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Impact of different temperature abuse scenarios on sensory quality and off-odour formation in ready-to-eat salad leaves

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Summary Packages of ready-to-eat (RTE) wild rocket and lettuce baby leaves were subjected during 8 days of cold storage to a chronic temperature abuse (CTA) at sub-optimal storage temperature (10 °C) or to a short-term (6 h) abuse at ambient temperature (STA) to evaluate the impact of two temperature abuse scenarios on gas composition within the packages, leaf sensory quality and volatile organic compounds (VOCs). In both species, the CTA scenario had a markedly higher impact on gas composition, sensory quality and off-odour formation than the STA, and the limit of sensory acceptability was reached in the CTA scenario 4 days or more earlier than in the STA. Sulphur compounds were the main responsible for off-odour perception in both leafy salads. Results from the present study may be useful in the assessment of critical points in the cold chain of RTE fresh produce and in prioritising actions towards improved cold-chain management.

Keywords Cold chain, fresh-cut, lettuce baby leaf, minimally processed, off-flavour, sensory quality, shelf life, temperature abuse, volatile organic compounds, wild rocket.

Introduction

Consumption of ready-to-eat (RTE) salads has been constantly increasing during last decades in all developed countries by virtue of their freshness, convenience and healthy attributes (Baselice *et al.*, 2017). The availability of both RTE vegetable and fruit on the market may facilitate an increased consumption of fruit and vegetable in the general population and, thus, may contribute to efforts towards the yet-to-be achieved goal of a daily consumption of 400 g per capita, as recommended by the WHO (World Health Organization, 2008). However, RTE salads, in particular, are highly perishable products due to their fresh nature and, additionally, to the processing operations to which they are subjected before packaging, when freshly harvested salads may be subjected to trimming, cutting, washing and mixing of different vegetables. All these operations may damage the natural protection of the epidermis and impair the internal compartmentalisation that separates enzymes from substrates, thus favouring microbial proliferation and inducing an increased respiration rate by the vegetable, which results in faster metabolic rate and tissue senescence. In this way, processing operations promote both microbial spoilage and physiological spoilage, which are associated with the development of off-odours, loss of texture and colour of the fresh vegetable, and thus make RTE salads particularly prone to the degradation of both microbiological and sensory quality during the shelf life (Beaulieu, 2011; Ragaert *et al.*, 2011). In particular, the decay of sensory quality, that may occur even before the deterioration of nutritional and microbiological characteristics, compromises the perception of product freshness and liking by consumers (Dinnella *et al.*, 2014).

Temperature is by far the most important environmental factor in order to control microbial growth and tissue senescence of RTE vegetables during the shelf life. For this reason, a tight control of the cold chain is prescribed by food legislation in all developed countries for RTE vegetables (USDA, 2013; Söderqvist, 2017). However, data from several field studies conducting time–temperature measurements on chilled food products along the cold chain revealed

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that temperature abuses are far from being rare events (Mercier *et al.*, 2017; Ndraha *et al.*, 2018). When considering, in particular, RTE salads, it has been noted that in real supply chain conditions, temperature abuse is likely to occur during retail storage, when the refrigeration system is temporarily shut down during cleaning and sanitisation of the cold room (Zeng *et al.*, 2014), during display at retail in open-refrigerated display cases, due to the large heterogeneity of the temperature according to the position inside the cabinet (Atilio de Frias *et al.*, 2015; Kou *et al.*, 2015), and during transport by consumers and domestic storage (Tsironi *et al.*, 2017). Temperature abuse occurring during storage or display at retail has been shown to significantly promote the growth of both spoilage and pathogenic bacteria, as well as the loss of visual quality in baby spinach (Atilio de Frias *et al.*, 2015; Kou *et al.*, 2015) and romaine lettuce mix (Zeng *et al.*, 2014). The impact of temperature abuse and improper shelf life treatment on bacterial loads, volatile organic compound (VOC) and phytochemical content in RTE wild rocket has been recently investigated by Yahya *et al.* (2019), who evaluated the effects of different fluctuating temperature regimes to simulate 'real world' post-processing supply chain conditions. On the contrary, at present no data are available about the effects of temperature abuses on the sensory quality of RTE lettuce baby leaves.

The aim of this study was to evaluate the impact of two different temperature abuse scenarios during post-processing cold storage on the quality of commercially produced and processed RTE wild rocket and lettuce baby leaves. The objective of the study was to investigate simulated abuse scenarios that could be representative of common faults in the cold chain of RTE products. The first scenario entailed a chronic temperature abuse (CTA) at a sub-optimal constant cold storage temperature (10 °C) and was designed in a way to mimic temperature abuse situations occurring during storage or display at retail, and possibly followed by home storage with a poor control of the refrigerator temperature. Indeed, a large survey on the retail sector in southern Spain recently found that average temperature of vegetables displayed at half and top shelves of display cabinets, during summer, was close to 10 °C (Baldera Zubeldia *et al.*, 2016), while in field test carried out in the supply chain of RTE leafy salads storage temperatures within the range from 8 to 10 °C have been observed after purchase, during domestic storage by consumers (Tsironi *et al.*, 2017). The second abuse scenario involved a relatively short-term abuse (STA) at ambient temperature (6 h at 25 °C), followed by storage at an appropriate temperature (6 °C), and was designed to simulate a relatively short breach of the cold chain due to failures in the management of the logistic operations, such as those occurring during truck loading and unloading, which represent another typical case of cold-chain interruption (Mercier *et al.*, 2017). The two abuse scenarios were evaluated with reference to a no temperature abuse scenario (NTA), where RTE salad leaves were stored at 6 °C along the entire storage time. Product quality was assessed by the measurements of gas composition within the packages, which is a key indicator of quality degradation of RTE vegetables along storage, by sensory evaluation of salad leaves and analysis of VOCs released within the bags. As regards sensory quality, defects that affect perception of freshness both before and after package opening was measured, because the former may influence consumer preference at purchase (Dinnella *et al.*, 2014), whereas the latter may play a role in repurchase behaviour (Løkke *et al.*, 2012). In addition, VOCs were determined because off-odour development is a major sensory defect in some RTE salad types, such as, in particular, rocket, where due to the presence of glucosinolates a remarkable level of unpleasant sulphur volatiles may be formed as a result of microbial and physiological spoilage (Løkke *et al.*, 2012; Spadafora *et al.*, 2016).

Materials and methods

Plant materials and experimental treatments

Freshly processed packages of wild rocket (*Diplotaxis tenuifolia* L.) leaves and lettuce (*Lactuca sativa* L.) baby leaves were obtained from a local processor. All plant materials were grown, harvested and processed according to standard commercial practices commonly adopted in Italy. Briefly, processing of the freshly harvested leaves involved washing with cold water containing sodium hypochlorite as sanitiser, then rinsing with tap water, drying and packaging under passive Modified Atmosphere Packaging (MAP) conditions by using polypropylene film bags. Packages of wild rocket and lettuce contained 100 and 125 g of leaves, respectively. Just after packaging, the bags were immediately transported to the CREA laboratories under cold-chain conditions. Then, the packages were subjected to three different storage conditions: a CTA condition, in which the bags were stored at a sub-optimal constant chilling temperature (in a fridge at 10 °C ± 2 °C); a condition with a relatively short-term (6 h) abuse at ambient temperature (25 °C ± 2 °C) during the first day of storage, followed by storage at a correct cold storage temperature (in a climatic chamber at 6 °C ± 1 °C) (STA); and a condition satisfying the requirements of the cold chain with NTA along the entire post-processing product life (in a climatic chamber at 6 °C ± 1 °C) (NTA). The cumulative thermal effect over time, as expressed by the accumulated thermal unit (ATU), where 1 ATU is equal to 1 °C for 1 day, amounted for the CTA, STA and NTA scenarios, to 40, 25.75 and 24 ATU, respectively, after 4 days, to 60, 37.75 and 36 ATU after 6 days and to 80, 49.75 and 48 ATU after 8 days. Packages of RTE wild rocket and lettuce baby leaves were analysed at arrival to the laboratory and then, for each condition, after 4, 6 and 8 days of storage. For each condition and storage time, six packages were used for the analyses, three for the sensory evaluation and three for the analysis of VOCs, whereas all the six packages were analysed for gas composition. Each bag represented one experimental replicate, both for sensory and chemical analyses. As a whole, 60 bags (six bags at arrival to the laboratory + 54 bags of stored product, corresponding to six bags × 3 conditions × 3 storage times) were analysed for both wild rocket and lettuce baby leaves.

Chemical and sensory analyses

Gas composition within packages

Oxygen and carbon dioxide levels within the sealed packages were determined by an Abyss Analyser LS210-LS212 (Dansensor Italia, srl). Analyses were carried out by inserting a small needle into the package through an adhesive rubber septum. For each condition and storage time, six bags were analysed. Results were expressed as % concentration of O_2 and CO_2.

Sensory evaluation

Sensory quality of RTE wild rocket and lettuce baby leaves was evaluated at the sensory laboratory of CREA – Research Centre for Food and Nutrition, which was designed according to the ISO guidelines and was equipped with individual booths and the FIZZ software v. 2.40 (Biosystemes, Couternon, France) to record analytical data. The sensory panel was formed by nine trained members. Prior to the start of the storage experiment, training sessions were performed to recognise and describe sensory defects of RTE wild rocket and lettuce baby leaves related to visual quality, off-odour and texture. For the storage experiment, three bags were analysed for each condition and storage time, representing three analytical replicates. Each sealed bag was coded with random three-digit numbers, after covering the expiration date label with dark adhesive tape, and then presented to the panellists who, one at a time, evaluated the product for visual quality defects (‘visual defects packaged’). Each single bag was then opened by the laboratory staff members and the product split into individual portions placed in plastic plates, one for each panellist, and the plates were presented to the panellists for the evaluation of visual quality defects after bag opening (‘visual defects opened’), off-odour development and texture defects. Off-odour was evaluated by smelling the intact leaves in the plate, without crushing them, whereas texture was evaluated simply by visual examination, without handling the leaves. The considered sensory defects were measured based on 5-point scales such as those used by Spadafora *et al.* (2016), but with reversed numerical values, because in the present study the focus was on the development of sensory defects (Table S1). Based on reported definition of sensory attribute scores, a score of 3 was assumed as the limit of sensory quality over which product acceptability by consumers would be severely compromised.

Analysis of volatile organic compounds (VOCs)

Volatile organic compounds were isolated by application of the headspace solid-phase microextraction (HS-SPME) technique to the salad leaf headspace within sealed packages, according to a method described elsewhere (Raffo *et al.*, 2020). The needle of a SPME fibre holder was inserted into a sealed bag via a foam rubber septum glued directly on the bag, and care was taken to avoid that the fibre touched the leaves or the package material. The SPME fibre (a 2-cm 50/30 µm DVB/CAR/PDMS fibre, purchased from Supelco, Sigma-Aldrich, Milan, Italy) was then exposed to the leaf headspace for 30 min while keeping the package at 6 °C. At the end of the extraction time, the fibre was immediately inserted into the GC split–splitless injection port, for the desorption step, and the GC run was started.

GC-MS analyses were performed on an Agilent 6890 GC 5973N MS system equipped with a quadrupole mass filter for mass spectrometric detection (Agilent Technologies, Palo Alto, CA, USA). Desorption of extracted volatiles from the fibre was carried out for 5 min within the GC injector, operating at 260 °C by the splitless mode. GC separation was achieved on a DB1-MS (0.25 mm i.d. × 60 m, 0.25-µm film thickness) manufactured by J&W, Agilent Technologies. GC operating conditions were as follows: oven temperature programme from 40 °C (1 min) to 150 °C at 6 °C min^{-1}, and then to 290 °C at 30 °C min^{-1} (10 min), total run time of 34 min, constant flow of He carrier gas was set at 1.5 mL min^{-1}, corresponding to a linear velocity of 31 cm s^{-1}, and transfer line at 300 °C. The MS detector was operated in the electronic ionisation mode at 70 eV; source and quadrupole temperatures were set, respectively, at 230 and 150 °C. Detection was performed by the full-scan mode, over the mass range 30–300 amu. For each volatile compound, a semi-quantitative determination of the level within the headspace of the sealed bags was obtained by calculating the area of chromatographic peaks obtained by extracting the signal of selected m/z ions (Table S2) from the total ion current signal. Identification of compounds was carried out as described elsewhere (Raffo *et al.*, 2020). For each condition and storage time, three replicates (three distinct bags) were analysed.

Statistical analysis

Data on gas composition and VOCs obtained from all experimental samples of each product, wild rocket and lettuce bay leaves, were subjected to one-way analysis of variance (ANOVA, $P < 0.05$), whereas Tukey's multiple comparison test ($P < 0.05$) was performed to identify mean values that were significantly different from each other. To evaluate statistical significance of differences in sensory data between salad bags subjected to different storage conditions and analysed at the same storage time, the Kruskal–Wallis test ($P < 0.05$) was performed, along with the Dunn multiple comparison test, by applying the correction of the significance level proposed by Bonferroni. A data set formed by gas composition, sensory and VOC mean values, was analysed by principal component analysis (PCA), after data pre-treatment (transformation by/standard deviation (n-1)). All analyses were performed by using the XLSTAT software package (2020.1.1 version, Addinsoft, Paris, France).

Results and Discussion

RTE wild rocket leaves

Gas composition within packages

Data on gas composition inside the wild rocket packages demonstrated that the different temperature abuse scenarios had a significant effect on the respiration rate of rocket leaves (Table 1). The CTA showed a significantly stronger impact on gas composition than the STA. Just after 4 days of storage, the O_2 level in the CTA scenario bags was markedly lower than in STA bags, which, in turn, showed a significantly lower level than the bags from the NTA scenario, and these differences endured after 6 days, whereas after 8 days significant differences were no longer observed. These effects paralleled those observed on CO_2 levels, with higher levels in CTA, with respect to STA and NTA. In a previous study, the low oxygen limit (LOL), the oxygen level below which anaerobic respiration begins to dominate over aerobic respiration, was estimated in wild rocket to be approximately 2.1% (Luca *et al.*, 2016). This limit was reached approximately after 4 days of storage in the CTA scenario, after 6 days in the STA and after 8 days in the NTA scenario. Interestingly, results of the present study confirmed the previous finding (Yahya *et al.*, 2019) that a CTA at a sub-optimal cold storage temperature had a higher impact on leaf respiration rate and gas composition changes, than a short-term temperature abuse, provided that the bags were returned to appropriate cold-chain conditions just after the STA.

Sensory evaluation

The effects of the abuse scenarios on the sensory quality of RTE wild rocket leaves paralleled those observed on gas composition changes within the bags. At all storage times all sensory defects in visual quality, off-odour and texture were more pronounced in bags subjected to CTA conditions than in STA and NTA scenarios (Fig. 1 and Table S3), whereas no significant differences were observed between STA and NTA. Bags exposed to CTA conditions showed a clear increase in all defects already in the first 4 days of storage, when the limit of sensory quality, over which product acceptability would be compromised, was nearly reached for off-odour, whereas in the following 2 days a sharp increase in all sensory defects made the product definitely unacceptable. Bags subjected to the STA scenario reached the limit after 8 days, primarily due to off-odour development, whereas at the end of the storage experiment (8 days) average sensory scores for all defects were lower than the limit of sensory quality in NTA bags. These results largely confirmed those previously obtained by Spadafora *et al.* (2016) on RTE rocket salad at 5 °C, whereas a shorter shelf life was observed by Amodio *et al.* (2015), who also found that two temperature abuses of 24 h at 13 °C, applied on day 2 and 6 of storage at 5 °C, reduced the shelf life of RTE rocket from 5.8 to 5.25 days. This kind of abuse was intermediate in extent when compared to the two scenarios investigated in the present study and suggested that a sub-optimal cold storage temperature, such as 13 °C, may have a significant impact on product shelf life even if applied for intermittent time intervals during storage, instead of as a CTA condition, as investigated in the present study.

Volatile organic compounds (VOCs)

In the present study, the analysis of VOCs was justified by the interest in the characterisation of off-odour, and thus, VOC isolation was carried out within bags of intact leaves, without crushing the leaves before VOC extraction, as done in previous studies (Luca *et al.*, 2016). The practice of crushing the leaves would have promoted the quick and extensive formation of volatiles produced by the lipoxygenase pathway and glucosinolates hydrolysis, which are responsible for the typical aroma of fresh rocket (Raffo *et al.*, 2018), but this would have made more troublesome the detection of the compounds responsible for the off-odour. Clear effects of the different storage scenarios on the formation of most of the determined VOCs were highlighted by experimental data (Table 1). A group of sulphur

Table 1 Gas composition within packages (%) and levels of VOCs (peak area*) in RTE wild rocket bags subjected to the three storage conditions, NTA, STA and CTA, during 8 days of storage. Average values and results of statistical analysis

	RTE wild rocket samples										
	Storage time first day	Storage time 4 days			Storage time 6 days			Storage time 8 days			*P* value
Parameter	**T0**	**NTA 4**	**CTA 4**	**STA 4**	**NTA 6**	**CTA 6**	**STA 6**	**NTA 8**	**CTA 8**	**STA 8**	ANOVA
Gas composition											
O_2	16.51 a	7.64 b	1.83 d	5.06 c	3.84 c	1.38 d	1.76 d	0.84 d	1.55 d	0.87 d	<0.0001
CO_2	4.0 f	10.4 e	14.7 ab	12.5 d	13.0 cd	15.6 a	14.1 bc	14.8 ab	14.2 ab	14.1 bc	<0.0001
Volatile organic compounds											
DMS	3015 c	17 997 c	1 108 331 ab	115 102 c	230 666 c	1 151 400 ab	569 393 bc	1 074 014 ab	1 421 557 ab	1 007 472 ab	<0.0001
DMDS	718 c	223 026 c	4 935 455 a	1 128 447 bc	2 152 576 bc	711 446 c	2 978 520 ab	2 145 359 bc	985 040 bc	738 630 c	<0.0001
Acetic acid	1344	54	198	97	1826	12 164	1591	3728	130 856	27 017	0.082
Methanethiol	0 b	1210 b	4146 b	2381 b	3360 b	50 248 a	3526 b	2363 b	40 619 a	12 316 b	<0.0001
Ethanol	0 b	0 b	0 b	0 b	0 b	1 132 675 a	0 b	0 b	1 196 378 a	256 326 b	<0.0001
Carbon disulphide	420 238 b	584 047 b	1 099 571 b	749 646 b	436 236 b	3 114 409 a	629 120 b	722 586 b	4 134 587 a	135 9777 b	<0.0001
Acetone	13 093 b	22 575 ab	73 926 ab	62 675 ab	61 481 ab	84 118 ab	200 068 a	131 872 ab	122 099 ab	154 887 ab	0.048
Methanol	36 d	270 d	3893 d	59 d	1662 d	568 580 b	5960 d	42 500 d	804 693 a	29 7516 c	<0.0001
Octane	8262 d	15 246 d	14 612 d	11 663 d	10 466 d	127 810 b	12 412 d	38 831 cd	196 723 a	81 100 c	<0.0001
Carbonyl sulphide	0 b	0 b	290 b	64 b	33 b	538 925 b	0 b	1980 b	1 458 839 a	243 140 b	0.0002
2,4-Dithiapentane	0 b	0 b	739 b	0 b	0 b	4005 ab	682 b	4408 ab	7010 a	5216 ab	0.0002
Tetrahydrothiophene	0 b	0 b	0 b	0 b	0 b	2149 b	0 b	0 b	140 124 a	1651 b	0.0125
Unknown 1	9802 c	34 154 bc	37 517 bc	30 771 bc	40 502 b	46 170 b	38 248 bc	32 654 bc	79 555 a	52 784 ab	<0.0001
Unknown 2	145 847 c	539 789 a	354 930 ab	401 743 ab	431 199 ab	264 679 bc	343 449 ab	297 084 bc	355 049 ab	355 250 ab	0.0003
Unknown 3	6524 d	14 329 bc	8913 cd	14 100 bc	9210 cd	13 209 bc	11 396 bcd	9310 cd	22 965 a	15 996 b	<0.0001
Unknown 4	105 893 d	278 684 a	147 150 bcd	216 469 ab	154 541 bcd	118 731 cd	161 218 bcd	124 858 cd	179 043 bcd	203 985 abc	0.0001

Lowercase letters near values indicate statistically different values according to one-way ANOVA and Tukey's multiple comparison test ($P < 0.05$).
*Area of chromatographic peaks obtained by extracting the signal of selected *m/z* ions (Table S2) from the total ion current signal.

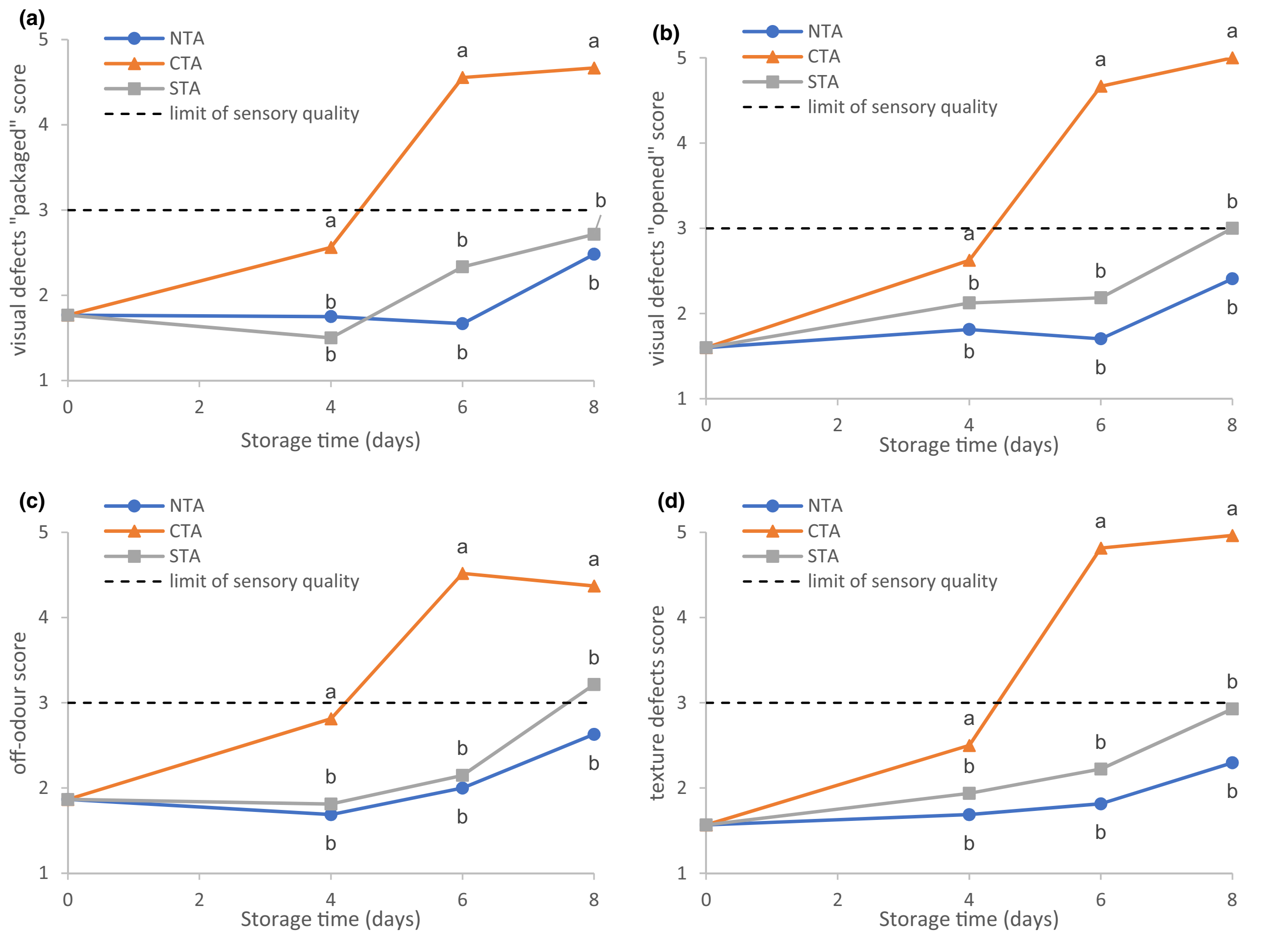

Figure 1 Sensory defects of RTE wild rocket bags subjected to the three storage conditions, NTA, STA and CTA, during 8 days of storage: scores[1] for (a) 'visual defects packaged[2]', (b) 'visual defects opened[3]', (c) 'off-odour development', (d) 'texture defects'. Notes. [1]A score of 3 was considered the limit of sensory quality over which product acceptability would be compromised. Lowercase letters indicate statistically different values between the different storage conditions at each storage times, according to the Kruskal–Wallis test (in all cases P was < 0.01) and the Dunn multiple comparison test, by applying the correction of the significance level proposed by Bonferroni. The mean values are obtained by the individual evaluations of nine panellists; each panellist evaluated three replicates (three bags) for each treatment/storage time. So, for each experimental point (treatment/storage time) mean values were obtained from 9×3 individual scores. [2] Visual quality as assessed before package opening. [3] Visual quality as assessed after package opening.

compounds, such as the prominent dimethyl sulphide (DMS), but also other compounds detected at a lower level, such as carbon disulphide, methanethiol, carbonyl sulphide and 2,4-dithiapentane, showed trends of variation that clearly resembled the development of off-odour as perceived by sensory panellists along the storage time. A clear association between the level of these VOCs and the sensory score of off-odour was also highlighted by significant Pearson's correlation coefficients (Table S4). It has to be underlined here that the analytical determination of VOCs performed in the present study did not allow to know the absolute concentration of the VOCs within the packages, but only to compare their level between different storage conditions and times. Thus, it was not possible to relate their concentration level to their odour detection threshold and, in that way, to estimate their actual contribution to the off-odour as perceived by the panellists. Nevertheless, while absolute evidence was lacking, the presented data, such as relatively high peak areas and significant correlations, made the contribution of these compounds to the perceived off-odour of the evaluated RTE wild rocket leaves likely. Moreover, three of the above listed compounds, DMS, methanethiol and 2,4-dithiapentane, for which such statistical association was observed, were identified among the major odour-active compounds contributing to off-odour notes in commercial bags of RTE rocket in a previous study carried out in our laboratory by GC-O analyses (Raffo *et al*., 2020), and results from the present study seemed to confirm their role in the perception of off-odour. For all these compounds, the level detected within the bags subjected to the CTA condition was markedly higher than the other storage conditions starting from the sixth day of storage, and for DMS from the fourth day of storage. Interestingly, a different trend of variation with respect to the previously mentioned sulphur compounds was observed for the other prominent sulphur compound commonly detected in rocket leaf headspace, dimethyl disulphide (DMDS). As can be observed in Table 1, its level quickly increased in the first 4 days of storage in the CTA bags and then it sharply decreased. Somewhat similarly, in STA bags, it increased, though less markedly, up to the sixth day of storage then declined. These trends strictly confirmed the finding of Luca *et al*. (2016), who observed that DMDS levels gradually increased as package O_2 levels decreased during storage, but sharply decreased as O_2 levels dropped below the LOL. This finding was also in agreement with results reported by Yahya *et al*. (2019), who observed higher levels of DMDS in bags exposed to relatively low storage temperature than in bags subjected to stronger temperature abuse, after 5 days of storage. According to the conjecture proposed by Luca *et al*. (2016), this consistently observed pattern of variation could be due to the reduction in a specific microbial or enzymatic activity related to the formation of DMDS when the partial pressure of O_2 drops to very low levels. This suggestion may be supported by evidence that, first, DMDS is formed through rapid oxidation of the very unstable methanethiol (Toivonen, 1997), and, second, that some enzyme activity related to food microbial flora, such as methioninase activity obtained from *Pseudomonas putida*, promotes the conversion of methanethiol to DMDS under aerobic conditions, whereas under anaerobic conditions this conversion does not occur (Di Pentima *et al*., 1995). Indeed, in our experiment methanethiol increased to the highest detectable levels only after anoxic conditions were established (Table 1). In any case, our data suggested the occurrence of a distinct biochemical pathway, or a distinct regulation of the same pathway, that controls the formation of the two sulphur compounds, DMS and DMDS, that were recognised as the major responsible for off-odour in packaged rocket in previous studies (Spadafora *et al*, 2016; Yahya *et al*., 2019; Raffo *et al*., 2020). As for VOCs other than sulphur compounds, interestingly, octane showed in all storage conditions a steady increase that was triggered by the occurrence of anoxic conditions, thus confirming a previous observation by Luca *et al*. (2016). A similar trend of variation was observed for the alcohols ethanol and methanol (Table 1). Release of ethanol is explained by degradation of pyruvate to acetaldehyde and, then, to ethanol when aerobic respiration switches to anaerobic respiration. Results of the present study confirmed that the formation of ethanol can be considered an accurate biomarker of the establishment of restricted O_2 conditions in RTE rocket, as previously suggested (Luca *et al*., 2016). As regards methanol, its formation may be related to enzymatic degradation of pectin by pectin methylesterase, that is associated with loss of texture attributes of the fresh tissue (Pelloux *et al*., 2007). As regards four unknown compounds, previously detected on RTE rocket salad headspace and characterised for their olfactory notes in our laboratory (Raffo *et al*., 2020), it was observed that the structurally related unknown compounds 1 and 3 followed a pattern of variation similar to that of sulphur compounds but different to that followed by unknown compounds 2 and 4.

A PCA of the global data set clearly highlighted that the CTA scenario produced a much more marked departure from the fresh state (T_0) than the STA scenario, in terms of sensory quality and off-odour formation, while the optimal storage condition of the NTA scenario allowed to limit the development of defects to a minimum extent (Fig. S1). Interestingly, all sensory defects appeared to be closely correlated with each other, as confirmed by significant Pearson's correlation coefficients (Table S4). Moreover, all sensory defects, gas composition and most of off-odour-related VOCs were linearly correlated with the cumulative thermal effect, as expressed by the ATU (Table S4), suggesting that the higher impact on product quality associated with the CTA with respect to the STA scenario corresponded to a significantly higher cumulative thermal effect in the former scenario.

RTE lettuce baby leaves

Gas composition within packages

As observed in the case of RTE wild rocket, the different abuse scenarios had a significant impact on gas composition inside bags of RTE lettuce baby leaves (Table 2). While the STA nearly had no effect on gas composition with respect to the NTA condition, a marked effect on product respiration rate was observed in the bags subjected to the CTA scenario, where noticeable decreases in O_2 and increases in CO_2 with respect to the other two conditions were detected. In this last scenario, O_2 levels sharply decreased after the fourth day of storage, creating anoxic condition at the end of the storage time, whereas at the same time, in the other two scenarios, O_2 and CO_2 levels amounted approximately to 10% and 7–8%, respectively. These last figures were in well agreement with results previously reported on cold-stored RTE lettuce baby leaves

(Martínez-Sánchez *et al.*, 2012), and confirmed that lettuce baby leaves were characterised by a markedly lower respiration rate than rocket leaves, and this corresponded to less active metabolism, slower deterioration rate and longer shelf life.

Sensory evaluation

As for RTE wild rocket leaves, the degradation of sensory quality closely reflected changes observed in gas composition within the bags of RTE lettuce baby leaves (Fig. 2 and Table S5). The STA did not produce significant effects in any of the considered sensory defects at all storage times with respect to the condition with NTA, whereas the CTA clearly boosted the appearance of all sensory defects. Under this last condition, all sensory quality attributes sharply degraded in the first 4 days of storage, and then, the process of leaf senescence slowed down in the following 2 days of storage, to speed up again in the last 2 days of the experiment. Interestingly, defects of visual quality, measured both before and after bag opening, exceeded the limit of sensory quality just before the fourth day of storage, whereas defects of odour and texture reached this limit slightly later. This result confirmed the previous finding that the shelf life of lettuce baby leaves is limited by defects in visual quality, mainly related to the occurrence of bruising, waterlogging and blackening of the leaves, rather than to the development of off-odour (Martínez-Sánchez *et al.*, 2012). Bags exposed to the other two scenarios showed a relatively slow rate of quality degradation, and none of the measured sensory attributes reached the limit of sensory quality within the time of the experiment. This was consistent with the relatively lower respiration rate of lettuce baby leaves when compared to wild rocket leaves and with a previous estimation of RTE lettuce baby leaf shelf life, ranging from 9 to 11 days under correct cold storage conditions (Martínez-Sánchez *et al.*, 2012).

Volatile organic compounds (VOCs)

To the best of our knowledge, the present study is the first one reporting data on VOCs isolated in the headspace of RTE lettuce baby leaves. Results showed the formation of a smaller number of VOCs than in the headspace of wild rocket leaves (Table 2). DMS was the main compound in the volatile fraction, and its level was correlated with the intensity of off-odour as perceived by the sensory panel (Table S4). Considering its low odour detection threshold in air (0.002–0.03 ppm) (van Gemert, 2003) and its unpleasant rotten leaf olfactory note, it was plausible that it could contribute to the perception of the off-odour in lettuce baby leaves, even though in the absence of absolute concentration data it was not possible to establish this with certainty. DMDS, which showed great variability

Table 2 Gas composition within packages (%) and levels of VOCs (peak area*) in RTE lettuce baby leaf bags subjected to the three storage conditions, NTA, STA and CTA, during 8 days of storage. Average values and results of statistical analysis

	RTE lettuce baby leaf samples										
	Storage time first day	Storage time 4 days			Storage time 6 days			Storage time 8 days			P value
Parameter	T0	NTA 4	CTA 4	STA 4	NTA 6	CTA 6	STA 6	NTA 8	CTA 8	STA 8	ANOVA
Gas composition											
O_2	18.64 a	14.31 b	11.23 b	13.06 b	12.31 b	4.28 c	11.25 b	9.79 b	0.73 c	10.53 b	<0.0001
CO_2	2.22 c	5.43 b	7.52 b	6.32 b	6.88 b	11.92 a	7.25 b	7.85 b	14.50 a	7.37 b	<0.0001
Volatile organic compounds											
DMS	31 493 f	40 5026 ef	401 7949 bc	169 4631 cdef	126 6670 def	624 1926 ab	319 2785 cd	151 9695 cdef	794 4069 a	283 1883 cde	<0.0001
DMDS	0	5617	30 7954	40 973	12 061	236 662	314 060	15 175	292 558	28 224	0.130
Methanol	28 149 bc	19 734 bcd	24 469 bcd	8663 d	19 050 bcd	34 071 ab	11 480 cd	11 392 cd	52 387 a	18 585 bcd	<0.0001
1-Undecene	2192 c	2621 c	20 299 bc	4381 c	4809 c	43 064 ab	5910 bc	9503 bc	79 365 a	7429 bc	<0.0001
Trichloromethane	11 4857 cd	14 9620 abcd	190 149 ab	206 658 a	127 248 bcd	175 141 abc	178 801 abc	97 476 d	161 831 abcd	189 760 ab	0.0004

Lowercase letters near values indicate statistically different values according to one-way ANOVA and Tukey's multiple comparison test ($P < 0.05$).

*Area of chromatographic peaks obtained by extracting the signal of selected m/z ions (Table S2) from the total ion current signal.

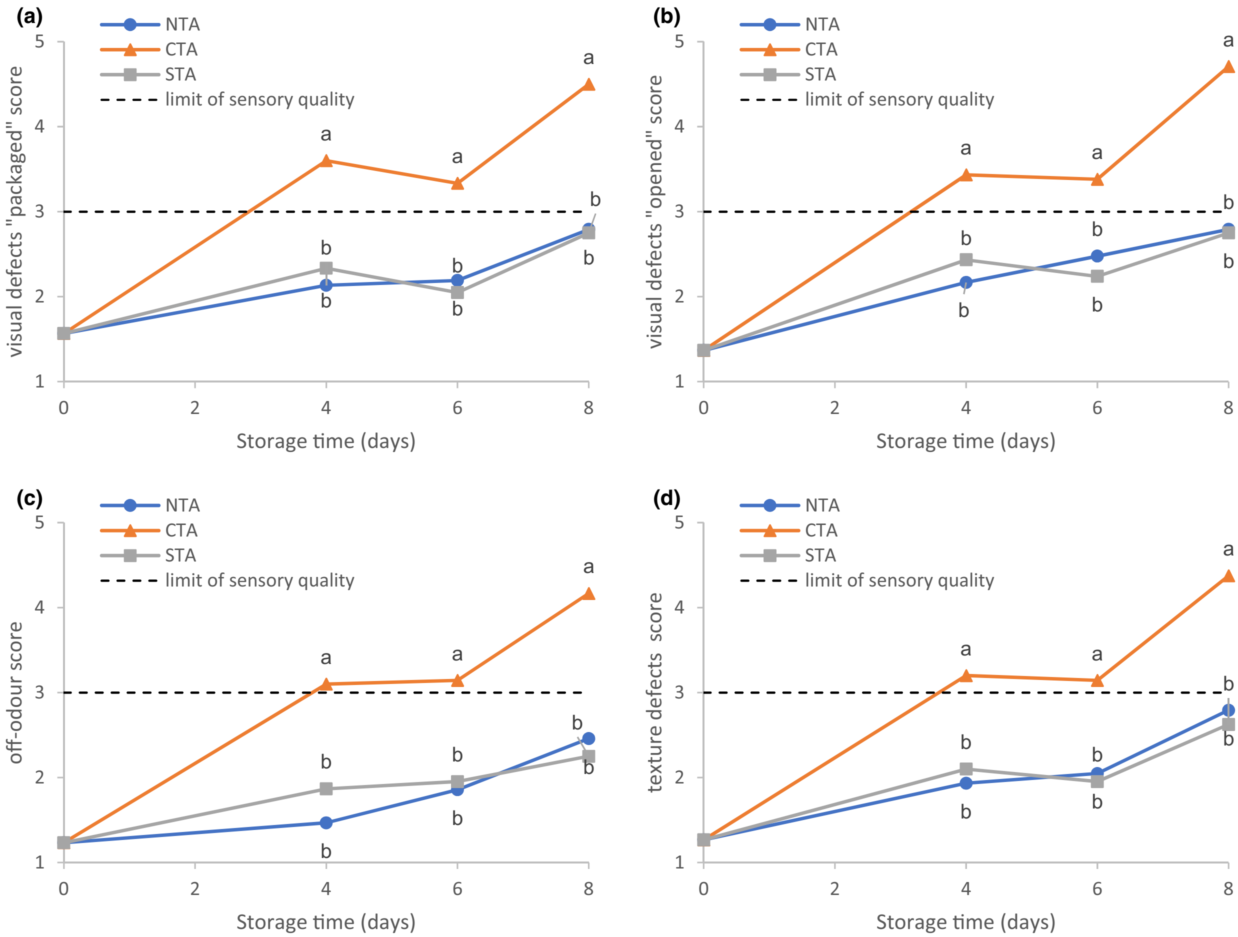

Figure 2 Sensory defects of RTE lettuce baby leaf bags subjected to the three storage conditions, NTA, STA and CTA, during 8 days of storage: scores[1] for (a) 'visual defects packaged[2]', (b) 'visual defects opened[3]', (c) 'off-odour development', (d) 'texture defects'. Notes. [1] A score of 3 was considered the limit of sensory quality over which product acceptability would be compromised. Lowercase letters indicate statistically different values between the different storage conditions at each storage times, according to the Kruskal–Wallis test (in all cases P was < 0.01) and the Dunn multiple comparison test, by applying the correction of the significance level proposed by Bonferroni. The mean values are obtained by the individual evaluations of nine panellists; each panellist evaluated three replicates (three bags) for each treatment/storage time. So, for each experimental point (treatment/storage time) mean values were obtained from 9×3 individual scores. [2] Visual quality as assessed before package opening. [3] Visual quality as assessed after package opening.

and no clear trend of variation, was detected at a quite lower level. Similarly to the case of wild rocket, sulphur compounds appeared to be plausible candidates for key odorants responsible for off-odour perception, even though, in this case, they were probably formed by degradation of sulphur-containing amino acids rather than of glucosinolates. Interestingly, the changes in the level of the aliphatic alkene 1-undecene were linearly correlated with the off-odour intensity, and other sensory defects, as perceived by the sensory panel (Table S6). However, it is plausible that it did not contribute to the perception of the off-odour, since it is characterised by a relatively high odour detection threshold, 570 ppm (van Gemert, 2003). 1-Undecene has not been previously reported in lettuce volatiles and its presence may be worth of investigation because it is known to be naturally produced by many strains of *Pseudomonas* bacteria (Rui *et al*., 2014), which are the dominating species in RTE lettuce in aerobic conditions (Ioannidis *et al*., 2018) and are known as the major cause of soft-rot, and, thus, of textural and visual defects in lettuce (Ragaert *et al*., 2011). Thus, 1-undecene could be an accurate marker of microbial spoilage in lettuce baby leaves. Moreover, trichloromethane, which is a disinfection by-product formed as a result of interaction of chlorine with organic matter during the washing step in fresh-cut salad processing (Shen *et al*., 2016), seemed not to be clearly influenced by the different storage conditions. In a previous study on RTE, mature iceberg lettuce only five VOCs were detected when lettuce leaves were stored under aerobic MAP conditions different from those applied in the present study, and in agreement with results of the present study, DMS and methanol were in this group, even though they both decreased along storage (Ioannidis *et al.*, 2018). While in that previous study it was found that ethanol could be a suitable spoilage indicator under anaerobic conditions, in the present study it was observed that ethanol did not form under aerobic conditions, as also observed by Ioannidis *et al.* (2018), and that DMS and 1-undecene could be appropriate volatile markers of quality degradation and off-odour formation that occurred under aerobic conditions, which are most common when RTE lettuce baby leaves are stored at a correct temperature. Identification of suitable volatile marker of spoilage and off-odour formation could be useful for the development of future intelligent packaging technologies aimed at monitoring sensory quality of fresh-cut produce during shelf life.

A PCA of all experimental data quickly showed the impact of the different scenarios on the sensory profile of lettuce baby leaves (Fig. S2). All sensory variables were closely associated with each other and also significantly correlated with DMS, 1-undecene and CO_2 levels and inversely correlated with O_2 concentration (Table S6). As observed for wild rocket, all these variables were linearly correlated with the ATU (Table S6), showing that the higher impact on RTE lettuce baby leaf quality associated with the CTA with respect to the STA scenario corresponded to a significantly higher cumulative thermal effect in the CTA scenario.

Conclusions

The present study, by integrating sensory evaluation of the leaves and chemical determination of gas composition and, by HS-SPME/GC-MS, of VOCs within the packages highlighted that a post-processing chronic thermal abuse at a sub-optimal cold storage temperature may have a stronger adverse impact on the sensory quality of RTE salad leaves than a relatively short-term breach of the cold chain at ambient temperature. The chronic thermal abuse investigated in the present study was characterised by a markedly higher cumulative thermal effect than the STA scenario, and this could have played a key role in determining a higher impact on product quality. In any case, storage and display at retail and domestic storage by consumers, when such CTAs at a sub-optimal temperature have been reported to occur, appear as the weak links of RTE salads cold chain where attention should be focused and actions prioritised. Implementation of most efficient refrigeration systems, such as those with display case with glass doors, is already a practicable option that may markedly improve product quality, reduce decay rate (Atilio de Frias *et al.*, 2015; Kou *et al.*, 2015) and, thus, food waste, while also allowing significant reduction in energy consumption, operational costs and CO_2 emissions in this sector of the cold chain.

In addition, actions addressed to consumers' education to increase their awareness of the perishable nature of RTE products could also preserve product quality and freshness by damages caused by incorrect handling after purchase. Moreover, while several sulphur compounds have been confirmed to be suitable marker of off-odour formation in RTE wild rocket, 1-undecene is worth of further investigation as a plausible marker of microbial spoilage and, thus, quality degradation of RTE lettuce baby leaves, at least under MAP aerobic conditions.

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Conflict of interest

The authors declare no conflict of interest.

Author Contributions

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Ethics approval was not required for this research.

Peer Review

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. 5-point scales used to quantify sensory defects in RTE wild rocket and lettuce baby leaves.

Table S2. Volatile organic compounds identified in the headspace of rocket or baby lettuce leaves.

Table S3. Gas composition within packages (%), sensory defects scores and levels of VOCs (peak area) in RTE wild rocket bags subjected to the three storage conditions, NTA, STA and CTA, during 8 days of storage.

Table S4. Pearson correlation coefficients[1] between experimental parameters determined on RTE wild rocket samples.

Table S5. Gas composition within packages (%), sensory defects scores and levels of VOCs (peak area) in RTE lettuce baby leaves bags subjected to the three storage conditions, NTA, STA and CTA, during 8 days of storage.

Table S6. Pearson correlation coefficients[1] between experimental parameters determined on RTE lettuce baby leaves samples.

Fig. S1. PCA biplot of gas composition, VOCs and sensory defects scores determined on wild rocket bags subjected to the three storage conditions, NTA, STA and CTA, during 8 days of storage.

Fig. S2. PCA biplot of gas composition, VOCs and sensory defects scores determined on lettuce baby leaves bags subjected to the three storage conditions, NTA, STA and CTA, during 8 days of storage.

