Evaluation of microbiological and physico-chemical parameters of retail ready-to-eat mono-varietal salads from packaging until expiry date

Raimondo Gaglio, Alessandro Miceli, Maria T. Sardina, Nicola Francesca, Giancarlo Moschetti, Luca Settanni*

Dipartimento Scienze Agrarie, Alimentari e Forestali, Università di Palermo, Viale delle Scienze 4, 90128 Palermo, Italy

*Corresponding author.

Email address: luca.settanni@unipa.it (L. Settanni).

Running title: **Shelf life parameters of mono-varietal salads**

Abstract

In this work, an integrated microbiological and physico-chemical approach was applied to monitor the decay of mono-varietal RTE escarole and red chicory during the refrigerated storage. Total mesophilic microorganisms, total psychrotrophic microorganisms and pseudomonads were detected at the highest cell densities in all samples just after packaging and at the expiry date in both procuts. The dominating microbial populations analysed by classical culture-dependent methods belonged to *Pseudomonas* and yeast groups. Illumina sequencing identified *Janthinobacterium lividum* and *Pseudomonas veronii* as main species. Regarding the physico-chemical quality until expiry date, the main differences were found for weight loss, higher for escarole, and increase of TA and L*, higher for red chicory. This work showed how the microbiological and physico-chemical shelf life parameters change over time for fresh escarole and red chicory when the cold chain is strictly applied.

Practical applications

The monitoring of the microbiological, chemical and physical decay of monovarietal escarole and red chicory during refrigeration indicated how the parameters considered changed over time. These findings are useful to predict the evolution of quality parameters of vegetables in mixed salads.

Keywords: Escarole; Fresh cut vegetables; Microbial biodiversity; Red chicory; Vegetable decay

1. INTRODUCTION

Due to their effects against several diseases (Chakraborty & Chattopadhyay, 2018), vegetables are no more considered just as side dish for many consumers. Vegetarian diet is based mainly on vegetables, while vegan diet excludes any source of animal origin. The modern life-style determined an increase of the request for vegetables with a high convenience of use. For this reason, it has been registered an increasing trend of minimally processed ready-to-eat (RTE) production

(Putnik et al., 2017). RTEs provide almost all characteristics of fresh vegetables and do not necessitate further treatments before consumption (Maffei, Alvarenga, Sant'Ana, & Franco, 2016).

Many leafy vegetables used for fresh cut salads belong to the *Asteraceae* family (Tsironi et al., 2017). Among these, escarole and red chicories, have been widely adopted for producing RTE salads as they resist to cut, are characterised by a long shelf life and can add colour to the mix of vegetables. They have also gained consumer attention as they are considered "healthier" foods (Dupont, Mondi, Willamson, & Price, 2000). Escarole (*Cichorium endivia* var. *latifolia*), also called broad-leaved endive, has wide leaves with light green colour and has a less bitter taste than other endives. This species supplies fibre content and phytochemicals with antioxidant properties (mainly vitamin C and polyphenols) (Llorach, Martínez-Sánchez, Tomás-Barberán, Gil, & Ferreres, 2008). Red chicories (*Cichorium intybus* var. *silvestre*) are a group of leafy vegetables with variegated red or white-veined red-leaved coloured leaves (red with white ribs) very popular in Italy. They are mostly consumed raw in salads to add color and zest due to their distinctive slightly bitter taste (Alfonzo et al., 2018). Red chicories are also consumed after cooking in many traditional culinary preparations. The interest of consumers towards this product is mainly due to the potential health benefit of its phytochemical content linearly correlated with the antioxidant capacity (Lavelli, 2008). Red chicories have been found to possess the highest polyphenol content among many fresh consumed leafy vegetables and a high content of anthocyanin pigments (Innocenti et al., 2005; Rossetto et al., 2005). Hence, the high amounts of these compounds in red chicory could encourage its consumption (Ninfali, Mea, Giorgini, Rocchi, & Bacchiocca, 2005).

The pre-harvest microbial contamination plays a defining role on the microbiological quality of minimally processed vegetables, because conventional surface sanitation methods can reduce the microbial load, but hardly eliminate pathogens if present (Olaimat & Holley, 2012). Cutting represents the main operation during the processing of RTE leafy vegetables with the consequence that fresh cut vegetables deteriorate faster than intact produce. This is due to the disrupted cells that release their content making available enough energy and nutrients to support microbial

proliferation (Alfonzo et al., 2018). The microbiota of fresh vegetables includes bacterial pathogens, such as *Aeromonas hydrophila*, *Bacillus cereus*, *Clostridium* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Campylobacter* spp. and *Yersinia enterocolitica* (Beuchat, 2002), and several spoilage microorganisms composed of bacterial genera (mainly *Erwinia*, *Pseudomonas*, *Xanthomonas* and *Pectobacterium*) and yeasts (Lavelli, Pagliarini, Ambrosoli, & Zanoni, 2009; Liao, Sullivan, Grady, & Wong, 1997).

Despite the sanitizing procedures applied during post-harvest manipulation (Gil et al., 2015), RTE leafy vegetables have been recently been implicated with several foodborne diseases (Alegbeleye, Singleton, & Sant'Ana, 2018). Thus, due to their economic and public health relevance, recently, these products have been object of investigations aimed to characterize the microbial populations, by means of culture-dependent and –independent tools, of retail salads mainly at the time of collection/purchasing (Higgins et al., 2018; Jackson, Randolph, Osborn, & Tyler, 2013). Furthermore, these products are generally investigated considering mix of vegetables, but very limited information are available on the bacterial evolution of mono-varietal salads to predict the microbial dynamics of each species.

The present work was aimed to evaluate the microbiological quality of mono-varietal RTEs of escarole and red chicory collected soon after processing directly from the production factory to keep under control the cold chain during the entire shelf life. In order to better compare the microbial communities of the two different vegetable systems, their microbial dynamics were correlated with the physico-chemical parameters registered at the time of production and at the expiry date indicated on the labels.

2. MATERIALS AND METHODS

2.1. Sample collection and storage

Mono-varietal RTE escarole and red chicory were collected directly from a factory (Rosone S.p.a., Palermo, Italy) just after packaging. Both minimally processed products were placed into a portable

fridge to be kept under refrigeration during transport (approximately 30 min) and transferred to the laboratories of "Agricultural Microbiology" and "Vegetable Analysis" (Department of Agricultural and Forestry Science, University of Palermo) to carry out the chemico-physical and microbiological determinations, respectively at T_0. Sealed packs were stored at 4 °C for 9 d (T_9) that represented the expiry dates reported on the plastic envelopes and the RTEs subjected to the same analyses of T_0. The collection of RTEs was carried out in duplicate at 2- week interval.

2.2. Microbiological analyses

Twenty-five grams of each RTE sample were added with 225 mL Ringer's solution (SigmaeAldrich, Milan, Italy), homogenised with the stomacher BagMixer® 400 (Interscience, Saint Nom, France) at the highest speed for 2 min, and then subjected to the serial decimal dilution. The microbiological investigation included the enumeration of the following populations: total mesophilic microorganisms (TMM) on plate count agar (PCA), incubated at 30 °C for 72 h; total psychrotrophic microorganisms (TPM) on plate count agar (PCA), incubated at 7 °C for 7 d; pseudomonads on *Pseudomonas* agar base (PAB) added with CFC supplement, incubated at 25 °C for 48 h; members of the *Enterobacteriaceae* family on violet red bile glucose agar (VRBGA), incubated at 37 °C for 24 h; total coliforms on violet red bile agar (VRBA), incubated at 37 °C for 24 h; enterococci on kanamycin aesculin azide (KAA) agar, incubated at 37 °C for 24 h; coagulase-positive and coagulase-negative staphylococci (CPS and CNS) on Baird Parker (BP) added with RPF supplement, incubated aerobically at 37 °C for 48 h; *L. monocytogenes* on *Listeria* Selective Agar Base (LSAB) added with SR0140E supplement, incubated at 37 °C for 48 h; yeasts on yeast extract peptone dextrose (YPD) agar supplemented with 0.1 g/L chloramphenicol to avoid bacterial growth, incubated at 30 °C for 48 h. Microbiological counts were carried out in triplicate and the results expressed as log CFU/g.

2.3. Isolation and grouping of bacteria

After growth, five identical colonies (or fewer if five were not available or showed confluent growth) were collected from the highest plated dilutions for each morphology (color, margin, surface and elevation) of bacteria detected. All isolates were purified by successive sub-culturing on the optimal growth media. The purity of bacteria, as well as their cell morphologies and motility were determined microscopically. Preliminary characterization of bacterial species was carried out with the method of Gregersen (1978) determined by transferring fresh colonies from a petri dish to a glass slide and adding 3% of KOH water solution to determine the type of cell wall and with the evaluation of the presence of catalase by addition of H_2O_2 (5%, w/v) to the colonies. Spore formation was investigated as follows: the cell suspensions of the pure cultures were treated at 85 °C for 15 min and, subsequently, inoculated in the same media used for isolation and purification and incubated at the optimal growth conditions.

2.4. Genotypic differentiation and identification of bacteria

Overnight grown bacterial isolates were subjected to the DNA extraction by means of the InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) following manufacturer's instructions. The cell extracts were used as templates for PCR.

The differentiation of bacterial cultures at strain level was performed by random amplification of polymorphic DNA (RAPD)- PCR. Each reaction mix (25 μL) included single primers and the amplifications were carried out with the Swift™ MaxPro Thermal Cycler (Esco Micro Pte Ltd, Rome, Italy). PCRs were performed applying the protocol described by Gaglio et al. (2017) using three primers (M13, AB106 and AB111). The amplicons were run on 1.5% (w/v) agarose gels (Gibco BRL, Cergy Pontoise, France) for band separation. GeneRuler 100 bp Plus DNA ladder (M Medical Srl, Milan, Italy) was loaded as molecular size marker. The gels were stained with the SYBR® safe DNA gel stain (Molecular probes, Eugene, OR, USA) and visualized by UV trans-illumination. RAPD-PCR profiles were analyzed with the pattern analysis software package GelCompar II software version 6.5 (Applied-Maths, Saint-Marten-Latem, Belgium). Calculation of

146 similarities of band profiles was based on the Pearson product moment correlation coefficient.
147 Dendrograms were obtained by means of the unweighted pair group method using an arithmetic
148 average clustering algorithm.
149 All bacteria showing different RAPD-PCR profiles were analysed by 16S rRNA gene sequencing.
150 PCRs were performed as described by Weisburg, Barns, Pelletier, and Lane (1991) using the
151 primers rD1 (5'-AAGGAGGTGATCCAGCC-3') and fD1 (5'-AGAGTTTGATCCTGGCTCAG-
152 3'). DNA fragments were run and visualized as reported above. The PCR products were purified
153 using 10 U of exonuclease I and 1 U of shrimp alkaline phosphatase (Thermo Fisher Scientific).
154 DNA sequencing reactions were performed with a BigDye Terminator v3.1 cycle sequencing kit
155 (Applied Biosystems, Beverly, MA) with 5 µM of each of the primers used for PCR. Cycle
156 sequencing reactions were performed according to the manufacturer's instructions following
157 ethanol-EDTA-sodium acetate precipitation. Sequencing analyses were performed in an ABI Prism
158 3130xl genetic analyzer (Applied Biosystems) at the AGRIVET Centre (University of Palermo).
159 The sequences were compared with those available in the EzTaxon-e (http://eztaxon-
160 e.ezbiocloud.net/) database that compares a given sequence to those of type strains only (Chun et
161 al., 2007).
162
163 **2.5. Phenotypic and genotypic characterization of yeasts**
164 The different colonies of yeasts were collected at the highest cell suspension dilutions from the agar
165 plates, purified by several consecutive sub-culturing and phenotypically grouped based on their
166 cellular shapes.
167 The representative yeast cultures of each group were genetically processed. DNA extraction was
168 performed as described per bacteria. All isolates were differentiated through restriction fragment
169 length polymorphism (RFLP) analysis of the region spanning the internal transcribed spacers (ITS1
170 and ITS2) and the 5.8S rRNA gene as reported by Esteve-Zarzoso, Belloch, Uruburu, and Querol
171 (1999). The isolates representative of each RFLP group were identified at species level by

sequencing the D1/D2 region of the 26S rRNA gene to confirm the preliminary identification obtained by RFLP analysis. D1/D2 region was amplified and the PCR products visualized as described by Moschetti et al. (2016). The identity of the yeast sequences was determined by comparison with the sequences available in the GenBank/EMBL/DDBJ (http://www.ncbi.nlm.nih.gov) database (Altschul et al., 1997).

2.6. DNA amplification, Illumina sequencing and data analysis

Genomic DNA was extracted from mono-varietal salad samples just after packaging and after 9 d of refrigerated storage using QIAamp DNA Mini Kit and diluted to 5 ng/μL in 10 mM Tris pH 8.5. To amplify and sequence the V3-V4 hypervariable region (approximately 469 bp) of the 16S rRNA gene the Illumina protocol 16S Metagenomic Sequencing Library Preparation 15044223 (Rev. B) was used. The obtained libraries (approximately 630 bp in length) were normalized to 4nM, then pooled, and finally sequenced with MiSeq Reagent Kit v3, 600 Cycles sequencing kit (MS-102-3003) on MiSeq System (Illumina).

Sequences obtained from Illumina Sequencing were processed using QIIME2 software package version 2018.4 (Caporaso et al., 2010). Briefly, reads were demultiplexed and assigned to each sample according to the unique index. Sequences were filtered based on quality scores and the presence of ambiguous base calls using the *quality-filter q-score* options. Trimming was performed in order to trim sequences where quality score was less than 20 and, then, *representative sequences* were found using a 16S reference as positive filter as implemented in the *deblur denoise-16S* method. Finally, sequences were classified by taxon in Operational Taxonomic Units (OTUs) using a fitted classifier base on Greengenes 13.8 database. QIIME2 *taxa barplot* option was used for visualization of taxonomic composition of each sample. Alpha diversity analysis was performed using Chao1 estimator (Chao & Bunge, 2002) and observed OTUs in order to measure the community richness within samples.

198 **2.7. Physico-chemical determinations**

199 Four RTE samples at each collection time were weighted to evaluate weight loss. Samples of 50 g
200 (4 replicates) were homogenized with H_2O (1:2 w/v); the homogenates were centrifuged at 3500
201 rpm for 10 min and the supernatants were used for the analysis of soluble solids (SSC), titratable
202 acidity (TA), ascorbic acid and nitrate contents.

203 Soluble solid content (SSC) was determined with a digital refractometer (MTD-045nD, Three-In-
204 One Enterprises Co., Ltd., Taiwan) and expressed as °Brix. Titratable acidity (expressed as mg of
205 citric acid for 100g of fresh weight) was determined by titrating 10 mL of water extract with 0.1 M
206 NaOH up to pH 8.1. Ascorbic acid and nitrate contents (expressed as mg kg-1 of fresh weight) were
207 obtained using a Reflectometer RQflex10 Reflectoquant and the Reflectoquant ascorbic acid and
208 nitrate test strips (Merk, Germany) (procedures described in art. 1.16971.0001 and 1.16981.0001 by
209 Merk (http://www.merckmillipore.com/chemicals/).

210 Overall visual quality (OQ) was assessed by a panel made of 12 people (7 men and 5 women, aged
211 25-55) using a 1 to 5 scale, where 5 = excellent product with a fresh appearance and optimal
212 sensory acceptability (e.g. no colour modification, free from alterations), 3 = fair/limit of sensory
213 acceptability and marketability (e.g. minor defects), and 1 = poor/unmarketable, with discoloured
214 zones or severe defects.

215 The colour of escarole and red chicory leaves was evaluated at two points of the upper side of ten,
216 randomly selected, leaves for each replicate using a colorimeter (Chroma-meter CR-400, Minolta
217 corporation, Ltd., Osaka, Japan). L*, a* and b* parameters were recorded and used for hue angle
218 ($h° = \arctan(b^*/a^*)$ when $a^* > 0$ and $b^* > 0$, or as $h° = 180° + \arctan(b^*/a^*)$ when $a^* < 0$ and $b > 0$
219 (McGuire, 1992) and Chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) calculation . Total colour difference (ΔE) was
220 also calculated as $\Delta E = [(L^* - L_0) + (a^* - a_0) + (b^* - b_0)]^{1/2}$, where L_0, a_0 and b_0 are the control
221 values at the beginning of storage (T_0).

222

223 **2.8. Statistical analyses**

To determine the effect of storage on microbiological and physico-chemical parameters, a one-way ANOVA was carried out.

3. RESULTS

3.1. Levels of cultivable microorganisms

The results of plate counts of lettuce and red chicory samples just after production and at the 9th day of refrigerated storage are reported in Table 1. Tukey's test applied on the levels of microorganisms registered at T_0 and T_9 indicated that the populations of TPM, TMM, pseudomonads, total coliforms and yeasts were statistically different. For both vegetable productions the levels of TPM and TMM were comparable and represented the microbial groups found at the highest cell densities at T_0 as well as at T_9. Except for red chicory at T_9, the levels of pseudomonads were almost superimposable with those of the psychrotrophic populations. The members of *Enterobacteriaceae* family and yeasts did not exceed 4.3 and 5.3 log CFU/g, respectively, after 9 d. *Listeria* spp. were detected at both collection times. A slight decrease was registered in the levels of CPS from T_0 to T_9 for both produce. Enterococci and CPS were below the detection limits in all samples analysed.

3.2. Phenotypic differentiation of microorganisms

Four hundred and forty-five colonies were collected from the agar media used to retrieve the levels of the cultivable bacterial populations. All cultures were subjected to microscopic inspection and preliminary biochemical characterization. The combination of the characteristics evaluated for the phenotypic differentiation of the isolates allowed their separation into four groups (Table 2). Basically, three different cell morphologies were observed, with straight rods included in Groups I and IV, while cocci were further distinguished based on their cell disposition in short chain (Group II) or cluster (Group III). Only Group IV included Gram negative bacteria and motile cells. Regarding catalase test, Groups I and II were negative, while Groups III and IV were negative. The

249 vast majority of isolates (more than 72%) were included in Group IV. After colony and cell
250 morphology recognition, 44 yeasts were selected for further investigations.
251
252 **3.3. Typing, identification and distribution of bacteria and yeasts**
253 Following a common procedure applied for strain differentiation, about 40% of the isolates of each
254 phenotypic group was subjected to RAPD-PCR analysis and only the different strains (all isolates
255 sharing the same RAPD pattern were considered the same strain) were further analysed by 16S
256 rRNA gene sequencing. The resulting dendrogram (Fig. 1) showed that the bacteria found at the
257 highest cell density were represented by eight Gram positive strains and 18 Gram negative strains.
258 The most numerous genera were *Pseudomonas* and *Staphylococcus* among Gram negative and
259 Gram positive bacteria, respectively. The 26 dominant strains belonged to 21 species with
260 *Pseudomonas extremaustralis* and *Staphylococcus saprophiticus* being the most abundant.
261 The combination of the length of the bands from 5.8S-ITS and the RFLP profiles allowed the
262 identification of five yeast species belonging to five different genera (Table 3).
263 The distribution of the viable bacteria and yeasts species among the two RTE vegetables at T0 and
264 T9 was also investigated (Table 4). The highest species biodiversity was found in the samples of
265 RTE escarole at T_0 (15 bacterial and four yeast species), while the lowest diversity was registered
266 for red chicory at T_9 (three bacterial and two yeast species). In general, the number of both bacterial
267 and yeast species diminished during storage. Regarding the most numerous species, both *Ps.*
268 *extremaustralis* and *St. saprophyticus* were isolated from escarole for the entire monitoring period.
269 It is worth noting that the colonies grown on LSAB were identified as *Staphylococcus* showing a
270 low specificity of this media for *Listeria*. A low medium specificity was also observed for KAA,
271 since some colonies collected from this substrate and considered presumptive enterococci were
272 identified as *Lactobacillus paracasei* and *Lactococcus lactis.*
273
274 **3.4 Culture-independent analysis**

After quality filters with DEBLUR method, 57,745 reads hit the reference with a mean value of 14,436 per sample. Chloroplast and mitochondria sequences were removed from the dataset. *Janthinobacterium lividum* and *Pseudomonas veronii* were the only two species directly identified from the OTUs analysed (Results not shown). In particular the former detected only in red chicory at 9 d, while the latter in both salads at 9 d. Thus, in order to retrieve information at species level, the rest of the OTUs that could not be directly allotted into given species were manually blasted against the NCBI database. All four samples showed the presence of OTUs identified at genus level as *Streptococcus* which were assigned to the species *Streptococcus thermophilus*. Members of *Flavobacterium* genus, identified at species level as *Flavobacterium dongtanense*, were found only in red chicory at 9 d, while members of the family *Oxalobacteraceae*, identified as *Massilia brevitalea* were detected in both red chicory and escarole at 9 d. No significant differences were found between observed and predicted (Chao1estimator) OTUs. Therefore, the majority of OTUs present in each sample were captured.

3.5. Evolution of physico-chemical parameters of fresh cut produce

Both RTE escarole and red chicory consistently retained their water content until the end of the storage period. The higher weight loss (2.77 g/100 g f.w.) was registered for escarole. Storage at 4 °C for 9 days did not affected SSC and nitrate content neither in escarole nor in red chicory (Table 5).

TA and ascorbic acid content increased during storage (Table 5). The increase was higher in red chicory (+37.0%) than escarole (+31.1%) for TA, while the raise of ascorbic acid content was comparable between the two trials (+21.5% on average).

Storage induced some changes of colour parameters (Table 5). Lightness (L*) of leaf was not affected by storage in escarole, while significantly increased in red chicory. The changes in a* and b* parameters determined a significant decrease of colour saturation (-7.7% and -8.1% for chroma values in escarole and red chicory, respectively). Hue angle showed an opposite trend in escarole

301 with values increasing from 117.7 at day 0 to 120.3 at day 9, while no change was registered for red
302 chicory. The analysis of colour variation against the colour of leaves at day 0, showed a significant
303 effect of storage on total colour difference (ΔE). At the end of storage this parameter was 3.5 in
304 escarole and 4.4 in red chicory.
305 Scores for overall visual quality decreased slightly, but significantly, during storage. Nevertheless,
306 samples of escarole and red chicory were characterized by an acceptability score well above the
307 limit of marketability after 9 days of storage (Table 5).
308

309 **4. DISCUSSION**

310 Vegetables are important components of a healthy diet (FAO/WHO 2004); they provide a wide
311 variety of nutrients (vitamins, carbohydrates, and proteins) and, due to their phytochemicals, exert
312 several beneficial effect to the human body (Tango et al. 2018). Nowadays, vegetables are available
313 in several forms from fresh unprocessed to cooked or precooked, but due to the modern frenetic
314 life-style and the need of healthy foods characterized by a high convenience of use, fresh-cut
315 vegetables represent the sector that is increasing more rapidly (Miceli & Miceli 2014; Putnik et al.,
316 2017).
317 RTE salads are basically constituted of leafy vegetables whose surfaces and internal tissues are
318 colonized by bacterial communities (Jackson et al., 2013). In fact, several soil bacteria are able to
319 internalize and can contaminate fresh vegetables from the inside (Settanni, Miceli, Francesca,
320 Cruciata, & Moschetti, 2013) making inefficient the decontamination by post-harvest washing
321 treatments. In past, the microbiological quality of commercial RTE salads has been approached
322 considering mix of vegetables (Jackson et al., 2013; Leff & Fierer, 2013) but very limited
323 information are available on the bacterial evolution of a given mono-varietal vegetable (Alfonzo et
324 al., 2018), in order to predict the microbial dynamics of commercial leafy vegetable salads.
325 In the present work, escarole and red chicory were chosen as mono-varietal vegetable salads for
326 their different growth habitus and antioxidant content that represent two different ecosystems for

the development of the microbial communities. Both salads were collected directly at a production plant in order to keep under control the entire storage period. This strategy allowed the determination of the quality parameters soon after packaging, a step generally not trackable when RTE vegetables are purchased in retail markets. The last sampling was performed after 9 d of refrigerated storage as indicated on the labels for the expiry date. The shelf-life of RTE vegetable products or salads established by manufacturers usually ranges between 7 and 14 d depending on the type of fresh produce selected (Garcia-Ginemo & Zurera-Cosano, 1997).

Plate counts indicated that the dominant populations of both red chicory and escarole were represented by pseudomonads during the entire period of monitoring. The maximum level of TMM registered was around 10^7 CFU/g at the end of observation, that is below 10^8 CFU/g reported for green and red leaf vegetables by Jackson et al. (2013). Yeasts did not exceed 5.3 log CFU/g, after 9 d. At the same sampling members of *Enterobacteriaceae* family were 2 log cycles lower than pseudomonads. In general, the microbial groups mostly associated with the spoilage of RTE vegetables are pseudomonads and yeasts (Lavelli et al., 2009; Liao et al., 1997; Tsironi et al., 2017). *Listeria* spp. were detected at both collection times in both salads confirming the need for surveillance of *Listeria monocytogenes* in fresh cut vegetables (D'Aoust, 2007; Potter, Murray, Lawson, & Graham, 2012; Tsironi et al., 2017).

The identification of the cultured dominant isolates indicated that just after packaging *Pseudomonas extremaustralis* was the most abundant species among the Gram negative community, while *Staphylococcus saprophiticus* within the Gram positive bacteria. *Pseudomonas extremaustralis* has been, recently, detected in red chicory subjected to different cutting operation (Alfonzo et al., 2018). *Staphylococcus saprophiticus* is commonly associated with vegetables (Amaral, 2018) and is the causing agent of infection of the urinary tract (Kuroda et al., 2005). Both species were not detected at dominant levels after 9 d of refrigerated storage, but the group of pseudomonads was registered at the highest cell counts. In particular, at the end of the monitoring process, the main species found in both salads was *Pseudomonas grimontii*, object of recent investigations for its food

353 concerns (Cunault et al., 2018). Among the yeast community, *Candida intermedia*, *Cryptococcus*
354 *flavescens*, *Meyerozyma guilliermondii*, *Pichia fermentans* and *Trichosporon moniliiforme* were
355 identified, of which only *Candida* genus includes species responsible for candidal infections
356 (Jencson, Cadnum, Piedrahita, & Donskey, 2017).
357 Culture-independent analysis revealed the presence of Betaproteobacteria, Gammaproteobacteria,
358 Firmicutes and Bacteroidetes among the main bacterial lineages. Previous studies evidenced similar
359 results, Rastogi et al. (2012) identified Bacteroidetes, Firmicutes and Proteobacteria in romaine
360 lettuce, Lopez-Velasco, Welbaum, Boyer, Mane, and Ponder (2011) detected Firmicutes and
361 Proteobacteria as dominant bacterial groups in spinach, while Gammaproteobacteria (mainly
362 *Enterobacteriaceae* family members) prevailed in several vegetables as revealed by Leff and Fierer
363 (2013). Gammaproteobacteria and Betaproteobacteria were also found to dominate the bacterial
364 community of leaf vegetable as reported by Jackson et al. (2013).
365 Regarding the chemical and physical parameters of the minimally processed escarole and red
366 chicory, weight loss occurring during storage may negatively change the appearance and quality of
367 leafy vegetables, especially in those subjected to the cut operation as fresh cut products (Toivonen
368 & DeEll, 2002). Nevertheless, minimally processed vegetables are generally packed in sealed
369 plastic films that have low permeability to water vapour determining a very high RH inside the
370 sealed bags (almost 100% RH) (Alfonzo et al., 2018; Miceli & Miceli, 2014; Miceli, Romano,
371 Moncada, D'Anna, & Vetrano, 2015; Watada & Qi 1999), so dehydration is not a main issue as we
372 found for fresh cut escarole and red chicory. Moreover, products stored in sealed plastic bags at low
373 temperature usually have a low respiration rates (Alfonzo et al., 2018), as confirmed by the very
374 small reduction of SSC. Titratable acidity increased significantly during storage, probably due to
375 tissues breakdown that vegetables suffer during storage. This increase could be explained by the
376 high levels of CO_2 which build up in the atmosphere inside the packs that may cause a drop in pH
377 (Daniels, Krishnamurthi, & Rivdi, 1985; Farber, 1991). The TA increase corresponded to an
378 increase in ascorbic acid content. The amount of this very labile compound is often related to the

379 nutritional value of vegetables and may also provide indications of product degradation during
380 storage. We observed that ascorbic acid content increased about 21% during 9 days of storage at 4
381 °C. Similar variations during the initial days of cold storage were also reported for Swiss chard
382 (Miceli & Miceli, 2014), carrots (Howard, Wong, Perry, & Klein, 1999), green asparagus (Esteve,
383 Farre, Frigola, & Clemente, 1995) and broccoli (Eheart & Odland 1972; Wu et al. 1992).
384 The amount of nitrate accumulated in leafy vegetables can determine a negative effect on human
385 health. Escarole and red chicory were not affected by cold storage and had an average nitrate
386 content of 1065.0 and 445.0 mg/kg f.w. respectively, as also found by other authors (Alfonzo et al.,
387 2018; Santamaria, 2006).
388 Visual quality has a great importance in determining product acceptance and marketability. The
389 colour changes that leafy vegetables may undergo during storage can be an index of freshness loss
390 and microbiological decay. Colour modifications during storage were recorded in escarole and red
391 chicory with different extent for L*, a* and b* parameters. Total colour difference (ΔE) recorded
392 after 9 days of storage at 4 °C can be classified as very distinct ($\Delta E > 3$; Adekunte, Tiwari, Cullen,
393 Scannell, & O'Donnell, 2010) for both escarole (3.5) and red chicory (4.4) but did not determined
394 reduction of the overall appearance below the sensory acceptability. Overall appearance of RTE
395 escarole and red chicory changed significantly during storage, but after 9 days at 4 °C OQ scores
396 were still above the limit of marketability thus confirming that these vegetables have a shelf life
397 longer than the expiration date when stored continuously at low temperature (Cefola et al., 2016;
398 Alfonzo et al, 2018).
399 In conclusion, the decay affecting mono-varietal RTE escarole and red chicory was followed
400 through an integrated microbiological and physicochemical approach. The main microbial group
401 detected just after packaging and, then, at the expiry date was represented by pseudomonads. The
402 highest biodiversity in terms of species was found by the classical culture-dependent approach
403 rather than next generation sequencing. At the end of the observation period, *Ps. grimontii*
404 dominated in both RTE products. Although both matrices retained consistently their water content,

a higher weight loss was found for escarole. A higher increase of TA and L* was registered in red chicory after 9 d.

This study showed that the strict application of the cold chain determines the global quality retention for escarole and red chicory.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Luca Settanni http://orcid.org/0000-0001-7019-3598

REFERENCES

Adekunte, A., Tiwari, B., Cullen, P., Scannell, A., & O'Donnell, C. (2010). Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chemistry, 122*, 500–507.

Alegbeleye, O. O., Singleton, I., & Sant'Ana, A. S. (2018). Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. *Food Microbiology, 73*, 177–208.

Alfonzo, A., Gaglio, R., Miceli, A., Francesca, N., Di Gerlando, R., Moschetti, G., & Settanni, L. (2018). Shelf life evaluation of fresh-cut red chicory subjected to different minimal processes. *Food Microbiology, 73*, 298–304.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., & Miller, W. (1997). Grapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research, 25*, 3389–3402.

Amaral, J. P. D. (2018). Identification and susceptibility of the genus staphylococcus isolated from vegetables and legumes of economic interest. *Global Journal of Molecular Biology, 1*.

Beuchat, L. R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection, 4*, 413–423.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Huttley, G.A. (2010). QIIME allows analysis of highthroughput community sequencing data. *Nature Methods, 7*, 335–336.

Cefola, M., Carbone, V., Minasi, P., & Pace, B. (2016). Phenolic profiles and postharvest quality changes of fresh-cut radicchio (*Cichorium intybus* L.): nutrient value in fresh vs. stored leaves. *Journal of Food Composition and Analysis, 51*, 76-84.

Chakraborty, I., & Chattopadhyay, A. (2018). Pre- and post-harvest losses in vegetables. In B. Singh, S. Singh, & T. K. Koley (Eds.), *Advances in Postharvest Technologies of Vegetable Crops* (pp. 25–82). Boca Raton, London, New York: CRC Press Taylor & Francis.

Chao, A., & Bunge, J. (2002). Estimating the number of species in a stochastic abundance model. *Biometrics, 58*, 531–539.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K., & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *International Journal of Systematic and Evolutionary Microbiology, 57*, 2259–2261.

Cunault, C., Faille, C., Briandet, R., Postollec, F., Desriac, N., & Benezech, T. (2018). *Pseudomonas* sp. biofilm development on fresh-cut food equipment surfaces e a growth curve e fitting approach to building a comprehensive tool for studying surface contamination dynamics. *Food and Bioproducts Processing, 107*, 70–87.

D'Aoust, J.-Y. (2007). Current foodborne pathogens: salmonella. In M. Storrs, M.-C. Devoluy, & P. Cruveiller (Eds.), *Food safety handbook: Microbiological challenges* (pp. 128–141). France: BioMérieux Education.

448 Daniels, J. A., Krishnamurthi, R., & Rivdi, S. S. H. (1985). A review of effect of carbon dioxide on microbial growth
449 and food quality. *Journal of Food Protection, 48*, 532–537.
450 Dupont, S., Mondi, Z., Willamson, G., & Price, K. (2000). Effect of variety, processing, and storage on the flavonoid
451 glycoside and composition of lettuce and chicory. *Journal of Agricultural and Food Chemistry, 48*, 3957–3964.
452 Eheart, M. S., & Odland, D. (1972). Storage of fresh broccoli and green beans. Effect of ascorbic acid, sugars, and total
453 acids. *Journal of the American Dietetic Association, 60*, 402–406.
454 Esteve, M. J., Farre, R., Frigola, A., & Clemente, G. (1995). Changes in ascorbic acid content of green asparagus during
455 the harvesting period and storage. *Journal of Agricultural and Food Chemistry*, 43, 2058–2061.
456 Esteve-Zarzoso, B., Belloch, C., Uruburu, F., & Querol, A. (1999). Identification of yeasts by RFLP analysis of the 5.8S
457 rRNA gene and the two ribosomal internal transcribed spacers. *International Journal of Systematic Bacteriology,*
458 *49*, 329–337.
459 FAO/WHO. 2004. United Nations Food and Agriculture Organization/World Health Organization: Fruit and Vegetables
460 for Health. Report of a Joint FAO/WHO Workshop, 1-3 September Kobe, Japan, 2004. Available at
461 http://www.who.int/dietphysicalactivity/fruit/en/index1.html/ Accessed 13 September 2018.
462 Farber, J. M. (1991). Microbiological aspects of modified atmosphere packaging technology. A Review. *Journal of*
463 *Food Protection, 54*, 58–70.
464 Gaglio, R., Francesca, N., Di Gerlando, R., Mahony, J., DeMartino, S., Stucchi, C., … Settanni, L. (2017). Enteric
465 bacteria of food ice and their survival in alcoholic beverages and soft drinks. *Food Microbiology, 67*, 17–22.
466 Garcia-Ginemo, R. M., & Zurera-Cosano, G. (1997). Determination of ready to eat vegetable salad shelf life.
467 *International Journal of Food Microbiology, 36*, 31–38.
468 Gil, M. I., Selma, M. V., Suslow, T., Jacxsens, L., Uyttendaele, M., & Allende, A. (2015). Pre-and postharvest
469 preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables.
470 Critical Reviews in Food Science and Nutrition, 55, 453–468.
471 Gregersen, T. (1978). Rapid method for distinction of gram-negative from gram-positive bacteria. *Applied*
472 *Microbiology and Biotechnology*, *5*, 123–127.
473 Higgins, D., Pal, C., Sulaiman, I. M., Jia, C., Zerwekh, T., Dowd, S. E., & Banerjee, P. (2018). Application of high-
474 throughput pyrosequencing in the analysis of microbiota of food commodities procured from small and large retail
475 outlets in a US metropolitan area–A pilot study. *Food Research International, 105*, 29-40.
476 Howard, L. A., Wong, A. D., Perry, A. K., & Klein, B. P. (1999). β-Carotene and ascorbic acid retention in fresh and
477 processed vegetables. *Journal of Food Science, 64*, 929–936.
478 Innocenti, M., Gallori, S., Giaccherini, C., Ieri, F., Vincieri, F. F., & Mulinacci, N. 2005. Evaluation of the phenolic
479 content of the aerial parts of different varieties of *Cichorium intybus* L. *Journal of Agricultural and Food*
480 *Chemistry, 53*, 6497–6502.
481 Jackson, C. R., Randolph, K. C., Osborn, S. L., & Tyler, H. L. (2013). Culture dependent and independent analysis of
482 bacterial communities associated with commercial salad leaf vegetables. *BMC Microbiology, 13*, 1–12.
483 Jencson, A. L., Cadnum, J. L., Piedrahita, C., & Donskey, C. J. (2017). Hospital sinks are a potential nosocomial source
484 of Candida infections. *Clinical Infectious Diseases, 65*, 1954–1955.
485 Kuroda, M., Yamashita, A., Hirakawa, H., Kumano, M., Morikawa, K., Higashide, M., … Kuhara, S. (2005). Whole
486 genome sequence of Staphylococcus saprophyticus reveals the pathogenesis of uncomplicated urinary tract
487 infection. *Proceedings of the National Academy of Sciences, 102*, 13272-13277.
488 Lavelli, V. (2008). Antioxidant activity of minimally processed red chicory (*Cichorium intybus* L.) evaluated in
489 xanthine oxidase-, myeloperoxidase-, and diaphorase-catalyzed reactions. *Journal of Agricultural and Food*
490 *Chemistry, 56*, 7194–7200.
491 Lavelli, V., Pagliarini, E., Ambrosoli, R., & Zanoni, B. (2009). Quality of minimally processed red chicory (*Cichorium*
492 *intybus* L.) evaluated by anthocyanin content, radical scavenging activity, sensory descriptors and microbial
493 indices. *International Journal of Food Science & Technology, 44*, 994–1001.
494 Leff, J. W., & Fierer, N. (2013). Bacterial communities associated with the surfaces of fresh fruit and vegetables. *PLoS*
495 *ONE, 8*, e59310.
496 Liao, C.-H., Sullivan, J., Grady, J., & Wong, L.-J. C. (1997). Biochemical characterization of pectate lyases produced
497 by fluorescent pseudomonads associated with spoilage of fresh fruits and vegetables. Journal of Applied
498 Microbiology 83, 10–16.
499 Llorach, R., Martínez-Sánchez, A., Tomás-Barberán, F. A., Gil, M. I., & Ferreres, F. (2008). Characterisation of
500 polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chemistry, 108*, 1028–1038.

Lopez-Velasco, G., Welbaum, G. E., Boyer, R. R., Mane, S. P., & Ponder, M. A. (2011). Changes in spinach phylloepiphytic bacteria communities following minimal processing and refrigerated storage described using pyrosequencing of 16S rRNA amplicons. *Journal of Applied Microbiology*, *110*, 1203–1214.

Maffei, D. F., Alvarenga, V. O., Sant'Ana, A. S., & Franco, B. D. (2016). Assessing the effect of washing practices employed in Brazilian processing plants on the quality of ready-to-eat vegetables. *LWT - Food Science and Technology*, *69*, 474–481.

McGuire, R.G. (1992). Reporting of objective color measurements. *HortScience*, *27*, 1254–1255.

Miceli, A. & Miceli, C. (2014). Effect of nitrogen fertilization on the quality of Swiss chard at harvest and during storage as minimally processed produce. *Journal of Food Quality*, *37*, 125–134.

Miceli, A., Romano, C., Moncada, A., D'Anna, F., & Vetrano, F. (2015). Effect of cold storage on the quality of minimally processed cauliflower. *Carpathian Journal of Food Science and Technology*, *7*, 70–74.

Moschetti, G., Corona, O., Gaglio, R., Squadrito, M., Parrinello, A., Settanni, L., … Francesca, N. (2016). Use of fortified pied de cuve as an innovative method to start spontaneous alcoholic fermentation for red winemaking. *Australian Journal of Grape and Wine Research*, *22*, 36–45.

Ninfali, P., Mea, G., Giorgini, S., Rocchi, M., & Bacchiocca, M. (2005). Antioxidant capacity of vegetables, spices, and dressings relevant to nutrition. *British Journal of Nutrition*, *93*, 257−266.

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: a review. *Food Microbiology*, 32, 1–19.

Potter, A., Murray, J., Lawson, B., & Graham, S. (2012). Trends in product recalls within the agri-food industry: empirical evidence from the USA, UK and the Republic of Ireland. *Trends in Food Science & Technology*, *28*, 77–86.

Putnik, P., Kovačević, D. B., Herceg, K., Roohinejad, S., Greiner, R., Bekhit, A. E. D. A., & Levaj, B. (2017). Modelling the shelf-life of minimally-processed fresh-cut apples packaged in a modified atmosphere using food quality parameters. *Food Control*, *81*, 55–64.

Rastogi, G., Sbodio, A., Tech, J. J, Suslow, T.V., Coaker, G. L., & Leveau, J. H. J. (2012). Leaf microbiota in an agroecosystem: Spatiotemporal variation in bacterial community composition on field-grown lettuce. *ISME Journal*, *6*, 1812–1822.

Rossetto, M., Lante, A., Vanzani, P., Spettoli, P., Scarpa, M., & Rigo, A. (2005). Red chicories as potent scavengers of highly reactive radicals: A study on their phenolic composition and peroxyl radical trapping capacity and efficiency. *Journal of Agricultural and Food Chemistry*, *53*, 8169–8175.

Santamaria P. (2006). Nitrate in vegetables: toxicity, content, intake and EC regulation. *Journal of the Science of Food and Agriculture*, *86*, 10–17.

Settanni, L., Miceli, A., Francesca, N., Cruciata, M., & Moschetti, G. (2013). Microbiological investigation of *Raphanus sativus* L. grown hydroponically in nutrient solutions contaminated with spoilage and pathogenic bacteria. *International Journal of Food Microbiology*, *160*, 344–352.

Tango, C. N., Wei, S., Khan, I., Hussain, M. S., Kounkeu, P. F. N., Park, J. H., … Oh, D. H. (2018). Microbiological quality and safety of fresh fruits and vegetables at retail levels in Korea. *Journal of Food Science*, *83*, 386–392.

Toivonen, P. M. A., & DeEll, J. R. (2002). Physiology of fresh-cut fruits and vegetables. In: O. Lamikanra (Eds.), *Physiology of Fresh-cut Fruits and Vegetables: Science, Technology, and Market* (pp. 91–123). Boca Raton, London, New York: CRC Press Taylor & Francis.

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. *International Journal of Food Microbiology*, *240*, 131–140.

Watada, A. E., & Qi, L., 1999. Quality of fresh-cut produce. *Postharvest Biology and Technology*, *15*, 201–205.

Weisburg, W., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, *173*, 697–703.

Wu, Y., Perry, A. K., & Klein, B. P. (1992). Vitamin C and β-carotene in fresh and frozen green beans and broccoli in a simulated system. *Journal of Food Quality*, *15*, 87–96.

Table 1. Microbial loads of RTE samples[a]

Microbial group	Escarole		Statistical significance[b]	Red chicory		Statistical significance[b]
	0 d	9 d		0 d	9 d	
TPM	5.5 ± 0.4^{A}	7.0 ± 0.2^{B}	***	5.9 ± 0.5^{A}	7.4 ± 0.3^{B}	***
TMM	5.9 ± 0.5^{A}	7.4 ± 0.3^{B}	***	6.0 ± 0.3^{A}	7.0 ± 0.2^{B}	**
Pseudomonads	5.5 ± 0.1^{A}	6.7 ± 0.3^{B}	**	5.6 ± 0.2^{A}	6.7 ± 0.3^{B}	**
Enterobacteriaceae	3.8 ± 0.3^{A}	4.3 ± 0.5^{A}	N.S.	4.1 ± 0.2^{A}	4.3 ± 0.5^{A}	N.S.
Total coliforms	2.6 ± 0.4^{A}	3.5 ± 0.5^{B}	**	2.5 ± 0.3^{A}	3.5 ± 0.5^{B}	**
Enterococci	$<2^{A}$	$<2^{A}$	N.S.	$<2^{A}$	$<2^{A}$	N.S.
Yeasts	4.4 ± 0.3^{A}	5.3 ± 0.2^{B}	**	4.4 ± 0.3^{A}	5.3 ± 0.2^{B}	**
Listeria spp.	2.2 ± 0.3^{A}	2.5 ± 0.3^{A}	N.S.	2.0 ± 0.1^{A}	2.5 ± 0.3^{A}	N.S.
CPS	<2	$<2^{A}$	N.S.	$<2^{A}$	$<2^{A}$	N.S.
CNS	3.2 ± 0.4^{A}	2.8 ± 0.1^{A}	N.S.	3.5 ± 0.6^{A}	3.4 ± 0.7^{A}	N.S.

[a] Units are log CFU/g. Results indicate mean values ± S.D. of four plate counts (carried out in duplicate for two different productions).

[b] Data within a line followed by the same letter for the escarole and red chicory at 0 d (soon after purchasing) and 9 d (expiry date) are not significantly different according to Tukey's test. P value: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; N.S., not significant.

Abbreviations: TPM, total psychrotrophic microorganisms; TMM, total mesophilic microorganisms; CPS, coagulase-positive staphylococci; CNS, coagulase-negative staphylococci.

Table 2. Phenotypic grouping of bacteria isolated from ready-to-eat salads.

Characters	Groups			
	I (n = 16)	II (n = 21)	III (n = 102)	IV (n = 321)
Cell morphology	Straight rod	Coccus (short chain)	Coccus (cluster)	Straight rod
Gram reaction	Positive	Positive	Positive	Negative
Catalase test	Negative	Negative	Positive	Positive
Motility	–	–	–	+
Spore formation	–	n.d.	n.d.	–

Abbreviations: n, number of isolates; n.d., not determined.

Table 3. Molecular identification of yeasts.

Species	Strain	5.8S-ITS PCR (bp)	Size of restriction fragments (bp)			% similarity[a]	Acc. No.*
			*Cfo*I	*Hae*III	*Hin*fI		
Candida intermedia	4G67	390	190+200	390	190+200	99% KU708236.1	MK028822
Cryptococcus flavescens	4G300	540	230+310	540	165+275	100% EU386724.1	MK028823
Meyerozyma guilliermondii	4G58	600	275+325	400	280+320	100% KX792967.1	MK028824
Pichia fermentans	4G140	450	100+175	390	195+255	99% KM655842.1	MK028825
Trichosporon moniliiforme	4G101	540	300	540	240+280	99% KT895976.1	MK028826

[a] According to BlastN search of D1/D2 26S rRNA gene sequences in NCBI database.
*The Submission code will be replaced later with the assigned GenBank Accession Numbers.

595 **Table 4.** Speciographic distribution of bacteria and yeasts among RTE vegetables.

Species	RTE vegetables			
	T_0 escarole	T_0 red chicory	T_9 escarole	T_9 red chicory
Bacteria				
E. ludwigii	■		■	
H. alvei	■		■	
H. paralvei	■			
Lb. paracasei			■	
Lc. lactis	■	■		
L. amnigena	■			
P. agglomerans			■	
P. vagans		■		
Ps. azotoformans	■			
Ps. extremaustralis	■	■		
Ps. grimontii	■		■	■
Ps. koreensis		■		■
Ps. marginalis	■		■	
Ps. trivialis		■		
Ps. weihenstephanensis	■		■	
R. variigena	■			
S. fonticola				■
St. epidermidis	■			
St. fleurettii		■		
St. saprophyticus	■	■		
St. stepanovicii	■			
Yeasts				
C. intermedia	■	■	■	
Cr. flavescens	■	■	■	■
M. guilliermondii	■			
Ph. fermentans	■	■	■	■
T. moniliiforme		■		

596 Abbreviations: *C., Candida; Cr., Cryptococcus; E., Enterobacter; H., Hafnia; Lb., Lactobacillus; Lc., Lactococcus; L., Lelliottia; M., Meyerozyma;*
597 *P., Pantoea; Ph., Pichia; Ps., Pseudomonas; R., Rahnella; S., Serratia; St., Staphylococcus; T., Trichosporon.*

598

599

600 **Table 5.** Chemical and physical parameters of RTE vegetables.

Storage (d at 4 °C)	Weight loss (g $100g^{-1}$ f.w.)	SSC (°Brix)	TA[a] (mg $100g^{-1}$ f.w.)	Ascorbic Acid (mg kg^{-1} f.w.)	N-NO_3 (mg kg^{-1} f.w.)	L*	Chroma	Hue angle	ΔE	OQ[b]
	Escarole									
0		3.4	15.4b	22.8b	1120.0	56.9	36.5a	117.7b		5.0a
9	2.77	3.1	21.1a	27.8a	1010.0	55.6	33.7b	120.3a	3.5	4.1b
	Red chicory									
0		5.5	18.3b	78.5b	395.0	30.9b	32.1a	9.1		5.0a
9	1.87	5.2	24.0a	95.0a	495.0	34.7a	29.5b	8.2	4.4	3.9

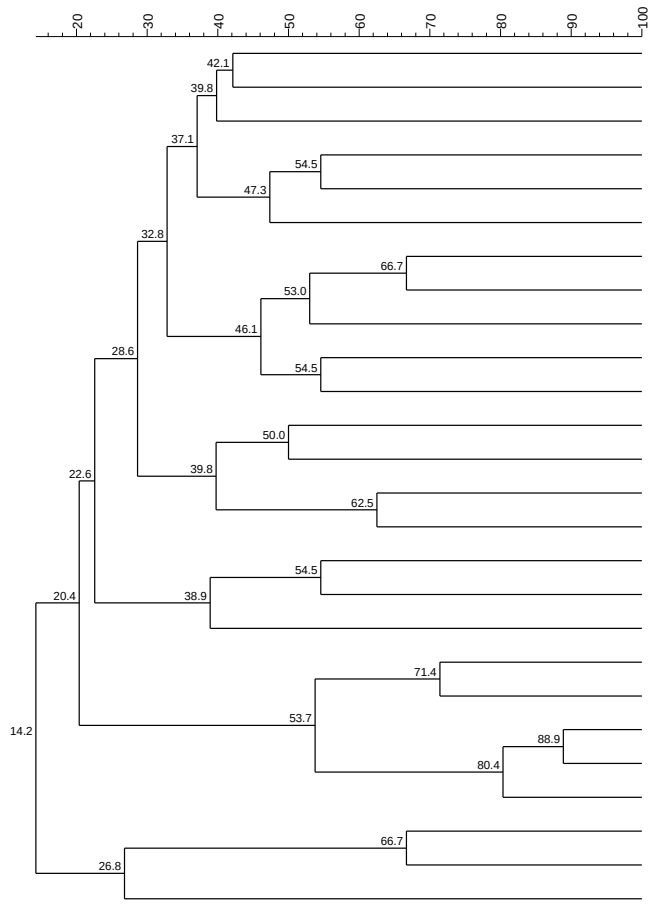
601
602 Data within a column for each vegetable followed by the same letter are not significantly different at P<0.05 according to ANOVA.
603 [a] Tritatable acidity expressed as citric acid.
604 [b] Overall visual quality 5: excellent or having a fresh appearance; 3: average - limit of marketability; 1: unmarketable

605 **Legend to figures**

606 **Fig. 1.** Dendrogram of bacteria obtained with combined RAPD patterns. Abbreviations: *E.*,
607 *Enterobacter*; *H.*, *Hafnia*; *Lb.*, *Lactobacillus*; *Lc.*, *Lactococcus*; *L.*, *Lelliottia*; *P.*, *Pantoea*; *Ps.*,
608 *Pseudomonas*; *R.*, *Rahnella*; *S.*, *Serratia*; *St.*, *Staphylococcus.*

609

Fig. 1.



Strain	Phenotypic group	Species	Acc. No.
4G292	IV	*Ps. extremaustralis*	MK026819
4G255	IV	*Ps. extremaustralis*	MK026817
4G278	IV	*Ps. extremaustralis*	MK026818
4G149	IV	*P. vagans*	MK026815
4G321	IV	*P. agglomerans*	MK026814
4G14	IV	*R. variigena*	MK026826
4G124	IV	*Ps. grimontii*	MK026820
4G177	IV	*Ps. grimontii*	MK026821
4G130	IV	*Ps. trivialis*	MK026824
4G91	IV	*Ps. marginalis*	MK026823
4G71	IV	*Ps. azotoformans*	MK026816
4G271	IV	*Ps. weihenstephanensis*	MK026825
4G88	IV	*Ps. koreensis*	MK026822
4G117	IV	*S. fonticola*	MK026827
4G145	IV	*E. ludwigii*	MK026808
4G44	IV	*H. alvei*	MK026809
4G53	IV	*H. paralvei*	MK026810
4G55	II	*Lc. lactis*	MK026812
4G5	III	*St. epidermidis*	MK026828
4G47	IV	*L. amnigena*	MK026813
4G155	III	*St. saprophiticus*	MK026831
4G31	III	*St. saprophiticus*	MK026830
4G163	III	*St. saprophiticus*	MK026833
4G156	III	*St. fleurettii*	MK026829
4G159	III	*St. stepanovicii*	MK026832
4G330	I	*Lb. paracasei*	MK026811