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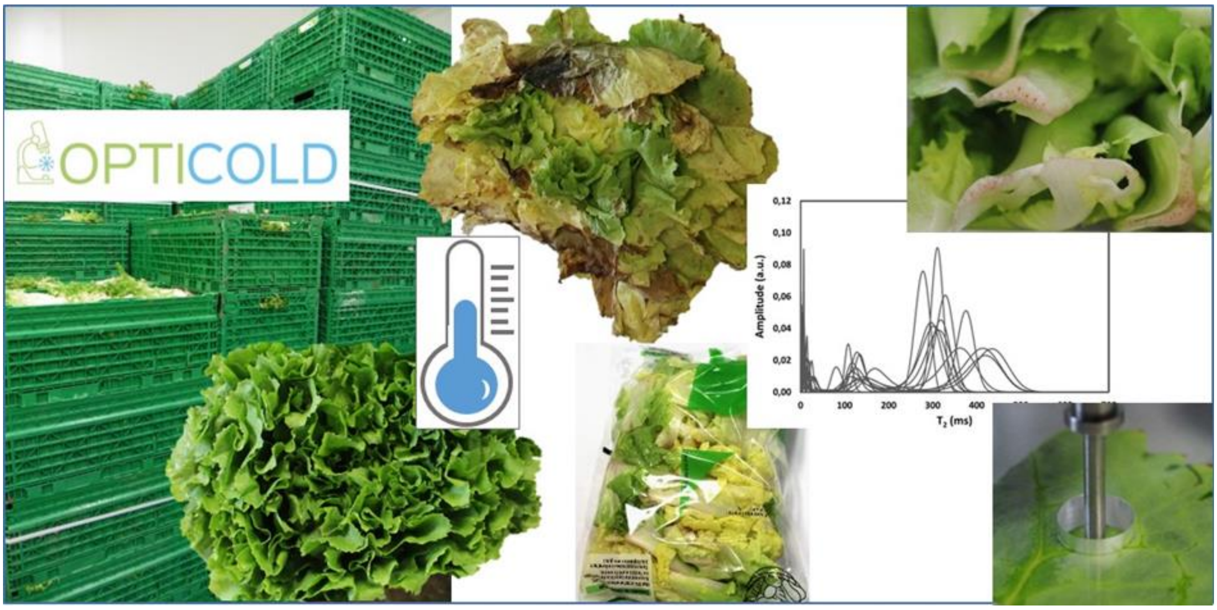
Highlights

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

Graphical abstract



ABSTRACT

The impact of temperature and storage time of escarole salad heads on the quality of processed, fresh-cut salad was investigated. Based on temperatures recorded in a fresh-cut processing plant, salad heads were stored for four different storage times (0, 5, 9, and 12 d) at four temperatures (4, 7, 10, and 12 °C) before being processed. A range of quality criteria (technical yield, global visual aspect, pink cut surfaces, mechanical texture, aerobic microflora, respiration rate, atmosphere composition in fresh-cut salad pouches, nuclear magnetic resonance transverse relaxation time) were measured on processing day, after 7 d storage at 4 °C of the fresh-cut salad packaged in pouches, and 1 d after pouch opening. The results are presented for salads grown in southeast France and early-season harvest, validated by experimental repetitions with a late-season harvest and salads from another geographical origin. Storage of salad heads caused deterioration of all quality attributes except for total aerobic bacteria. The global visual aspect was the most sensitive to changes in storage conditions of salad heads (significant reduction in quality for 5 d over 7 °C). In contrast, mechanical texture (maximum load for the shear test) was only significantly different for the fresh-cut salad prepared from salad heads stored for 9 d at 12 °C. For all quality criteria, fresh-cut salads processed from salad heads stored for 5 d at 4 and 7 °C were not significantly different from those of non-stored salad heads. For storage time of 5 d or less, a temperature of 7 °C is likely as good as 4 °C for escarole salad-head storage intended for fresh-cut processing.

Keywords:

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

1. Introduction

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

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

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

2. Materials and methods

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

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

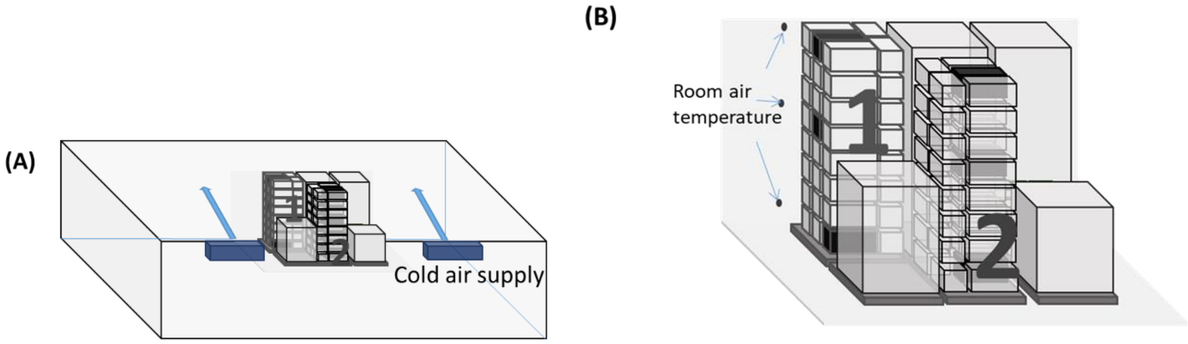


Fig. 1. Temperature monitoring in the factory cold room. (A) Disposition of the products in the cold room (real dimensions unknown). (B) Positions of the sensors for room air temperature and for product temperature measurements (black box) in the two pallets.

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

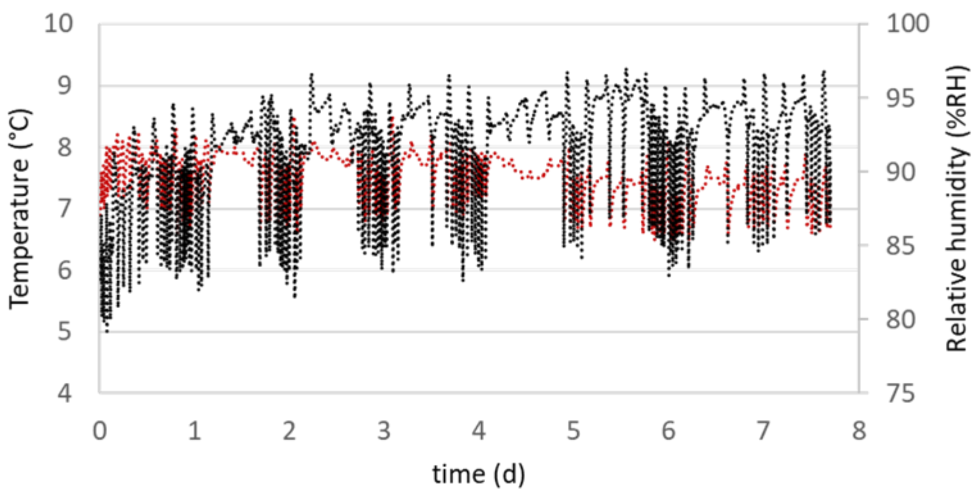


Fig. 2. Recording over eight days of cold room air temperature (red dotted line) and relative humidity (black dotted) in a fresh-cut processing plant.

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

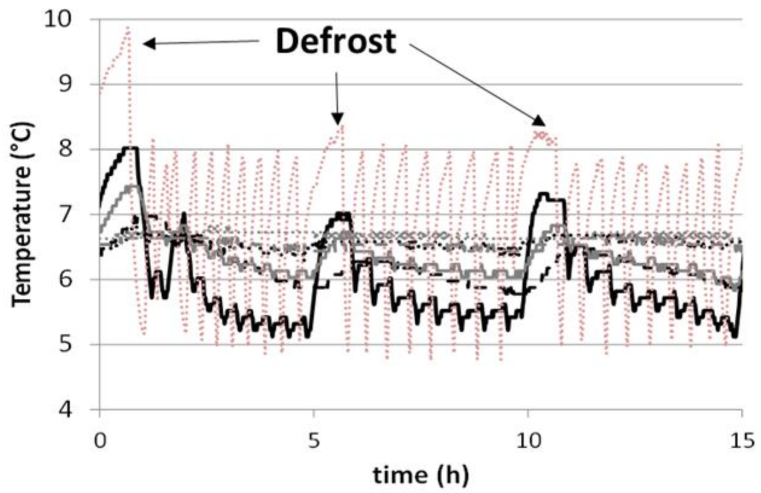


Fig. 3. Focus of a recording over one-day of air (red dotted line) and salad-head temperatures at different locations in the pallets (pallet 1: black lines, pallet 2: gray lines, top: solid lines, middle: dashed lines, bottom: dotted lines) in a fresh-cut processing plant.

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

- 4 °C was selected as the lowest temperature as it represents the temperature that should be achieved by the fresh-cut product at the end of the processing;
- 7 and 10 °C were selected as intermediate temperatures because these temperatures are close to the range of temperatures recorded in the partner factory;
- 12 °C was included as an extremely high temperature to ensure an impact on the quality and physiology of the processed product.

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

2.2. Plant material

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

Two repetitions of the experiment were conducted:

- One on escaroles harvested on June 4th 2018 (the end of the season) and purchased from the same producer.
- Another on open-field escaroles var. *latifolium* Leika (CLX 1001, Clause, France) purchased from a market gardener in northeast France (Balgau, Alsace) on June 25th (season beginning in this part of France) and sent to INRAE Avignon in refrigerated trucks.

2.3. Salad-head storage and fresh-cut processing

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

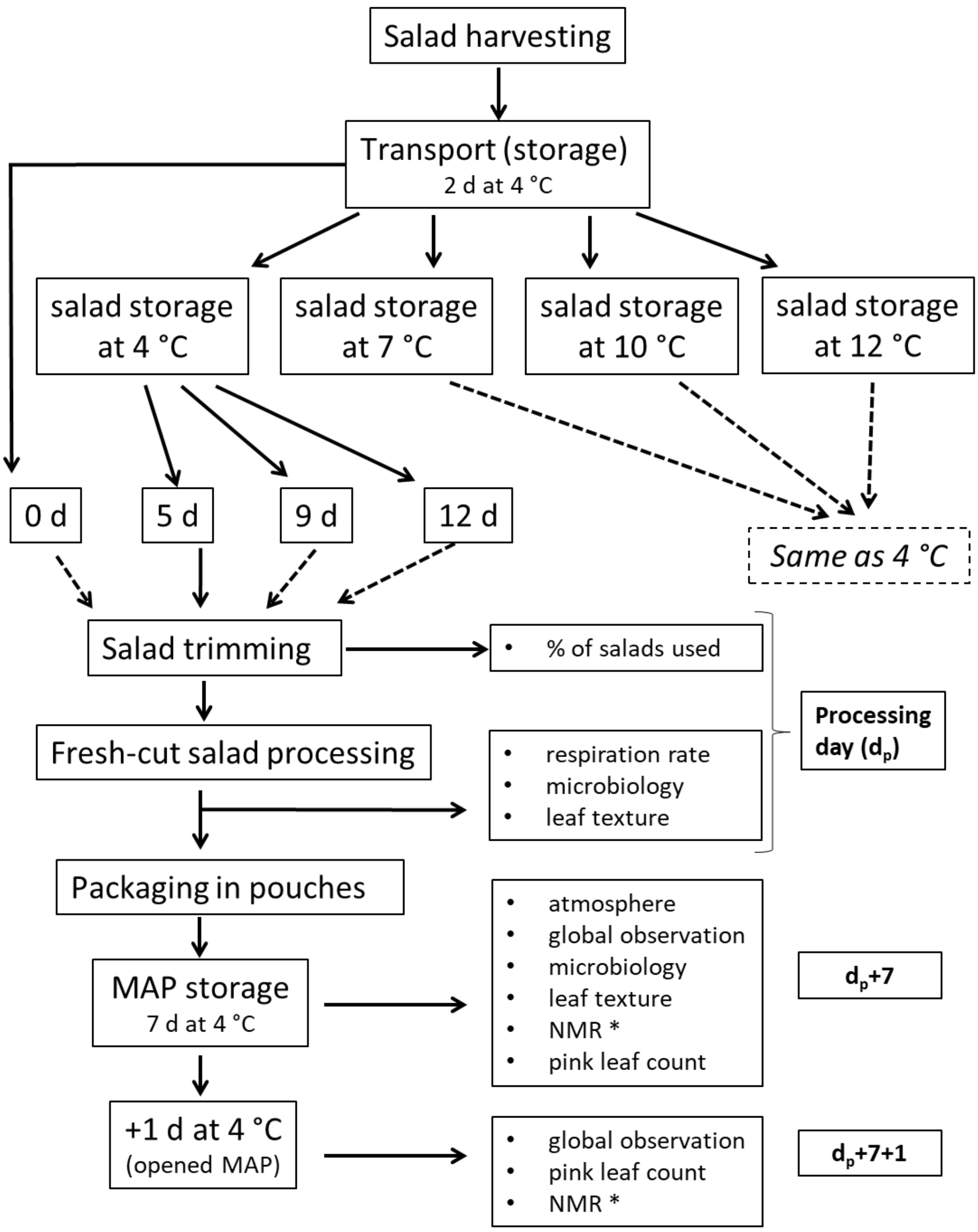


Fig. 4. Global design of the storage experiment and analysis carried out at each stage.
* Specific storage conditions: NMR was performed on fresh-cut leaves 5 d after processing from salads stored for 0.5, 6 or 11 d at 4, 7 or 12 °C.

At each storage time (0, 5, 9, or 12 d), six escarole heads for each temperature (4, 7, 10, or 12 °C) were processed into fresh-cut salad pouches in the laboratory, according to the procedure followed by the processing plant (d_p: processing day, Fig. 4). The salad heads were trimmed to remove external, withered, and damaged leaves. Furthermore, the bases and tips of the salad heads were removed; thereafter, the leaves were sliced into large pieces (approximately 10 × 6 cm) and immediately rinsed in pre-wash bath to remove the soil residues, followed by a washing bath (4 °C, 80 mg L^{-1} chlorine, 2 min shaking). Leaves were drained and dipped in a rinsing bath (4 °C, 1 min shaking), and then drained and spun using a manual salad spinner (10 turns; Dynamic 10 L, France). The washing and rinsing baths were changed for each temperature.

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

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

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

2.4. Characterization of fresh-cut salads

2.4.1. Raw material used after trimming

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

2.4.2. Microbiology

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

2.4.3. Respiration rate and atmosphere composition

The respiration rate (RR) of fresh-cut salads was measured at all d_p using the jar technique (Varoquaux et al. 2002). Leaves (30 to 45 g per jar) were placed in a tight gas jar (500 or 750 mL, three jars for each storage/temperature condition), and oxygen consumption and carbon dioxide production were measured at 20 °C (after temperature equilibrium). The results were expressed in mmol gas produced or consumed per h and per kg of leaves. The measurement of RR for both the gases allowed for the calculation of the respiratory quotient (RR_{CO2}/RR_{O2}) for all samples. Moreover, for escarole characterization, we calculated the Q_{10} (the multiplication factor for the RR for a temperature increase of 10 °C) in accordance with Gore's law (Varoquaux et al. 2002) by measuring the RR of fresh-cut leaves on 0 d of storage at 4, 7, 12, and 20 °C at d_p and d_p+1.

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

The atmosphere composition in the sealed jars and fresh-cut salad pouches was analyzed by gas chromatography using a µGC (Agilent 3000A). O_2 and CO_2 were separated on two capillary columns (MS-5A and Poraplot) under argon and helium, respectively, and quantified using a catharometric detector (TCD).

2.4.4. Global observations of stored fresh-cut salad pouches

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

2.4.5. Leaf texture

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

A puncture test was performed with a flat probe (3 mm in diameter) at a rate of 1 mm s^{-1} (50 N load cell) until the green leaf piece held in place with a pierced plate ruptured. The results were expressed as the maximum load (N), and the corresponding deflection (mm) for six replicate leaves of fresh-cut salads at d_p or for sixteen replicate leaf pieces at d_p+7 (four leaf pieces per pouch × four pouches per salad-head storage condition). To overcome the fluctuations at the start of the test (leaf tension), the zero value for the deflection was set at a load of 0.025 N. In addition, to better take into account the differences in the shape of the curve load=f(deflection) between the different treatments, curves were fitted to a binomial equation $y=ax^2+bx+c$, where y is the load, x is the deflection, and a, b, and c are the estimated parameters used to characterize each curve.

2.4.6. Nuclear magnetic resonance (NMR) relaxometry

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

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

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

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

2.5. Statistical analysis

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

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

3. Results

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

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

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

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

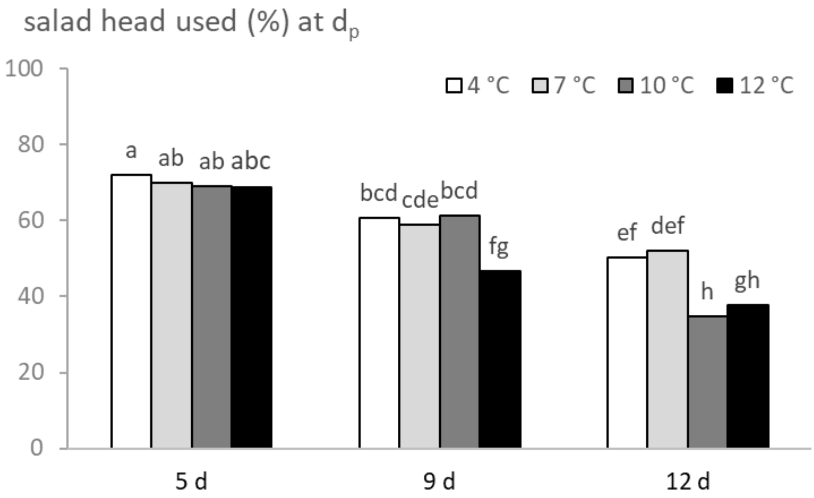


Fig. 5. Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on the percentage of salad used after trimming to process fresh-cut salads (d_p: processing day). Bars represent the average of the trimming carried out on six salad heads. ANOVA was performed on the twelve storage conditions,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

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

The salad heads stored at different times and temperatures were processed into fresh-cut salads; thereafter, all fresh-cut bags were stored for 7 d at 4 °C (d_p+7) and examined at the opening. Moreover, the fresh-cut bags were evaluated after 1 d of further storage at 4° C after opening (d_p+7+1). The global visual aspect was evaluated on an arbitrary scale of 0 (poorest) to 5 (best). The results are presented in Fig. 6A.

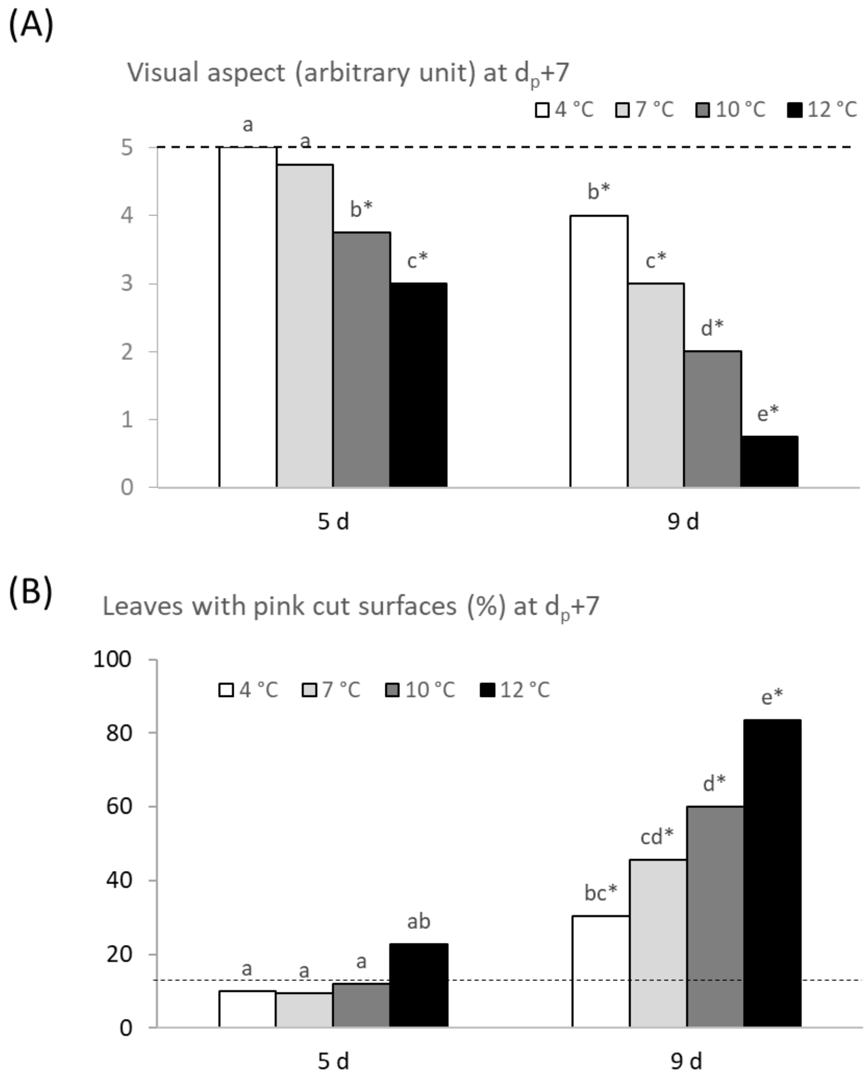


Fig. 6. Impact of salad-head storage conditions (5 or 9 d at 4, 7, 10 or 12 °C) on visual aspect (A) and the percentage of leaf pieces with pink discoloration of the cut surfaces (B) of the fresh-cut salad pouches stored for 7 d at 4 °C (d_p+7). With respect to visual aspect, 5 represented the best and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

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

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

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

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

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

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

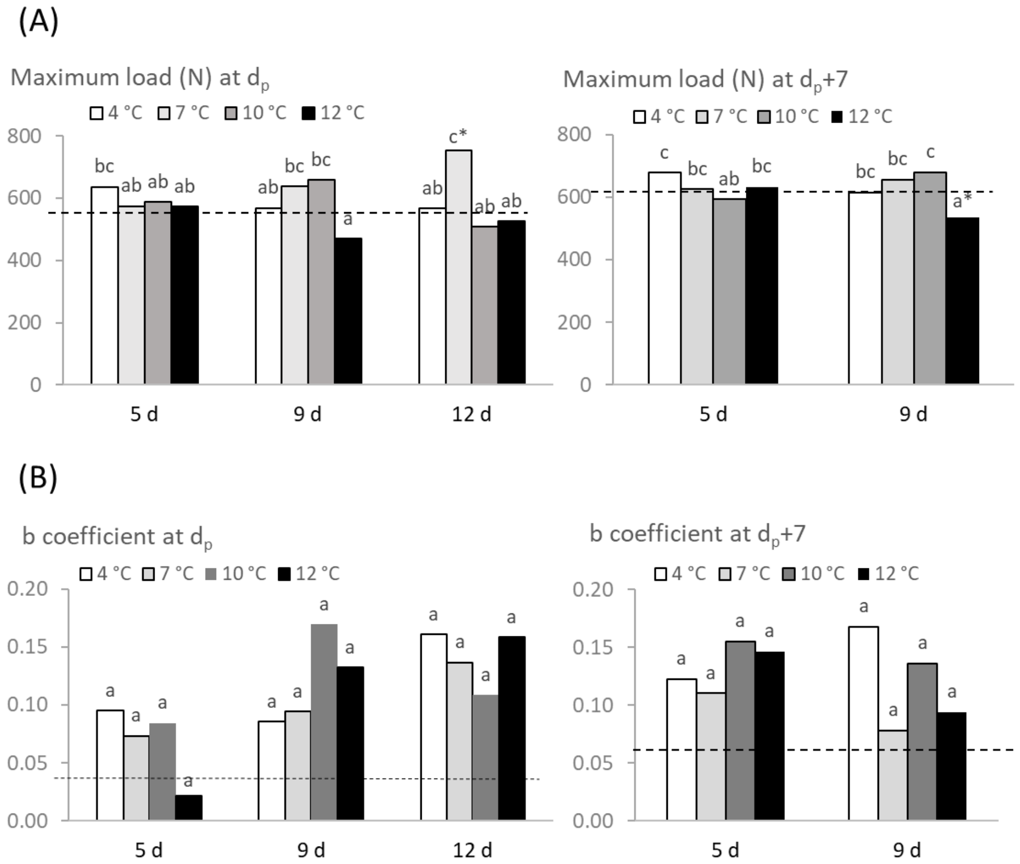


Fig. 7. Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on the leaf texture of fresh-cut salad just after processing (d_p) and after 7 d storage at 4 °C (d_p+7). (A) Maximum load (N) for the Kramer shear test. (B) Polynomial b factor obtained from puncture test spectra. Bars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

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

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

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

Total aerobic bacteria in fresh-cut salad leaves were counted at d_p and d_p+7. At d_p (washed-cut leaves ready to be packaged), the microbial counts of samples prepared from salad heads stored between 0 d and 12 d, at temperatures from 4 to 12 °C, ranged from 3.3 $\log_{10}$ CFU g^{-1} to 5.2 $\log_{10}$ CFU g^{-1}. ANOVA indicated that both storage time and temperature, as well as the interaction between the two factors, had a significant impact ($p<0.0001$) on total aerobic bacteria. Furthermore, it was observed that fresh-cut salads prepared from salads stored for 5 d at 4 °C and 12 d at 4 and 10 °C were not significantly different from those prepared using non-stored salads (Fig. 8). In accordance with the results of the Tukey HSD test, only fresh-cut salads prepared from salad heads stored for 12 d at 4 or 10 °C were significantly different from those prepared in other conditions (Fig. 8). Storage of salad heads tended to increase the counts of aerobic bacteria on the fresh-cut product at d_p (compared to non-stored salads), with some exceptions for the longest storage time (12 d). This phenomenon may be explained by the increased elimination of external dark green leaves during trimming after 12 d of storage.

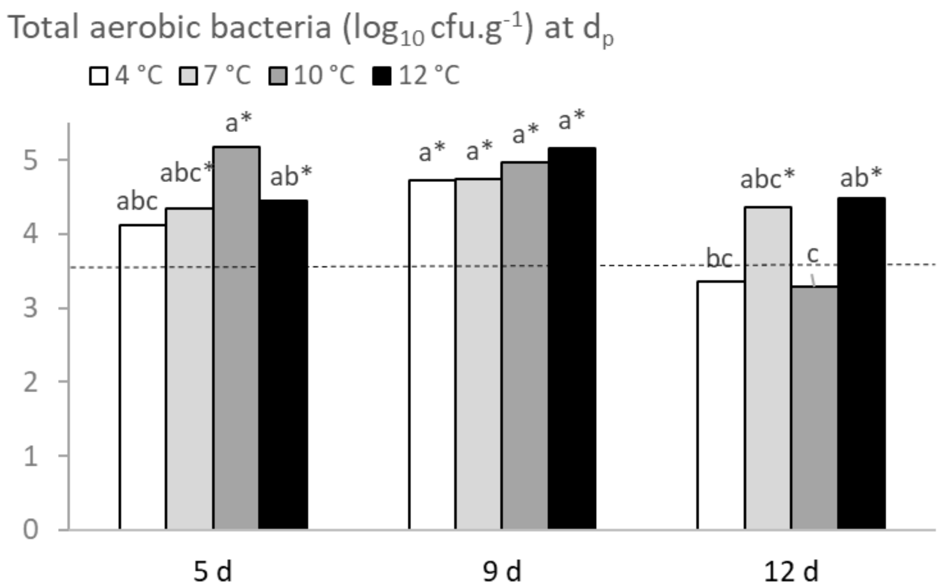


Fig. 8. Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on total aerobic bacteria of fresh-cut salad after processing, before pouches storage (d_p). Bars represent the means of four replicates. ANOVA was performed on the twelve storage conditions, and bars with different letters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

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

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

The Q_{10} (multiplying factor of the RR caused by a 10 °C increase) was calculated based on RRs measured at 4, 7, 12, and 20 °C. For fresh-cut salads made from non-stored salads (0 d), the Q_{10} value was 2.44 for RR_{O2} and 2.52 for RR_{CO2}. After 24 h, the RR of fresh-cut leaves decreased at the four temperatures. Moreover, a decrease in Q_{10} was observed for both RR_{O2} (1.95) and RR_{CO2} (2.16) (results not shown).

RR at 20 °C indicates the physiological activity of the salads. For all the samples, the respiratory quotient (RR_{CO2}/RR_{O2}) was 0.95±0.06, and no modification was observed in this value throughout the experiment. Therefore, only the RR_{CO2} results (mmol h^{-1} kg^{-1}) are presented. Storage of the salad heads increased the RR (measured at 20 °C) of fresh-cut salad leaves at d_p. The values for RR ranged from 2.4 mmol h^{-1} kg^{-1} for non-stored salads to 4.6 mmol h^{-1} kg^{-1} for salad heads stored for 12 d (Fig. 9).

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

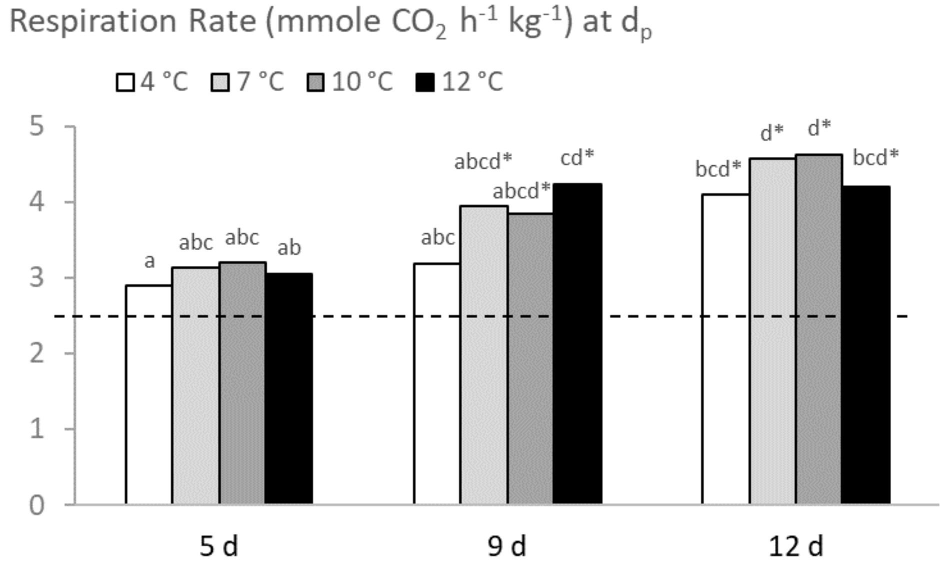


Fig. 9. Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on the respiration rate (RR_{CO2}) measured at 20 °C of fresh-cut leaves just after processing (d_p). Bars represent the means of three replicates. ANOVA was performed on the twelve storage conditions, and bars with different letters are significantly different ($p<0.05$) according to the Tukey HSD test. The dotted line corresponds to fresh-cut salads processed from non-stored salad heads. (*) indicates results significantly different from those of the non-stored salads according to the Dunnett test ($p<0.05$).

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

The atmospheres in fresh-cut salad pouches at d_p+7 for different storage conditions of salad heads ranged from 12.1 % O_2/5.9 % CO_2 to 3.2 % O_2/11.7 % CO_2. ANOVA revealed that the 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 composition, their interaction did not have any significant impact. Considering all the time× temperature combinations of salad-head storage (Fig. 10), no significant difference was observed among pouches prepared from salads stored for 5 d at all temperatures (4, 7, 10, and 12 °C). For salads stored for 9 d, the O_2 concentration was significantly lower in pouches with leaves from salads stored at 12 °C than those stored at 4 and 10 °C. The atmosphere compositions of pouches for salads stored for 9 d at 7 and 12 °C were significantly different (lower O_2 and higher CO_2) than those stored for 5 d at the same temperature. Compared to pouches prepared from non-stored salads (0 d, dotted line), only those from salad heads stored for the longest time and highest temperature (9 d at 12 °C) had a significantly different atmosphere composition and were the most modified (Fig. 10).

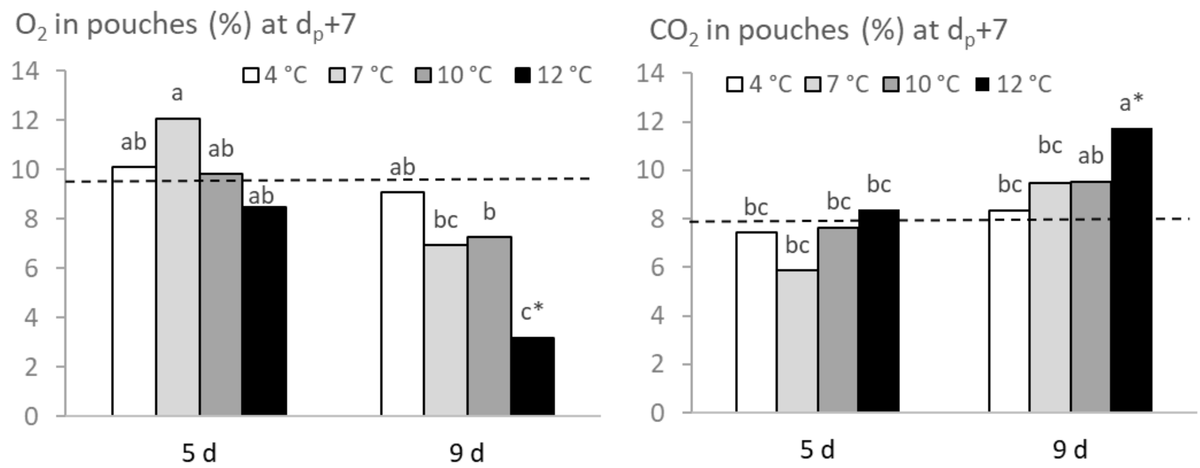


Fig. 10. Impact of salad-head storage conditions (5 or 9 d at 4, 7, 10 or 12 °C) on atmosphere composition (% O_2 and % CO_2) in the fresh-cut salad pouches after 7 d at 4 °C. Bars represent the mean of four replicates. ANOVA was performed on the eight storage conditions, and bars with different letters are significantly different ($p<0.05$) according to the Tukey HSD test. The dotted line corresponds to fresh-cut salads processed from non-stored salads (0 d). (*) indicates results significantly different from those of the non-stored salad according to the Dunnett test ($p<0.05$).

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

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

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

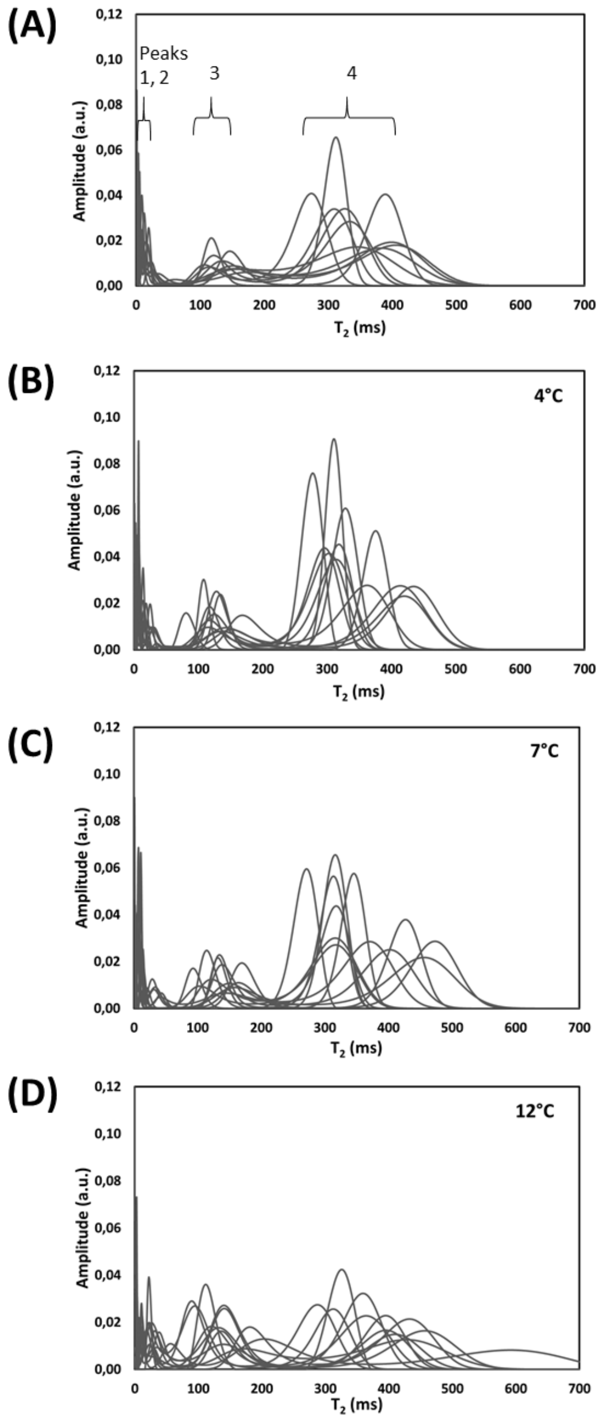


Fig. 11. T_2 (transverse relaxation time) spectra of non-stored salad leaves (A) and of fresh-cut leaves after 5 d at 4 °C (d_p+5), prepared from salad heads stored for 11 d at 4 °C (B), 7 °C (C) and 12 °C (D).

Fig. 12 depicts the mean T_2 of the two vacuolar peaks (3 and 4) in fresh-cut salad leaves at d_p+5, d_p+5+1, and for different storage conditions of the salad head (storage times and temperatures). For fresh-cut salads at d_p+5, $T_{2\text{-}3}$ remained constant over time for all conditions, with no significant differences among the various salad-head storage conditions (Fig. 12A). For salads stored at 4 and 7 °C, T_{2-4} did not significantly differ with the storage time (0.5, 6, or 11 d). Considering all salad-head storage temperatures and times, only the salads stored for 6 and 11 d at 12 °C showed a significant increase in T_{2-4} (compared to salads stored for 0.5 d at 4 °C). None of the other conditions were significantly different from each other.

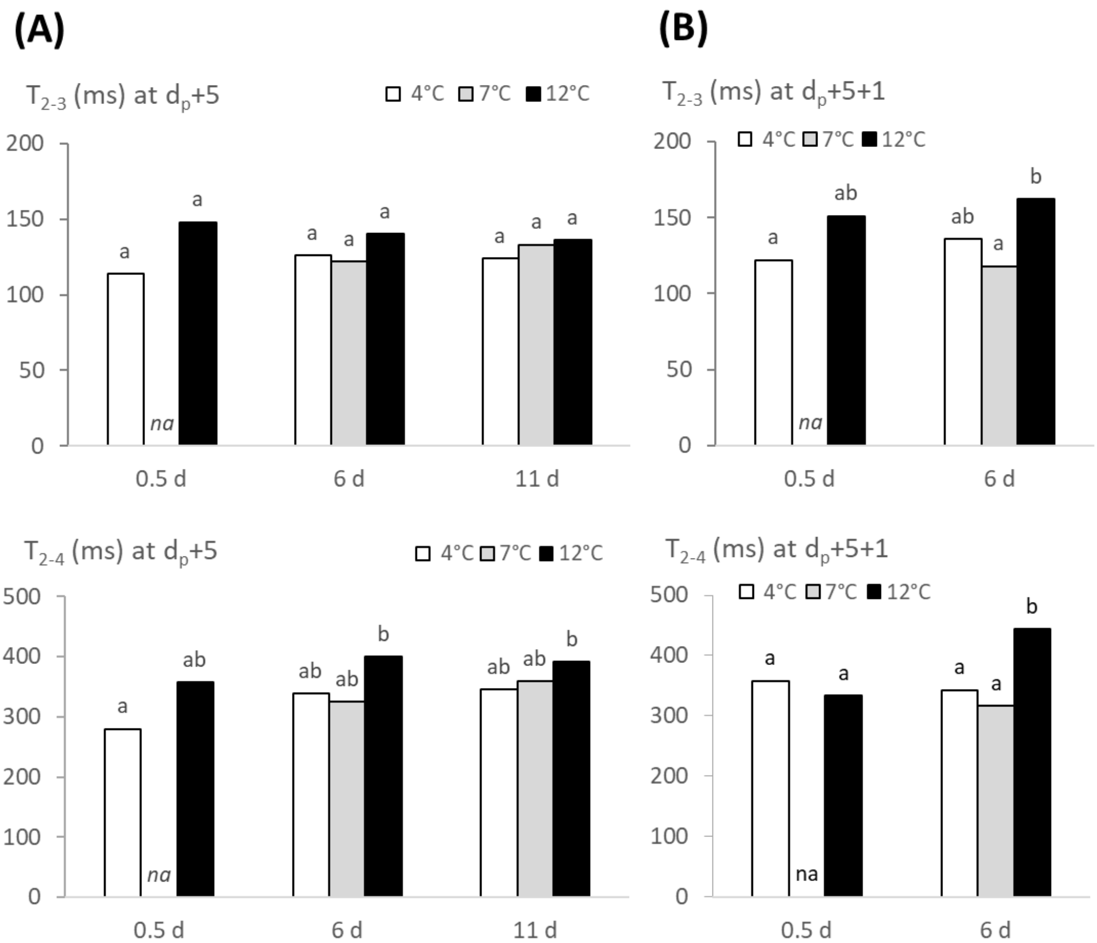


Fig. 12. T_2 (transverse relaxation time) of the two vacuolar peaks (3 and 4) for fresh-cut packaged leaves after 5 d at 4 °C (d_p+5) (A) and 1 d after pouch opening (d_p+5+1) (B), processed from salad heads stored 0.5, 6, and 11 d at 4, 7, and 12 °C. The results represent the mean of twelve replicates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

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

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

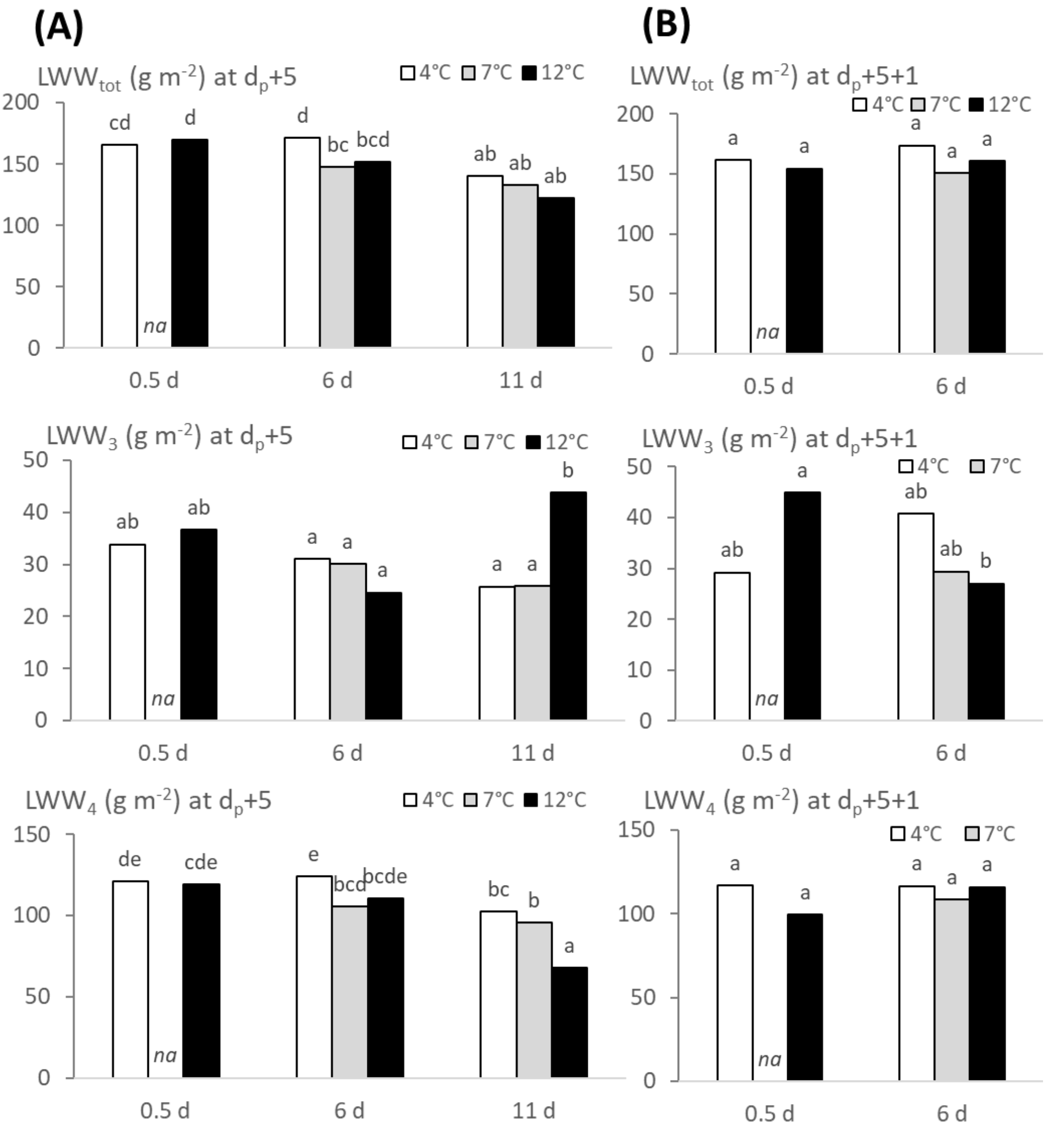


Fig. 13. LWW_{tot} (total leaf water weight) and specific LWW of the two vacuolar peaks (3 and 4) for fresh-cut packaged leaves after 5 d at 4 °C (d_p+5) (A) and 1 d after pouch opening (d_p+5+1) (B), processed from salad heads stored for 0.5, 6 and 11 d at 4, 7 and 12 °C. The results represent the mean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

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

The main experiment (presented in sections 3.1 to 3.6) was conducted on salads grown in southeast France and harvested in spring. The results were completed by two experiments, performed on salads grown in the same area but later in the season (end of June) and on salads grown in a different area (northeast of France, season beginning). ANOVA was performed on the results of these three experiments, considering the following factors: “experiment,” “storage time of salad head (d),” and “storage temperature of salad head (°C)”. The conditions selected for time and temperature were those common for all three experiments: 5, 9 and 12 d, and 4, 7 and 10 °C (12 °C was not repeated as it highly deteriorated the quality of the fresh-cut product). Variables included were the percentage of salad heads used to process the fresh-cut salads after trimming (at d_p), the visual aspects of the fresh-cut salads and the percentage of pink cut surfaces at the opening of the pouches (d_p+7), and the atmosphere composition (% CO_2) in the fresh-cut salad pouches at d_p+7. No significant difference was observed between salad-head storage conditions (see section 3.4); thus, the determination of total aerobic bacteria of the fresh-cut salads were not repeated.

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

CO_2); however, the values were similar at 4 and 7 °C, except for pink cut surfaces, which were significantly less important for fresh-cut leaves from salads stored at 4 than at 7 and 10 °C.

Table 1: Impact of experiments, times and temperatures of salad-head storages on the percentage of salads used after trimming to process fresh-cut salads (processing day, d_p) and on the visual aspect, percentage of pink cut surfaces and atmosphere composition (% CO_2) of the fresh-cut salad pouches after 7 d at 4 °C (d_p+7). Results of Tukey HSD test from an ANOVA with three factors (Experiment, Time, and Temperature), three conditions each and four variables.

Factors	**Conditions**	**Variables**							
		% used after trimming[1]		**Visual aspects**[2]		**Pink cut surface**[2]		**%CO_2**[2]	
		Mean value[3]	Tukey HSD groups[4]	Mean value[3]	Tukey HSD groups[4]	Mean value[3]	Tukey HSD groups[4]	Mean value[3]	Tukey HSD groups[4]
Experiment[5]	**south/early**	58.7	a	3.7	a	27.9	a	8.1	b
	south/late	62.4	b	2.1	b	76.2	b	7.9	b
	east/early	65.8	b	2.2	b	84.0	c	8.8	a
Time (d)[6]	**5**	71.9	a	3.1	a	56.6	a	7.6	b
	9	62.3	b	2.2	b	68.7	b	8.9	a
	12	52.6	c	nd	nd	nd	nd	nd	nd
Temperature (°C)[7]	**4**	63.6	a	3.0	a	58.0	a	8.0	b
	7	64.6	a	2.8	a	63.4	b	8.0	b
	10	58.6	b	2.2	b	66.6	b	8.7	a

[1]: Measured on processing day (d_p) at the end of the salad-head storage.
[2]: Measured after 7 d storage at 4 °C of the fresh-cut salad pouches (d_p+7).
[3]: For each condition of a factor, means were calculated from the values of all the conditions of the other factors.
[4]: Mean values from different Tukey HSD group are significantly different ($p<0.05$).
[5]: The three experiments were conducted with salads from different geographical origins and production periods.
[6]: Storage times of salad heads before processing of fresh-cut salads.
[7]: Storage temperatures of salad heads before processing of fresh-cut salads.

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

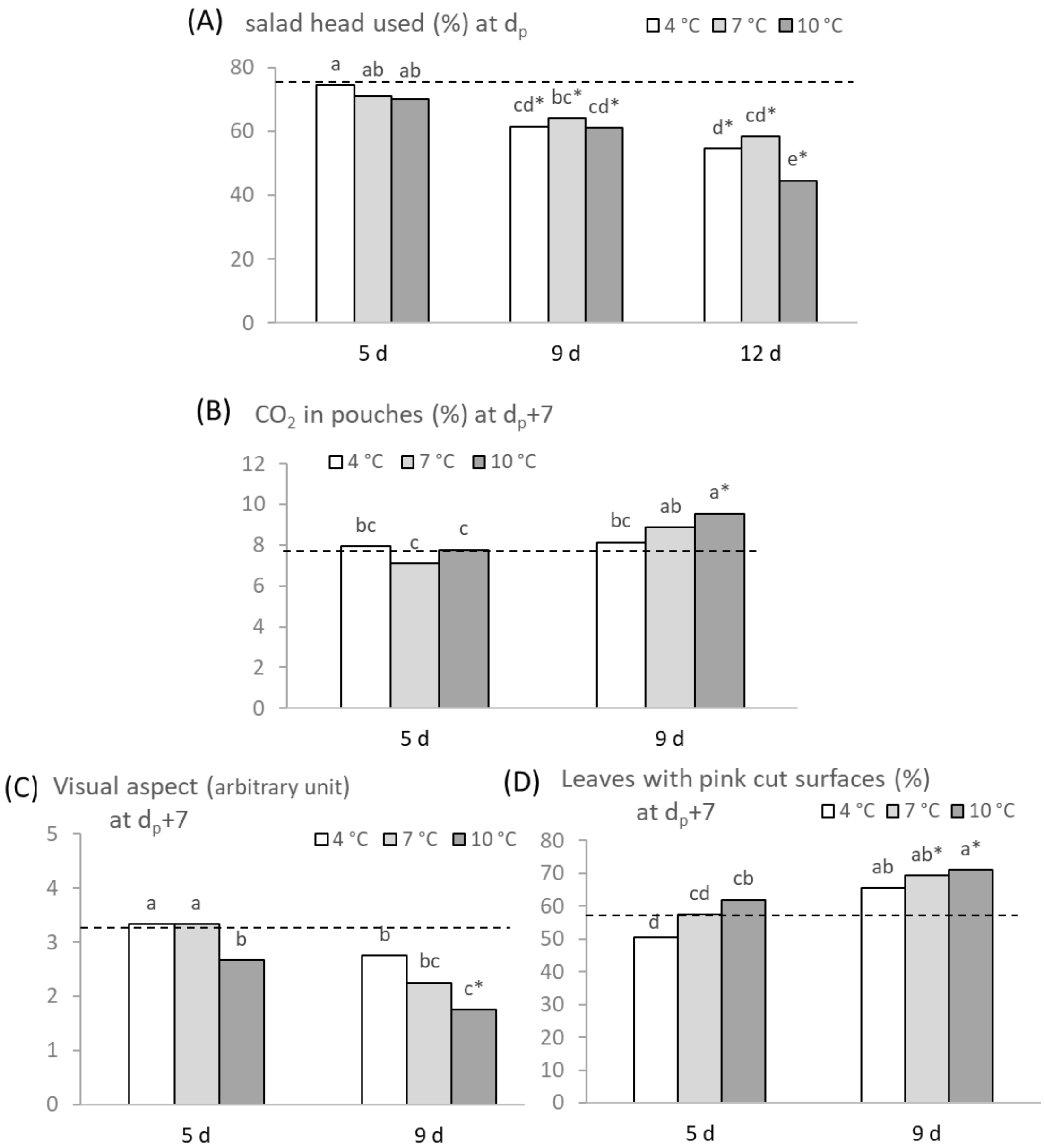


Fig. 14. Impact of salad-head storage conditions (5 or 9 d at 4, 7 or 10 °C) on the percentage of salads used after trimming to process fresh-cut salads (at d_p) (A) and on concentrations of CO_2 (B), global visual aspects (C), and percentages of leaves with pink cut surfaces (D) in the fresh-cut salad pouches after 7 d at 4 °C (d_p+7). Results represent the mean of three independent experiments conducted with salads from different geographical origins and harvested during different seasons, with six (A) and four replicates (B, C, D) per experiment. Bars with different letters are significantly different according to the Tukey HSD test ($p<0.05$). The dotted line represents fresh-cut leaves from non-stored salads. (*) indicates results significantly different from those of the non-stored salads according to the Dunnett test ($p<0.05$).

For a storage of 5 d of the salad heads, percentages of salads used were not significantly affected by the storage temperature (4, 7, and 10 °C) (Fig. 14A). Regardless of the storage temperature, these values were significantly higher than those of salads stored for 12 d; furthermore, they were similar to those of the non-stored salads. With respect to the atmosphere composition in the pouches at d_p+7 (Fig. 14B), no significant differences were observed between the three storage temperatures for 5 d salad storage time and 4 °C for the 9 d storage. For storage temperatures of 7 and 10 °C, atmosphere contained significantly more CO_2 in the pouches prepared from salads stored for 9 than for 5 d. Furthermore, the atmospheres of the pouches prepared from salads stored for 9 d at 10 °C were significantly different from those from non-stored salad heads.

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

4. Discussion

The variables used in the present study to characterize the impact of salad-head storage conditions covered a wide range of parameters in fresh-cut salad quality. In addition, the percentage of salads used for processing plays an important role in determining the economic and environmental performance of the process. After trimming the salad heads, regardless of the storage conditions, all leaves processed into fresh-cut salads were visually undamaged. Nevertheless, increasing the duration and temperature of salad storage globally reduced the technical yield, visual quality, and texture of fresh-cut salad at the end of shelf life (7 d at 4 °C). It also modified the physiology of the fresh-cut salad, as indicated by an increase in the RR. However, these parameters were not affected to the same extent by salad-head storage conditions.

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

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

Some components of the aerobic microflora can contribute to the spoilage of fresh-cut salad (Nguyen-the and Carlin 1994, Paillart et al. 2017). Furthermore, the total count of aerobic microflora has been included as a process hygiene criterion in the guide of good manufacturing practices in France (Anonymous 1988) and specification for the retailers' association in France (FCD 2019). In our study, we could not identify an association between total mesophilic bacteria in the fresh-cut product and salad-head storage conditions. Furthermore, no association with other quality parameters was observed. Some studies have shown a lack of correlation between total aerobic microflora during processing and spoilage with good quality fresh-cut salads at the end of storage, despite the high counts of aerobic microflora (Allende et al. 2008).

The texture of leaf pieces is an important quality attribute of fresh-cut salads; these leaf pieces should retain their crunchy texture until consumption. The texture is often assessed by measuring the mechanical properties of the leaf tissue (Martín-Diana et al. 2006, Tsironi et al. 2017). In our study, the maximum load of fresh-cut products in the Kramer shear test was affected by the long storage time of the salad heads. Moreover, we observed an increase in the b coefficient (puncture test) with salad-head storage time and temperature. This might indicate an increase in the deflection needed before the rupture of leaf tissues, thereby corresponding to a more elastic and less crunchy texture. This finding may explain the higher maximum load for the Kramer test at d_p under some salad-head storage conditions. In contrast, differences in leaf composition due to decay of the most external leaves for the most extreme salad-head storage conditions might have reduced the maximum load in the Kramer test; however, there were a few significant differences between the groups. Sorin et al. (2019) showed that an increase in NMR transverse relaxation times during the storage of salad heads was a sensitive method to detect changes in water status and distribution within leaf tissues, which may result in texture alteration. In the present study, NMR transverse relaxation times of fresh-cut products increased with the salad-head storage time and temperature; however, the results were significant only for the most extreme conditions tested, such as for mechanical texture tests. Previous studies have shown that texture is not the first quality parameter to deteriorate with increasing storage time and temperature of fresh-cut salads (Manolopoulo et al. 2010, Manzocco et al. 2017).

The visual aspect, global or pink discoloration, has a strong impact on the attractiveness of the product. The global visual aspect and pink cut surfaces of the stored fresh-cut salads were the most discriminant of the parameters studied, with a significant loss in visual quality and a significant increase in pink cut surfaces (except for the experiment with south/early season salads) when salad heads were stored over 7 °C or for more than 5 d. Our results are consistent with those reported by López-Gálvez et al. (1996), who found that storage of romaine salad heads for 7 d at 5 °C decreased visual quality and increased leaf edge browning. In contrast, the storage of baby spinach before processing had no effect on the visual quality of the processed product (Garrido et al. 2015). However, in this study, the maximum storage time tested was 48 h at 4 °C, which is shorter than in our study (Garrido et al. 2015). Global visual quality results from the combined effect of several phenomena, such as leaf senescence (Charles and Varoquaux 2016), microbial soft rot at the leaf margins (Nguyen-the and Punier 1989, Nguyen-the and Carlin 1994), and de-structuration of the leaf tissues leading to contact between enzymes and substrates, thereby resulting in discoloration (Charles and Varoquaux 2016), thus ensuring quality losses. In our results, the role of microbial spoilage in the loss of visual quality is questionable, as no consistent impact of salad-head storage on total mesophilic bacteria of fresh-cut product was observed. Castañer et al. (1999) found that the polyphenol oxidase (PPO), and phenolic compounds in the midribs, increased during the cold storage of romaine and baby lettuce. This suggests that, in our study, salad-head storage increased PPO and phenolic compounds in the leaf tissues, thereby leading to a higher discoloration potential, revealed after fresh-cut processing. The highest percentage of leaves with pink cut surfaces was observed in pouches made from salad heads stored for 9 d at 12 °C. This is surprising, as these pouches had the most modified atmosphere, which could have inhibited the discoloration. However, pouches were packaged under air, and the atmosphere was measured only once after 7 d; discoloration might have occurred prior the CO_2 accumulation.

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

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

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

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

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

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

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Declaration of Competing Interest

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

CRediT authorship contribution statement

Barbara Gouble: Investigation, Validation, Formal analysis, Writing-Review & Editing, Visualization **Maja Musse**: Investigation, Writing-Review & Editing **Steven Duret**: Investigation, Writing-Review & Editing **Patrice Reling**: Resources, Investigation **Evelyne Derens-Bertheau**: Investigation **François Mariette**: Investigation, Supervision **Clément Sorin**: Investigation **Erwann Hamon**: Resources, Investigation **Valérie Stahl**: Conceptualization, Investigation **Christophe Nguyen-The**: Investigation, Writing-Review & Editing, Supervision, Project administration, Funding acquisition

References

Allende, A., Selma, M.V., López-Gálvez, F., Villaescusa, R., Gil, M.I., 2008. Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce. Postharvest Biol. Technol. 49(1), 155-163. https://doi.org/10.1016/j.postharvbio.2007.12.010

Anonymous, 1988. Guide de bonnes pratiques hygiéniques concernant les produits végétaux dits de la « IV^{e} gamme ». Journal Officiel de la République française, ISSN 0767-4538, 1621, 29 p

Castañer, M., Gil, M.I., Ruíz, M.V., Artés, F., 1999. Browning susceptibility of minimally processed Baby and Romaine lettuces. Eur. Food Res. Technol. 209, 52-56. https://doi.org/10.1007/s002170050456

Charles, F., Varoquaux, P., 2016. From fruit and vegetables to fresh-cut products, in: Jeantet R., Croguennec T., Schuck P. and Brule G. (eds) Handbook of food science and technology 3, Wiley-ISTE, chapter 8, 297-320.

Couture, R., Cantwell, M.I., Ke, D., Saltveit, M.E. Jr. 1993. Physiological attributes related to quality attributes and storage life of minimally processed lettuce. HortScience 28(7), 723-725. http://dx.doi.org/10.21273/HORTSCI.28.7.723

Deltsidis, A.I., Tsouvaltzis, P., Brecht, J.K. 2012. Wound-induced respiration and ethylene production in fresh cut Romaine lettuce. Acta Hortic. 934, 613-618.

Duret, S., Hoang, H.-M., Guillier, L., Derens-Bertheau, E., Dargaignaratz, C., Oriol, S., Delahaye, A., Laguerre, O., Nguyen-the, C., 2021. Interactions between refrigeration temperatures, energy consumption in a food plant and microbiological quality of the food product: Application to refrigerated stuffed pasta. Food Control 126, 108076. https://doi.org/10.1016/j.foodcont.2021.108076

FCD (Fédération du Commerce et de la Distribution), 2019. https://www.fcd.fr/documentation-fcd/?thematic=83

FranceAgriMer, 2019. Achats de fruits et légumes frais par les ménages français. Données 2018. CTIFL et INTERFEL, 8p. https://www.franceagrimer.fr/fam/content/download/62829/

FranceAgriMer, 2020. Achats de fruits et légumes frais par les ménages français en 2019. CTIFL et INTERFEL, 12p. https://www.franceagrimer.fr/fam/content/download/63789/

Garrido, Y., Tudela, J.A., Gil, M.I., 2015. Time of day for harvest and delay before processing affect the quality of minimally processed baby spinach. Postharvest Biol. Technol. 110, 9-17. https://doi.org/10.1016/postharvestbio.2015.07.002

Guillier, L., Duret, S., Hoang, H.-M., Flick, D., Nguyen-Thé, C., Laguerre, O., 2016. Linking food waste prevention, energy consumption and microbial food safety: the next challenge of food policy? Curr. Opin. Food Sci. 12, 30-35. https://doi.org/10.1016/j.cofs.2016.06.006

Jacques, M.A., Kinkel, L.L., Morris, C., 1995. Population sizes, immigration, and growth of epiphytic bacteria on leaves of different ages and positions of field-grown endive (*Cichorium endivia* var. latifolia). Appl. Environ. Microbiol. 61(3), 899-906. http://dx.doi.org/10.1128/AEM.61.3.899-906.1995

Kim, J.G., Luo, Y., Gross, K.C., 2004. Effect of package film on the quality of fresh-cut salad savoy. Postharvest Biol. Technol. 32, 99-107. http://dx.doi.org/10.1016/j.postharvbio.2003.10.006

Koukounaras, A., Siomos, A.S., Sfakiotakis, E., 2018. Browning of fresh-cut lettuce as affected by preprocessing storage and heat treatments. Acta Hortic. 1209, 321-324. https://doi.org/10.17660/ActaHortic.2018.1209.46

López-Gálvez, G., Saltveit, M., Cantwell, M., 1996. The visual quality of minimally processed lettuces stored in air or controlled atmosphere with emphasis on romaine and iceberg types. Postharvest Biol. Technol. 8(3), 179-190. https://doi.org/10.1016/0925-5214(95)00002-X

Manolopoulo, H., Lambrinos, GR., Chatzis, E., Xanthopoulos, G., Aravantinos, E., 2010. Effect of temperature and modified atmosphere packaging on storage quality of fresh-cut Romaine lettuce. J. Food Qual. 33, 317-336. https://doi.org/10.1111/j.1745-4557.2010.00321.x

Manzocco, L., Alongi, M., Lagazio, C., Sillani, S., Nicoli M.C., 2017. Effect of temperature in domestic refrigerators on fresh-cut Iceberg salad quality and waste. Food Res. Int. 102, 129-135. http://dx.doi.org/10.1016/j.foodres.2017.09.091

Mariette, F., Guillement, J.P., Tellier, C., Marchal, P., 1996. Continuous relaxation time distribution decomposition by MEM, in: Rutledge, D.N. (Ed.) Signal Treatment and Signal Analysis in NMR, chapter 10, Elsevier. Data Handling in Science and Technology 18, 218-234. https://doi.org/10.1016/S0922-3487(96)80047-5

Martín-Diana, A.B., Rico, D., Frías, J., Henehan, G.T.M., Mulcahy, J., Barat, J.M., Barry-Ryan, C., 2006. Effect of calcium lactate and heat-shock on texture in fresh-cut lettuce during storage. J. Food Eng. 77, 1069-1077. https://doi.org/10.1016/j.jfoodeng.2005.08.037

Martínez, J.A., Chiesa, A., Tovar, F., Artés, F., 2005. Respiration rate and ethylene production of fresh cut lettuce as affected by cutting grade. Agric. Food Sci. 14(4), 354–361. https://doi.org/10.2137/145960605775897669

Monagham, J.M., Vickers, L.H., Grove, I.G., Beacham, A.M., 2016. Deficit irrigation reduces postharvest rib pinking in wholehead iceberg lettuce, but at the expense of head fresh weight. J. Sci. Food Agric. 97, 1524-1528. https://doi.org/10.1002/jsfa.7895

Monforti-Ferrario, F., Dallemand, J.F., Pinedo Pascua, I., Motola, V., Banja, M., Scarlat, N., Medarac, H., Castellazzi, L., Labanca, N., Bertoldi, P., Pennington, D., Goralczyk, M., Schau, E.M., Saouter, E., Sala, S., Notarnicola, B., Tassielli, G., Renzulli, P., 2015. Energy use in the EU food sector: State of play and opportunities for improvement. EUR 27247 EN, Luxembourg (Luxembourg), Publications Office of the European Union, JRC96121. 176 p. http://dx.doi.org/10.2790/266295

Nguyen-the, C., Prunier, J.P. 1989. Involvement of pseudomonads in deterioration of ready-to-use salads. Int. J. Food Sci. Technol. 24, 47-58. https://doi.org/10.1111/j.1365-2621.1989.tb00618.x

Nguyen-the, C., Carlin, F., 1994. The microbiology of minimally processed fresh fruits and vegetables. Crit. Rev. Food Sci. Nutr. 34(4), 371-401. https://doi.org/10.1080/10408399409527668

Paillart, M.J.M., van der Vossen, J.M.B.M., Levin, E., Lommen, E., Otma, E.C., Snels, J.C.M.A., Woltering, E.J., 2017. Bacterial population dynamics and sensorial quality loss in modified atmosphere packed fresh-cut iceberg lettuce. Postharvest Biol. Technol. 124, 91-99. http://dx.doi.org/10.1016/j.postharvbio.2016.10.008

Rico, D., Martín-Diana, A.B., Barat, J.M., Barry-Ryan, C., 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends Food Sci. Technol. 18 (7), 373-386. http://dx.doi.org/10.1016/j.tifs.2007.03.011

Rogers, G.S., Jobling, J., Titley, M.E., Giggins, B., Bauer B., 2006. The influence of trimming intensity and pre-processing storage on the self life of fresh cut Romaine lettuce (*Lactuca sativa* L.). Acta Hortic. 712, 231-235. https://doi.org/10.17660/ActaHortic.2006.712.24

Seefeldt, H.F., Løkke, M.M., Edelenbos, M., 2012. Effect of variety and harvest time on respiration rate of broccoli florets and wild rocket salad using a novel O_2 sensor. Postharvest Biol. Technol. 69, 7-14. https://doi.org/10.1016/j.postharvbio.2012.01.010

Sorin, C., Mariette, F., Musse, M., 2019. NMR study of fresh cut salads: Influence of temperature and storage time on leaf structure and water distribution in escarole. Magn. Reson. Chem. 4865, 1-12. http://dx.doi.org/10.1002/mrc.4865

Tsironi, T., Dermesonlouoglou, E., Giannoglou, M., Gogou, E., Katsaros, G., Taoukis, P., 2017. Shelf-life prediction models for ready-to-eat fresh cut salads: Testing in real cold chain. Int. J. Food Microbiol. 240, 131-140. http://dx.doi.org/10.1016/j.ijfoodmicro.2016.09.032

Tudela, J.A., Marín, A., Martínez-Sánchez, A., Luna, M.C., Gil, M.I., 2013. Preharvest and postharvest factors related to off-odours of fresh-cut iceberg lettuce. Postharvest Biol. Technol. 86, 463-471. https://doi.org/10.1016/j.postharvbio.2013.07.028

Tudela, J.A., Hernández, N., Pérez-Vicente, A., Gil, M.I., 2017. Growing season climates affect quality of fresh-cut lettuce. Postharvest Biol. Technol. 123, 60-68. http://dx.doi.org/10.1016/j.postharvbio.2016.08.013

Varoquaux, P., Mazollier, J., Albagnac, G., 1996. The influence of raw material characteristics on the storage life of fresh-cut butterhead lettuce. Postharvest Biol. Technol. 9, 127-139. https://doi.org/10.1016/S0925-5214(96)00042-7

Varoquaux, P., Gouble, B., Ducamp, M.N., Self, G., 2002. Procedure to optimize modified atmosphere packaging for fruit. Fruits 57, 313-322. https://doi.org/10.1051/fruits:2002028

Yahia, H.N., Lignou, S., Wagstaff, C., Bell, L., 2019. Changes in bacterial loads, gas composition, volatile organic compounds, and glucosinolates of fresh bagged Ready-To-Eat rocket under different shelf life treatments scenarios. Postharvest Biol. Technol. 148, 107-119. https://doi.org/10.1016/j.postharvbio.2018.10.021

Appendix A. Supplementary data

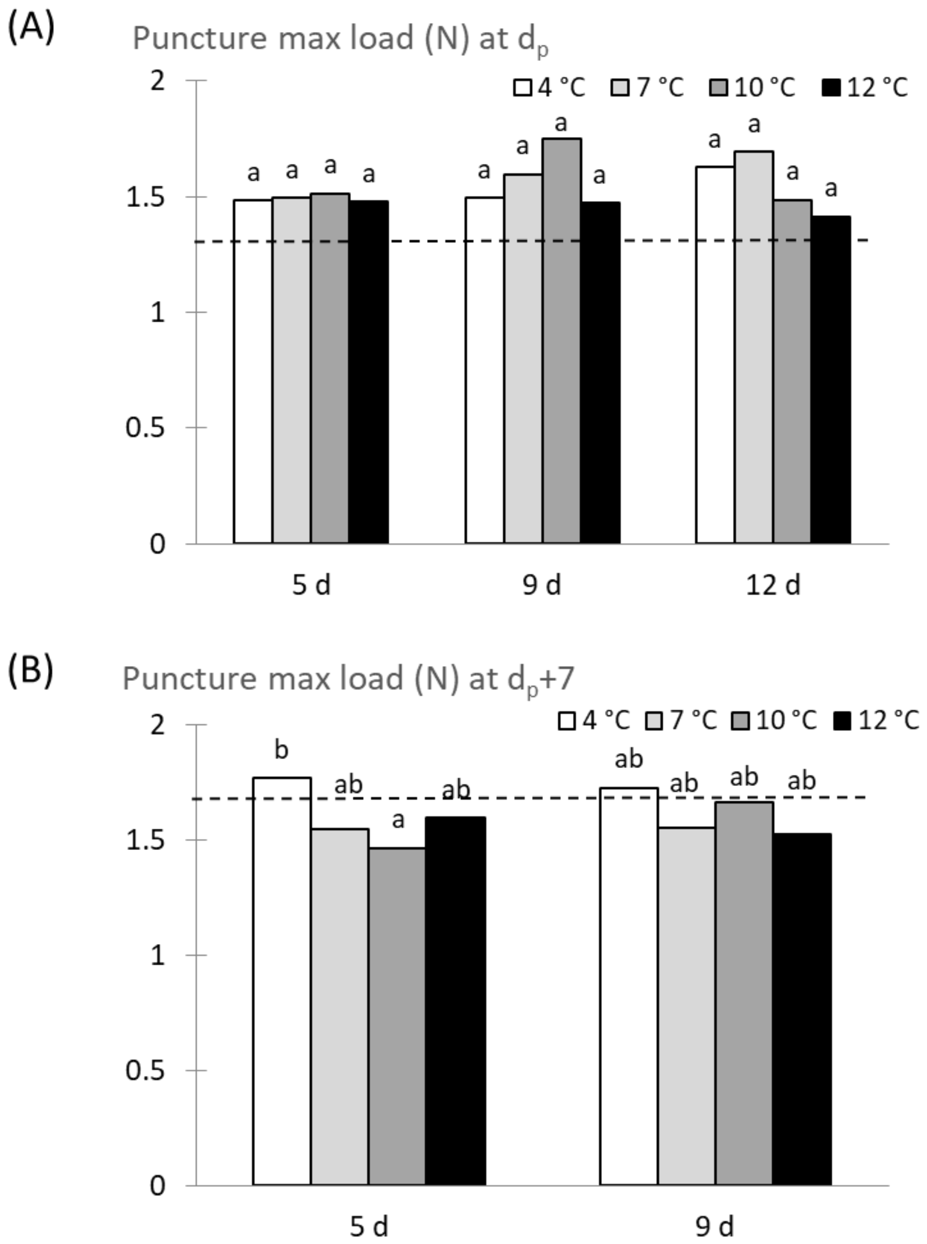

Fig. S1. Impact of salad-head storage conditions (5, 9 or 12 d at 4, 7, 10 or 12 °C) on maximum load (N) for puncture test on fresh-cut salad just after processing (d_p) (A) and after 7 d storage at 4 °C (d_p+7) (B). Bars represent means of six to sixteen replicates for puncture test. ANOVA was performed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

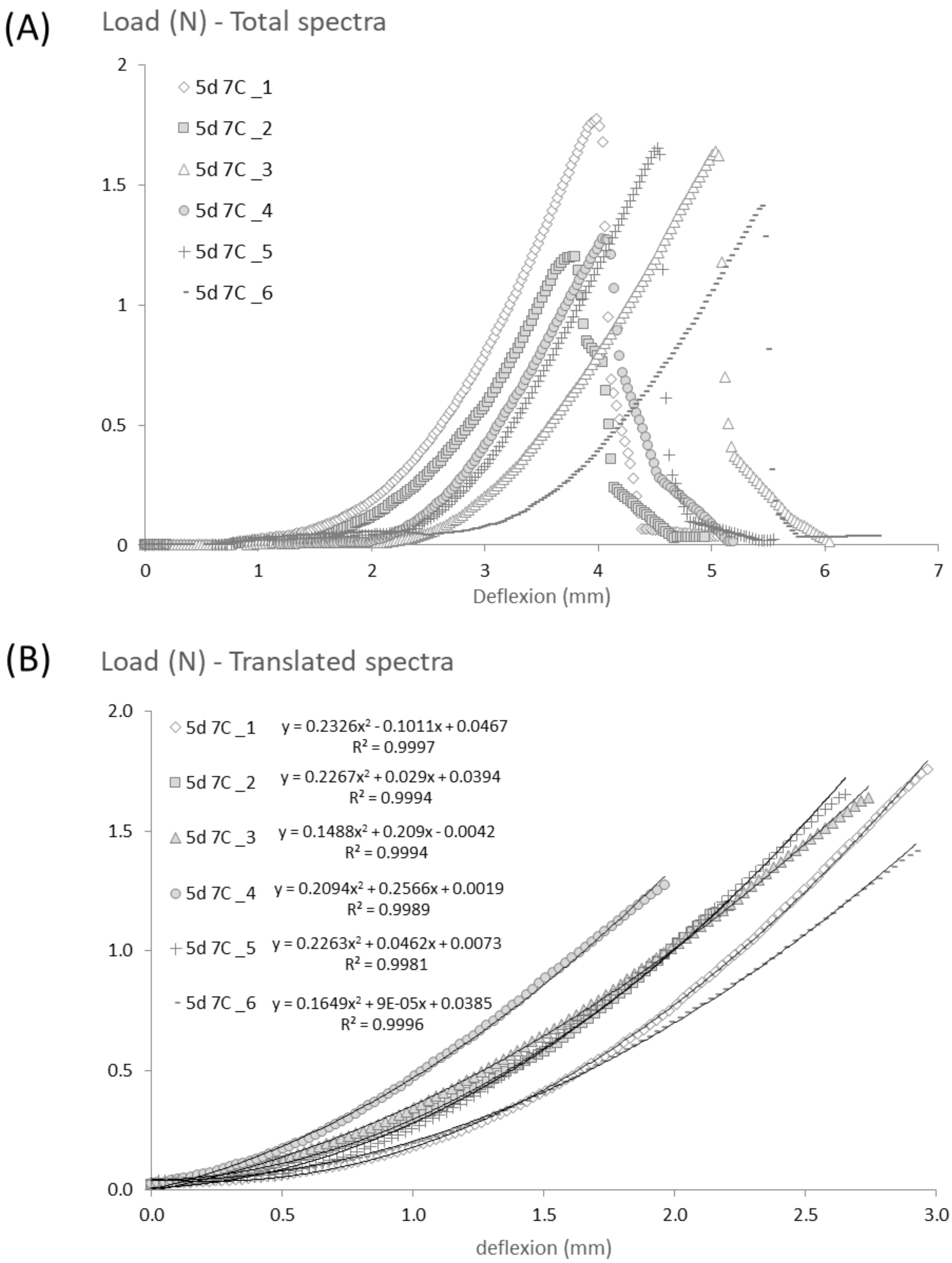

Fig. S2. Puncture test on six salad leaves after 5 d storage at 7 °C. (A) Total spectra (B) Translated spectra (from 0.025 N to maximum load) and polynomial equations.