8.0	b
	<b>7</b>	64.6	a	2.8	a	63.4	b	8.0	b
	<b>10</b>	58.6	b	2.2	b	66.6	b	8.7	a

574 <sup>1</sup>: Measured on processing day (d<sub>p</sub>) at the end of the salad-head storage.

575 <sup>2</sup>: Measured after 7 d storage at 4 °C of the fresh-cut salad pouches (d<sub>p</sub>+7).

576 <sup>3</sup>: For each condition of a factor, means were calculated from the values of all the conditions of the other  
 577 factors.

578 <sup>4</sup>: Mean values from different Tukey HSD group are significantly different (p<0.05).

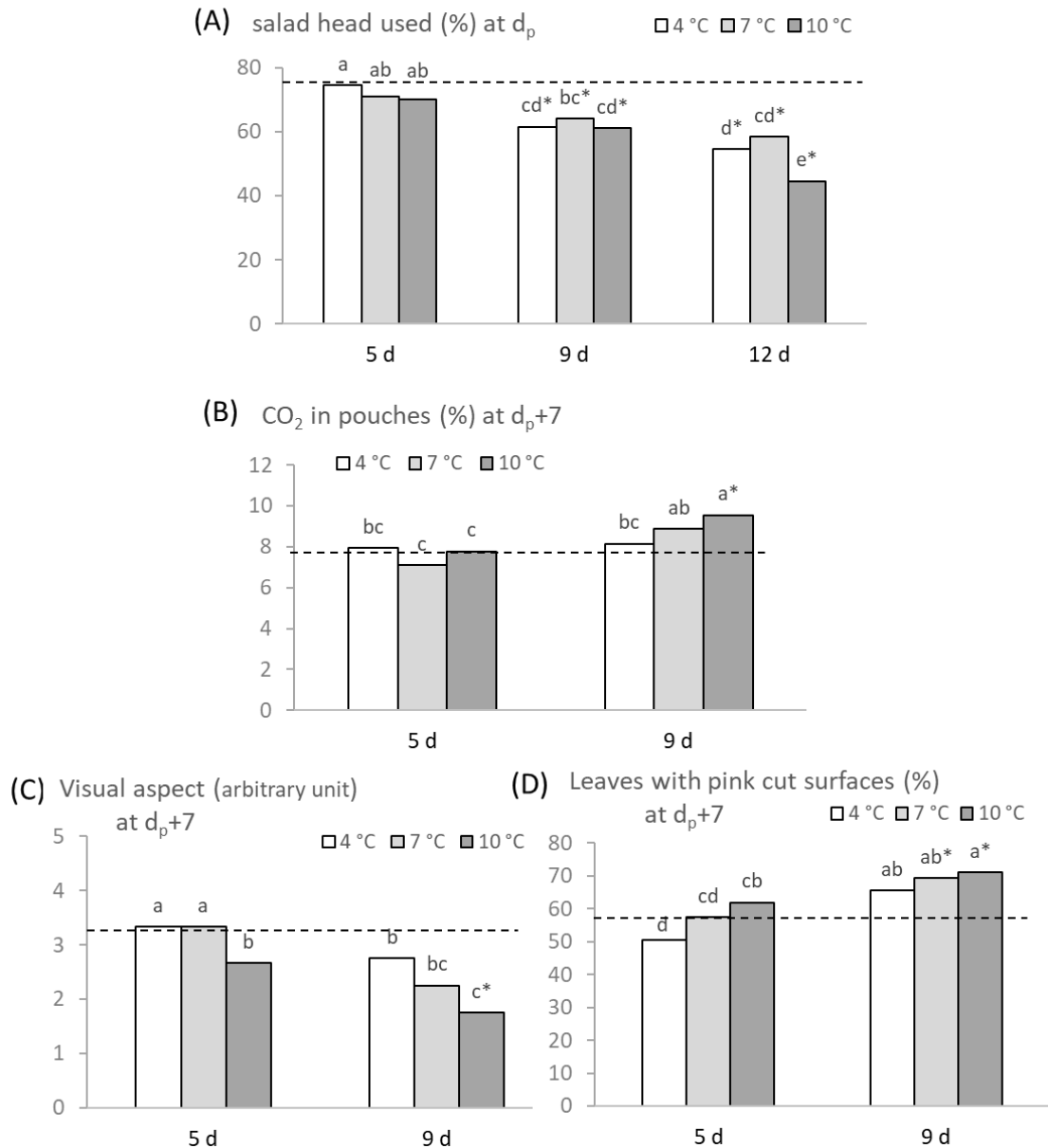
579 <sup>5</sup>: The three experiments were conducted with salads from different geographical origins and production  
 580 periods.

581 <sup>6</sup>: Storage times of salad heads before processing of fresh-cut salads.

582 <sup>7</sup>: Storage temperatures of salad heads before processing of fresh-cut salads.

583

584 The mean of the four variables over the three experiments for all combinations of salad-head  
 585 storage times and temperatures are shown in Fig. 14.



586

587 **Fig. 14.** Impact of salad-head storage conditions (5 or 9 d at 4, 7 or 10 °C) on the percentage of salads  
 588 used after trimming to process fresh-cut salads (at  $d_p$ ) (A) and on concentrations of CO<sub>2</sub> (B), global  
 589 visual aspects (C), and percentages of leaves with pink cut surfaces (D) in the fresh-cut salad pouches  
 590 after 7 d at 4 °C ( $d_p+7$ ). Results represent the mean of three independent experiments conducted with  
 591 salads from different geographical origins and harvested during different seasons, with six (A) and four  
 592 replicates (B, C, D) per experiment. Bars with different letters are significantly different according to  
 593 the Tukey HSD test ( $p<0.05$ ). The dotted line represents fresh-cut leaves from non-stored salads. (\*)  
 594 indicates results significantly different from those of the non-stored salads according to the Dunnett test  
 595 ( $p<0.05$ ).



596

597 For a storage of 5 d of the salad heads, percentages of salads used were not significantly  
598 affected by the storage temperature (4, 7, and 10 °C) (Fig. 14A). Regardless of the storage  
599 temperature, these values were significantly higher than those of salads stored for 12 d;  
600 furthermore, they were similar to those of the non-stored salads. With respect to the atmosphere  
601 composition in the pouches at  $d_p+7$  (Fig. 14B), no significant differences were observed  
602 between the three storage temperatures for 5 d salad storage time and 4 °C for the 9 d storage.  
603 For storage temperatures of 7 and 10 °C, atmosphere contained significantly more CO<sub>2</sub> in the  
604 pouches prepared from salads stored for 9 than for 5 d. Furthermore, the atmospheres of the  
605 pouches prepared from salads stored for 9 d at 10 °C were significantly different from those  
606 from non-stored salad heads.

607 Visual aspects of pouches at  $d_p+7$  (Fig. 14C) prepared from salads stored for 5 d at 4 and  
608 7 °C were not significantly different. Furthermore, the visual aspects of these salads were  
609 significantly better than those of the salads stored for longer periods and/or at higher  
610 temperatures. Pink cut surfaces (Fig. 14D) were more frequent in pouches prepared from salad  
611 heads stored for 9 than for 5 d; for each storage time, there was no significant difference among  
612 the three storage temperatures (4, 7, and 10 °C).

613

#### 614 **4. Discussion**

615 The variables used in the present study to characterize the impact of salad-head storage  
616 conditions covered a wide range of parameters in fresh-cut salad quality. In addition, the  
617 percentage of salads used for processing plays an important role in determining the economic  
618 and environmental performance of the process. After trimming the salad heads, regardless of  
619 the storage conditions, all leaves processed into fresh-cut salads were visually undamaged.  
620 Nevertheless, increasing the duration and temperature of salad storage globally reduced the

621 technical yield, visual quality, and texture of fresh-cut salad at the end of shelf life (7 d at 4 °C).  
622 It also modified the physiology of the fresh-cut salad, as indicated by an increase in the RR.  
623 However, these parameters were not affected to the same extent by salad-head storage  
624 conditions.

625 RR of the leaf tissues increases after wounds, cuts, and exposure to ethylene (Martínez et al.  
626 2005, Deltisdis et al. 2012). RR at 20 °C was measured at each  $d_p$  as an indicator of the  
627 physiological status of the fresh-cut salad leaves. Part of the RR was presumably due to the  
628 stress caused by cutting the leaves as respiration rate decreased after 24 h, as well as the  $Q_{10}$ ,  
629 presumably reflecting a transitory effect of cutting stress on RR, as previously observed by  
630 Martínez et al. (2005). Storage of salad heads for 9 d or more increased the RR of the fresh-cut  
631 salad; this suggests an increased impact of cutting stress. Furthermore, this stress may have  
632 contributed to the loss in quality of the fresh-cut products as a higher respiration rate has been  
633 linked to the shorter shelf life of fresh-cut salads (Varoquaux et al. 1996, Kim et al. 2004,  
634 Charles et Varoquaux 2016).

635 Fresh-cut salads were packaged in pouches of polymeric film, sealed under air. In this  
636 passive modified atmosphere packaging, the atmosphere composition results from the balance  
637 between product respiration and film permeability (Varoquaux et al. 2002). Any excessive  
638 modification of the atmosphere in the pouches can lead to a metabolic shift and/or  
639 phytotoxicity, thereby causing damage to the leaf tissue (Varoquaux et al. 1996, Kim et al.  
640 2004, Paillart et al. 2017). In contrast, atmosphere modification can reduce the browning and  
641 pink discoloration of cut leaf tissues (López-Gálvez et al. 1996). An increase in atmosphere  
642 modification was observed in the pouches made from salad heads stored for the longest time at  
643 the highest temperature.

644 Some components of the aerobic microflora can contribute to the spoilage of fresh-cut salad  
645 (Nguyen-the and Carlin 1994, Paillart et al. 2017). Furthermore, the total count of aerobic

646 microflora has been included as a process hygiene criterion in the guide of good manufacturing  
647 practices in France (Anonymous 1988) and specification for the retailers' association in France  
648 (FCD 2019). In our study, we could not identify an association between total mesophilic  
649 bacteria in the fresh-cut product and salad-head storage conditions. Furthermore, no association  
650 with other quality parameters was observed. Some studies have shown a lack of correlation  
651 between total aerobic microflora during processing and spoilage with good quality fresh-cut  
652 salads at the end of storage, despite the high counts of aerobic microflora (Allende et al. 2008).

653 The texture of leaf pieces is an important quality attribute of fresh-cut salads; these leaf  
654 pieces should retain their crunchy texture until consumption. The texture is often assessed by  
655 measuring the mechanical properties of the leaf tissue (Martín-Diana et al. 2006, Tsironi et al.  
656 2017). In our study, the maximum load of fresh-cut products in the Kramer shear test was  
657 affected by the long storage time of the salad heads. Moreover, we observed an increase in the  
658 b coefficient (puncture test) with salad-head storage time and temperature. This might indicate  
659 an increase in the deflection needed before the rupture of leaf tissues, thereby corresponding to  
660 a more elastic and less crunchy texture. This finding may explain the higher maximum load for  
661 the Kramer test at  $d_p$  under some salad-head storage conditions. In contrast, differences in leaf  
662 composition due to decay of the most external leaves for the most extreme salad-head storage  
663 conditions might have reduced the maximum load in the Kramer test; however, there were a  
664 few significant differences between the groups. Sorin et al. (2019) showed that an increase in  
665 NMR transverse relaxation times during the storage of salad heads was a sensitive method to  
666 detect changes in water status and distribution within leaf tissues, which may result in texture  
667 alteration. In the present study, NMR transverse relaxation times of fresh-cut products increased  
668 with the salad-head storage time and temperature; however, the results were significant only  
669 for the most extreme conditions tested, such as for mechanical texture tests. Previous studies

670 have shown that texture is not the first quality parameter to deteriorate with increasing storage  
671 time and temperature of fresh-cut salads (Manolopoulo et al. 2010, Manzocco et al. 2017).

672 The visual aspect, global or pink discoloration, has a strong impact on the attractiveness of  
673 the product. The global visual aspect and pink cut surfaces of the stored fresh-cut salads were  
674 the most discriminant of the parameters studied, with a significant loss in visual quality and a  
675 significant increase in pink cut surfaces (except for the experiment with south/early season  
676 salads) when salad heads were stored over 7 °C or for more than 5 d. Our results are consistent  
677 with those reported by López-Gálvez et al. (1996), who found that storage of romaine salad  
678 heads for 7 d at 5 °C decreased visual quality and increased leaf edge browning. In contrast, the  
679 storage of baby spinach before processing had no effect on the visual quality of the processed  
680 product (Garrido et al. 2015). However, in this study, the maximum storage time tested was  
681 48 h at 4 °C, which is shorter than in our study (Garrido et al. 2015). Global visual quality  
682 results from the combined effect of several phenomena, such as leaf senescence (Charles and  
683 Varoquaux 2016), microbial soft rot at the leaf margins (Nguyen-the and Punier 1989, Nguyen-  
684 the and Carlin 1994), and de-structuration of the leaf tissues leading to contact between  
685 enzymes and substrates, thereby resulting in discoloration (Charles and Varoquaux 2016), thus  
686 ensuring quality losses. In our results, the role of microbial spoilage in the loss of visual quality  
687 is questionable, as no consistent impact of salad-head storage on total mesophilic bacteria of  
688 fresh-cut product was observed. Castañer et al. (1999) found that the polyphenol oxidase (PPO),  
689 and phenolic compounds in the midribs, increased during the cold storage of romaine and baby  
690 lettuce. This suggests that, in our study, salad-head storage increased PPO and phenolic  
691 compounds in the leaf tissues, thereby leading to a higher discoloration potential, revealed after  
692 fresh-cut processing. The highest percentage of leaves with pink cut surfaces was observed in  
693 pouches made from salad heads stored for 9 d at 12 °C. This is surprising, as these pouches had  
694 the most modified atmosphere, which could have inhibited the discoloration. However, pouches

695 were packaged under air, and the atmosphere was measured only once after 7 d; discoloration  
696 might have occurred prior the CO<sub>2</sub> accumulation.

697 The quality variables measured for salad heads stored for 5 d were not significantly different  
698 between 4 and 7 °C. In addition, these values were not significantly different from those  
699 measured in fresh-cut salads processed from non-stored salad heads (0 d). Similarly,  
700 Koukounaras et al. (2018) found no impact on the browning of cut surfaces of lettuce heads  
701 stored for 3 and 6 d at 5 °C before processing. Rogers et al. (2006) noted that lettuce heads  
702 stored for 5 d at 4 °C before processing did not decrease the global quality index of the fresh-  
703 cut product in case of hard trimming.

704 For longer storage (e.g. 9 d), even the lowest of the temperatures tested (i.e. 4 °C) caused a  
705 significant decrease in several of the quality variables measured for the fresh-cut salads. This  
706 was particularly true for the technical yield, global visual quality, and pink discoloration.

707 The quality of fresh-cut salads is strongly affected by the production period, climate, and  
708 cultural practices used for the salad-head growing (Seefeldt et al. 2012, Tudela et al. 2013,  
709 Monaghan et al. 2016, Tudela et al. 2017). Monaghan et al. (2016) noted that less irrigation  
710 reduced the pink discoloration of ribs. Furthermore, heavy rainfall in spring 2018 in the  
711 southeast and northeast France might have contributed to the higher prevalence of pink  
712 discoloration in experimental replicates compared to that in the experiment with early season  
713 salads, thereby resulting in a relatively low quality at the end of shelf life. Despite these marked  
714 differences, the three experiments in unison confirmed the trend observed in the main  
715 experiment (sections 3.1 - 3.6).

716 In conclusion, for the range of salads tested in this study, storage of salad heads for up to 5 d  
717 at temperatures of up to 7 °C had no measurable impact on the quality of fresh-cut salad at the  
718 end of shelf life (compared to non-stored salads or salads stored at 4 °C). The range of  
719 temperature variations during salad storage—recorded in the partner processing plant (between

720 5 and 8 °C) and confirmed by the fresh-cut salad processor association—should therefore not  
721 cause any quality loss (compared to salads processed immediately), provided that this storage  
722 period does not exceed a few days.

723 Refrigeration of raw material at sufficiently low temperatures is necessary to maximize the  
724 quality of the fresh-cut produce. However, refrigeration represents an important part of energy  
725 consumption. Approximately 30 % of the electricity consumption in the EU food industry is  
726 attributed to cooling and freezing (Monforti-Ferrario et al. 2015). Therefore, it is important to  
727 optimize the refrigeration temperature with respect to the quality requirements of the food  
728 products (Guillier et al. 2016). For instance, in the case of a plant processing refrigerated fresh  
729 pasta, increasing the temperature of cold rooms from 4 °C to 6 °C reduced the absorbed  
730 electrical power by 12 % for cold rooms and by 7 % for the entire processing line (Duret et al.  
731 2021). For raw material storage not exceeding five days, storing raw salads at 7 °C before fresh-  
732 cut processing appeared as a good compromise that permits quality preservation while saving  
733 refrigeration energy (compared to 4 °C).

734

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744

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746 The authors declare that they have no known competing financial interests or personal  
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748

749 **CRedit authorship contribution statement**

750 **Barbara Gouble:** Investigation, Validation, Formal analysis, Writing-Review & Editing,

751 Visualization **Maja Musse:** Investigation, Writing-Review & Editing **Steven Duret:**

752 Investigation, Writing-Review & Editing **Patrice Reling:** Resources, Investigation **Evelyne**

753 **Derens-Bertheau:** Investigation **François Mariette:** Investigation, Supervision **Clément**

754 **Sorin:** Investigation **Erwann Hamon:** Resources, Investigation **Valérie Stahl:**

755 Conceptualization, Investigation **Christophe Nguyen-The:** Investigation, Writing-Review &

756 Editing, Supervision, Project administration, Funding acquisition

757

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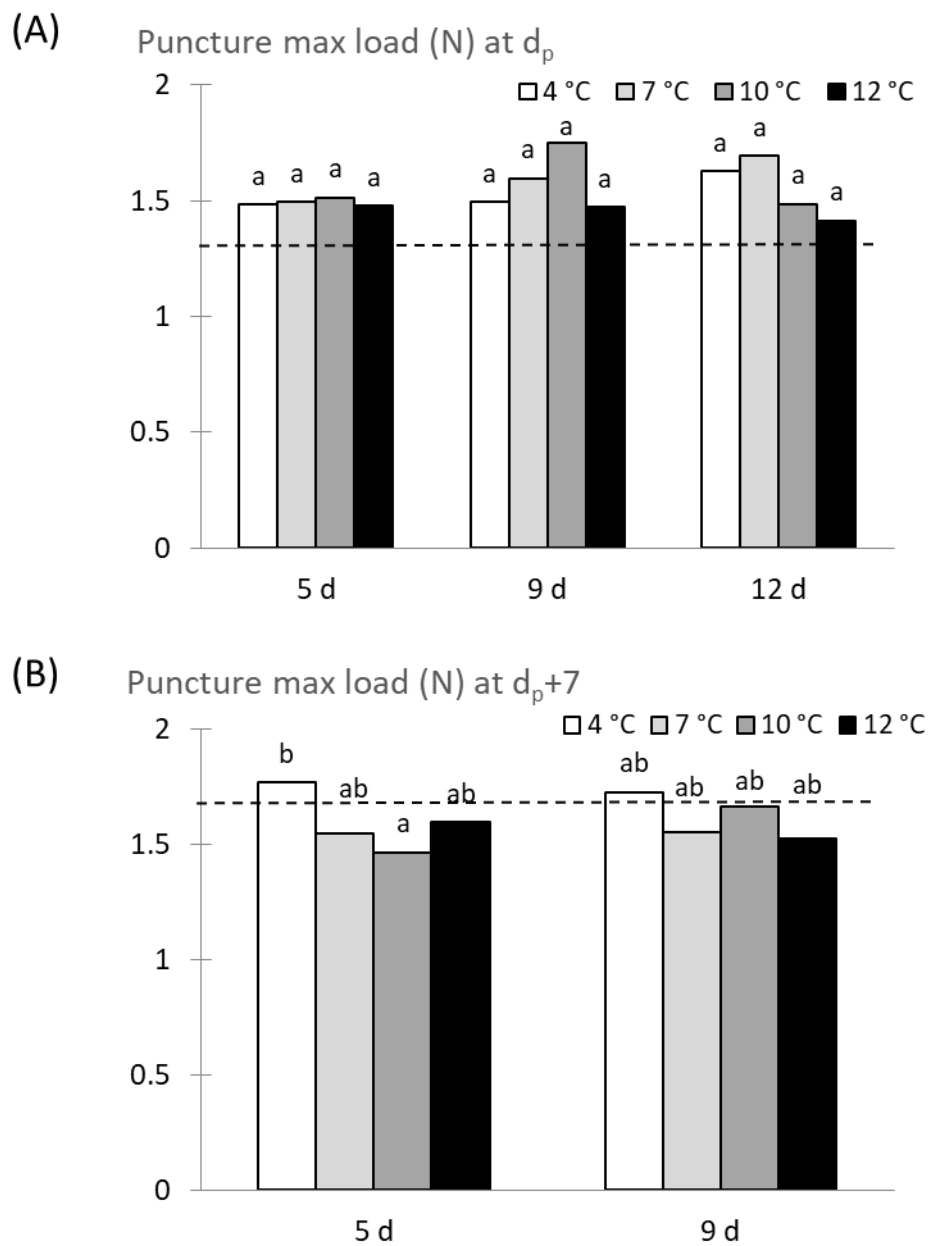
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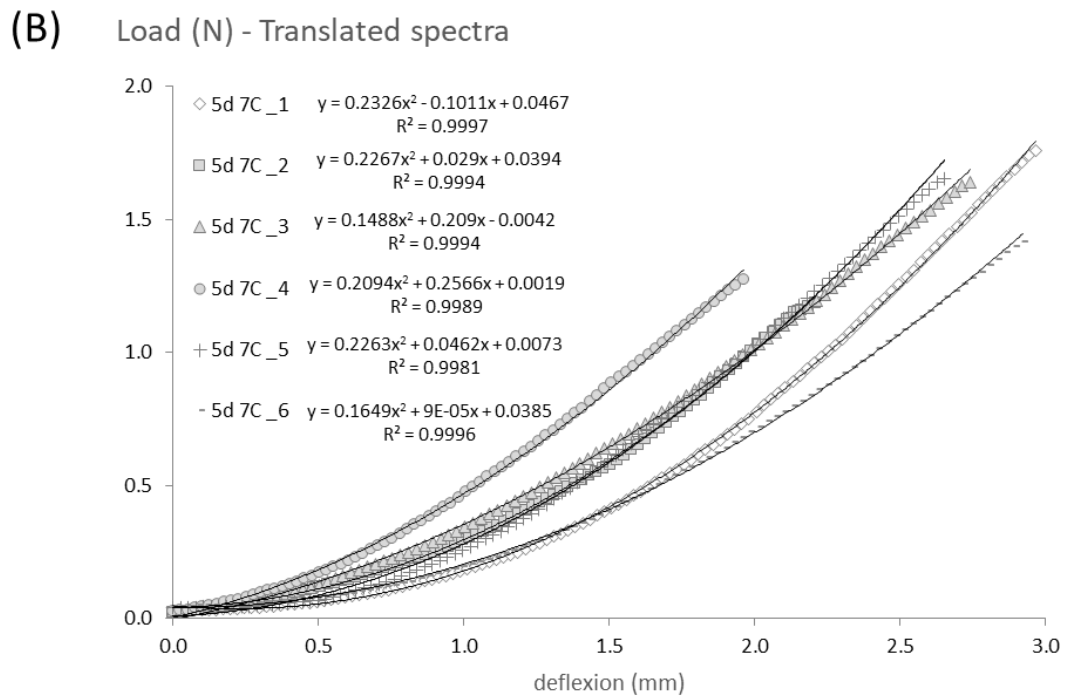
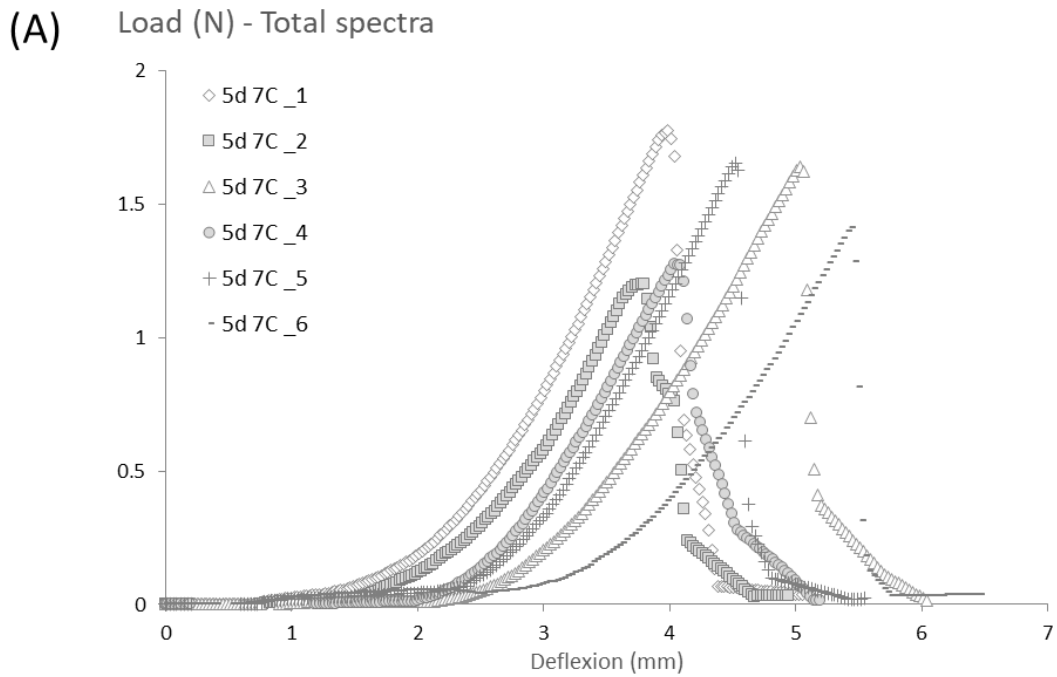


873

874 **Fig. S1.** Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on maximum load  
 875 (N) for puncture test on fresh-cut salad just after processing ( $d_p$ ) (A) and after 7 d storage at 4 °C ( $d_p+7$ )  
 876 (B). Bars represent means of six to sixteen replicates for puncture test. ANOVA was performed on the  
 877 twelve or eight storage conditions and bars with different letters are significantly different ( $p < 0.05$ )  
 878 according to the Tukey HSD test. Dotted line corresponds to fresh cut salads processed from non-stored  
 879 salads.

880

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882

883 **Fig. S2.** Puncture test on six salad leaves after 5 d storage at 7 °C. (A) Total spectra (B) Translated

884 spectra (from 0.025 N to maximum load) and polynomial equations.

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886