Shelf life evaluation of fresh-cut red chicory subjected to different minimal processes

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ABSTRACT

Microbiological, chemical and physical parameters of minimally processed red chicory (*Cichorium intybus* L.) subjected to two different transformation processes were investigated. A classic ready-to-eat (RTE) process (P1) and a production without cutting (P2) were monitored during refrigerated (4 °C) storage (15 d). Total mesophilic microorganisms, total psychrotrophic microorganisms and pseudomonads were detected at the highest cell densities in all samples. Presumptive *Pseudomonas* population dominated the cultivable microbial community of RTE red chicory and were characterized genetically. Twenty-two randomly amplified polymorphic DNA (RAPD) types were investigated by 16S rRNA gene sequencing, resulting in members of *Rahnella* and *Pseudomonas*. The identification of *Pseudomonas* species was further determined by sequencing of *gyrB*, *rpoB* and *rpoD* genes resulting in 16 species. A highest visual quality and a lower weight loss and colour variation were registered for P2, while soluble solid, nitrate and ascorbic acid contents were not affected by processing and storage. The integrated microbiological, chemical and physical approach applied in this study demonstrated the longer shelf-life of P2 red chicory.

1. Introduction

In the last decades, the increasing demand for vegetable convenience foods determined the availability of several ready-to-eat (RTE) products (Maffei et al., 2016). Most of them are obtained from leafy vegetables that are minimally processed, packaged and stored under refrigeration (Kennedy and Wall, 2007). RTE vegetables may represent a public health issue due to their potential transmission of bacterial, parasitic and viral pathogens (Abadias et al., 2008). The contamination of fresh cut products can take place during the pre- and post-harvest operations (Park et al., 2012; Verhoeff-Bakkenes et al., 2011) and during the administration to the final consumers. Fresh cut vegetables deteriorate faster than intact produce as a result of the wounding associated with processing, which leads to a number of physical and physiological changes affecting the viability and quality of the produce (Saltveit, 1997). Disrupted cells release their content and several microorganisms can easily grow. The main human pathogens associated to fresh cut vegetables are *Salmonella* spp., some serotypes of *Escherichia coli* and *Listeria monocytogenes* (Potter et al., 2012) that cause symptoms of gastroenteritis and even chronic infections (Francis et al., 1999). RTE vegetables host also several spoilage microorganisms such as *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Pectobacterium* and yeasts (Lavelli et al., 2009; Liao et al., 1997) causing textural changes and the appearance of off-odours and off-flavours (Liao et al., 1997).

Among leafy vegetables, chicory (*Cichorium intybus* L.) has gained attention for its content of phytochemicals with potential nutraceutical effects, such as phenolic acids and anthocyanins (Bais and Ravishankar, 2001). Red chicories (*C. intybus* var. *silvestre*) are very popular in Italy and are mostly consumed as raw salads characterized by a distinctive slightly bitter taste. The interest of consumers towards red chicory is mainly due to the health benefits provided by its phytochemical content correlated with the antioxidant capacity (Lavelli, 2008).

So far, only a few works have characterized the quality of fresh cut red chicory as affected by washing procedures (Wulfkuehler et al., 2015) and the packaging film (Lavelli et al., 2009). In this work, the effect of the cutting opertion on the microbiological, chemical and physical characteristics of RTE red chicory produced

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under controlled conditions were evaluated during a longer storage (15 d) than that generally considered for this kind of products (8–9 d).

2. Materials and methods

2.1. Plant material and experimental plan

Fresh red chicory, cv. Rosso di Chioggia, was purchased from a local market in Palermo and transported by a portable fridge to the Laboratory of Agricultural Microbiology (University of Palermo) where RTE vegetables were produced. Two different production processes were applied: process 1 (P1), represented the classical RTE vegetable production consisting of prewashing, cutting, washing, drying and packaging; in process 2 (P2), the cutting operation was excluded. Four heads of red chicory (approximately 2 Kg) were used for each production. After visual inspection, the external leaves were eliminated and the leaves suitable to be transformed were individually subjected to prewashing in cold (13–14 °C) tap water for 5 min. For P1, the prewashing step was followed by manual cutting performed with a sharp knife to approximately $3 \times 3 \text{ cm}^2$. After cutting (P1) or prewashing (P2), two consecutive washing steps were applied, the first with 0.2% (v/v) chlorine solution at ambient temperature (about 20 °C) for 2 min and the second with cold tap water for 2 min to remove chlorine. The ratio between red chicory and washing water or chlorine solution was kept at 1:5 (w/v). Fresh cut produce (P1) and entire leaves (P2) were dried with a manual centrifuge for 1 min at the maximum speed and 200 g of leaves for each production were packed into sterile plastic bags (Interscience, Saint Nom, France) thermally sealed with a hot bar (Laica VT3112, Vicenza, Italy) and refrigerated at 4 °C for 15 d. Samples were collected before treatment, after both processes and at 9, 12 and 15 d of storage. RTE vegetable productions were carried out in duplicate in two consecutive weeks during March 2016.

2.2. Microbiological analyses and isolation of the dominant microorganisms

Vegetable samples (25 g) from P1 and P2 were homogenized with a stomacher (BagMixer® 400, Interscience, Saint Nom, France) for 2 min at the highest speed in Ringer's solution (Sigma-Aldrich, Milan, Italy) (225 ml) and subjected to the decimal serial dilution. Different microbial groups were investigated as follows: total mesophilic microorganisms (TMM) and total psychrotrophic microorganisms (TPM) on plate count agar PCA; pseudomonads on Pseudomonas agar base (PAB) added with CFC supplement; members of the Enterobacteriaceae family on double-layered violet red bile glucose agar (VRBGA); total coliforms on double-layered violet red bile agar (VRBA); enterococci on kanamycin aesculin azide (KAA) agar; coagulase-positive and coagulase-negative staphylococci (CPS and CNS) on Baird Parker (BP) added with RPF supplement; L. monocytogenes on Listeria selective agar base (LSAB) added with SR0140E supplement; yeasts on yeast extract peptone dextrose (YPD) agar supplemented with 0.1 g/L chloramphenicol to avoid bacterial growth. The incubation conditions are described in Cruciata et al. (2018). All media and supplements were purchased from Oxoid (Milan, Italy). Microbiological counts were carried out in triplicate (three aliquots from the same bag) for all samples at each collection time.

Due to their relevant role in vegetable tissue degradation (Lavelli et al., 2009), pseudomonads were better characterized. After growth, presumptive Pseudomonas from PAB agar plates at the highest cell suspension dilutions were isolated. Almost five identical colonies (or fewer if five were not available or showed

confluent growth) were collected for each morphology (colour, margin, surface and elevation) detected. Bacterial isolates were purified by successive sub-culturing on PAB and their purity was checked microscopically. The preliminary characterization of the bacterial cultures was based on cell wall type determination by KOH test and production of catalase by addition of H_2O_2 (5%, w/v) to the colonies. Cell morphology and motility were evaluated microscopically.

2.3. Genetic characterization of pseudomonas

Genomic DNA from bacteria was prepared after overnight growth in Luria Bertani broth (Oxoid) at 25 °C. Cells were harvested and DNA was extracted using the InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) as described by the manufacturer. The cell extracts were used as templates for PCR.

The differentiation of the bacterial cultures 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 carried out as described by Gaglio et al. (2017) using three primers (M13, AB106 and AB111). RAPD-PCR profiles were analysed with the program GelCompar II 6.5 (Applied-Maths, Saint-Marten-Latem, Belgium). Calculation of similarities of band profiles was based on the Pearson's product moment correlation coefficient. Dendrograms were obtained by means of the unweighted pair group method using an arithmetic average clustering algorithm.

All bacteria showing different RAPD-PCR profiles were analysed by 16S rRNA gene sequencing as described by Weisburg et al. (1991) and gyrB, rpoD and rpoB gene sequencing following the methodology reported by Mulet et al. (2010). Sequencing analyses were performed in an ABI Prism 3130xl genetic analyzer (Applied Biosystems) at the AGRIVET Centre (University of Palermo, Italy). The resultant sequences were compared to sequenced bacteria with a BLAST search using the GenBank/EMBL/DDBJ database.

2.4. Physico-chemical analyses

The physico-chemical characteristics of red chicory transformed through P1 and P2 processes were investigated at the same intervals considered for microbiological analysis. Weight loss was evaluated by weighing four samples at each collecting time for each processing method. Samples of 50 g (4 replicates) were then homogenized with H_2O (1:2 w/v) and the water extracts were centrifuged at $3500 \times g$ for 10 min at 4 °C with the centrifuge Medifriger-BL (P Selecta, Barcelona, Spain). The supernatants were used for chemical determinations. Soluble solids concentration (SSC) was determined in °Brix using a digital refractometer (MTD-045nD, Three-In-One Enterprises Co., Ltd., Taiwan). Nitrate and ascorbic acid contents (mg/kg of fresh weight) were determined using a Reflectometer RQflex10 Reflectoquant and the Reflectoquant nitrate and ascorbic acid test strips (Merk, Germany) (procedures described in art. 1.16971.0001 and 1.16981.0001 by Merk (http://www.merckmillipore.com/chemicals/). Titratable acidity (TA) was determined by potentiometric titration with 0.1 M NaOH up to pH 8.1 using 10 ml of extract and expressed as mg of citric acid for 100 g of fresh weight.

Leaf colour was measured using a Chroma-meter CR-400 colorimeter (Minolta corporation, Ltd., Osaka, Japan) at two points of red tissue on the upper side of ten, randomly selected, entire leaves (P2) or leaf pieces (P1) for each replicate. Parameters L*, a* and b* were recorded. Hue angle (h°) and Chroma (C*) were calculated as h° = arctan (b*/a*) and C* = $(a^{*2} + b^{*2})^{1/2}$. Total colour difference (ΔE) was 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 values immediately after processing (T_0).

Overall visual quality (OQ) was evaluated by an informal panel made of nine people (4 men and 5 women, aged 35–55) using a 1 to 5 scale, with 5 = excellent or having a fresh appearance and full sensory acceptability (e.g. no browning or colour variation, free from handling defects and disease), 3 = fair/limit of sensory acceptability and marketability (e.g. presence of minor defects), and 1 = poor/unmarketable, with spots or extended browning, and severe defects.

2.5. Statistical analysis

Microbiological data were subjected to one-way variance analysis (ANOVA). Pair comparison of treatment means was achieved by Tukey's procedure at P < 0.05. To determine the effect of processing and storage time on physico-chemical parameters, a two-way ANOVA was carried out. Mean values were compared by the LSD multiple range test to identify significant differences among treatments and significant interactions between factors.

3. Results and discussion

3.1. Microbiological counts

The microbial loads of the red chicory samples collected during the application of P1 and P2 are reported in Table 1. Statistical significant differences were found for the levels of TPM and TMM and the presumptive pseudomonads, members of Enterobacteriaceae family, total coliforms, yeasts and CNS between the vegetables processed with P1 and P2. Putative enterococci were present only on the unprocessed red chicory, while presumptive L. monocytogenes and CPS were never detected. Except for raw material, the levels of TPM and TMM were almost superimposable indicating that the microbial communities of red chicory processed through P1 and P2 processes were mainly constituted of psychrotrophic populations. This observation was confirmed by the negligible differences found among the levels of TPM, TMM and presumptive Pseudomonas spp. for all samples. Since these three microbial groups were found at the highest cell densities, presumptive Pseudomonas was one of the dominating bacterial populations until 15 d of refrigerated storage for P1 and P2 RTE red chicory.

The involvement of pseudomonads in the deterioration or RTE salads is well known (Nguyen-the and Prunier, 1989). Pseudomonas spp. may account for 50–90% of the total microbial community of fresh vegetables (Wulfkuehler et al., 2015; Zagory, 1999). Among the previous works, only Wulfkuehler et al. (2015) estimated the concentration of Pseudomonas on fresh cut red chicory (cv. Rosso di Chioggia) reporting levels higher than 7 Log CFU/g at 10 d of conservation at 4 °C. In the present investigation, levels of 7.1 Log CFU/g were found only for P1 at 15 d, while lower levels were displayed by P2, even after 15 d of storage (6.0 Log CFU/g).

The levels of putative coliforms were significantly lower than those of potential Enterobacteriaceae members for all samples except P2 at 15 d. The last group was at least 1.3 Log CFU/g lower than the dominating population during the first days of manipulation until 2.9 and 2.2 Log cycles of difference for P1 and P2, respectively, at the end of the experimentation. The numbers of presumptive yeasts increased over time for both processes and the final counts were 2 Log cycles higher than those estimated soon after processing. Presumptive CPS and CNS were enumerated on the same agar medium (BP) and were differentiated by the formation of a clear halo around CPS colonies. CNS developed differently among P1 and P2. Even though after processing and at 9 d of storage their levels were below the detection limit for both processes, CNS appeared at 12 d of storage of P1 samples and increased to 3.0 Log CFU/g at 15 d, while in P2 they were only detected at the 15th day.

3.2. Phenotypic and genotypic characterization of pseudomonas

Due to the dominance of Pseudomonas populations revealed by culture-dependent assays, a total of 309 colonies were collected to be characterized. However, it cannot be excluded that different phyla and subphyla are identified by culture independent methods (Jackson et al., 2013). Phenotypic (morphological and biochemical) characterization confirmed that all isolates were Gram negative, catalase positive motile rods.

Due to the high number of isolates sharing the same phenotypic characteristics, about 30% of them (93 isolates), representative of each sample for each sampling time, was subjected to the genetic differentiation by RAPD-PCR analysis. By setting the cut-off for differentiation at a level of 61.5%, the population of presumptive Pseudomonas associated with the red chicory samples analysed was mainly represented by 22 RAPD types. However, the genetic investigation carried out only by 16S rRNA gene sequencing (sequence length ranging between 1422 and 1446 bp) indicated

Table 1
Microbial loads of red chicory samples.[a]

| Growth media[b] | Whole vegetables | Process 1 | | | | Process 2 | | | | Statistical significance[c] |
		0 d	9 d	12 d	15 d	0 d	9 d	12 d	15 d	P 1 * P 2
PCA 7 °C	6.0 ± 0.5	4.1 ± 0.4^A	5.7 ± 0.5^A	6.2 ± 0.2^A	6.9 ± 0.4^A	4.0 ± 0.1^A	4.9 ± 0.4^B	5.3 ± 0.2^B	5.6 ± 0.2^A	**
PCA 30 °C	6.8 ± 0.5	4.2 ± 0.5^A	5.5 ± 0.5^A	6.3 ± 0.2^A	6.9 ± 0.4^A	4.1 ± 0.2^A	4.9 ± 0.2^A	5.3 ± 0.1^B	5.6 ± 0.2^A	**
PAB	5.9 ± 0.6	4.1 ± 0.1^A	5.9 ± 0.3^A	6.2 ± 0.2^A	7.1 ± 0.3^A	3.9 ± 0.3^A	5.2 ± 0.2^A	5.3 ± 0.5^B	6.0 ± 0.2^A	**
VRBGA	4.3 ± 0.3	2.8 ± 0.2^A	3.3 ± 0.2^A	3.7 ± 0.2^A	4.2 ± 0.4^A	2.2 ± 0.2^B	2.5 ± 0.2^B	3.0 ± 0.1^A	3.8 ± 0.2^A	*
VRBA	4.0 ± 0.3	2.3 ± 0.3^A	2.6 ± 0.4^A	2.8 ± 0.2^A	3.4 ± 0.3^A	1.9 ± 0.2^B	2.1 ± 0.2^A	2.3 ± 0.3^A	3.5 ± 0.4^A	**
KAA	2.0 ± 0.3	<2^A	<2^A	<2^A	<2^A	<2^A	<2^A	<2^A	<2^A	N·S.
YPD	3.8 ± 0.3	2.2 ± 0.2^A	3.6 ± 0.6^A	4.7 ± 0.5^A	4.1 ± 0.5^A	2.2 ± 0.1^B	2.5 ± 0.3^B	3.9 ± 0.2^B	3.4 ± 0.6^A	***
BP (- halo)	2.4 ± 0.4	<2^A	<2^A	2.3 ± 0.2^A	3.0 ± 0.5^A	<1^A	<1^A	<1^A	2.1 ± 0.1^B	***

[a] Units are Log CFU/g. Results indicate mean values ± S.D. of four plate counts (carried out in duplicate for two independent productions). Data within a line followed by the same letter in the Process 1 and 2 at the same day are not significantly different according to Tukey's test.

[b] Correspondence between media and the presumptive microbial groups: PCA at 7 °C for total psychrotrophic microorganisms; PCA at 30 °C for total mesophilic microorganisms; PAB for pseudomonads; VRBGA for members of the Enterobacteriaceae family; VRBA for total coliforms; KAA for enterococci; YPD for yeasts; BP (- halo) for coagulase-negative staphylococci. The results of LSAB for L. monocytogenes and BP (+halo) for coagulase-positive staphylococci are not shown because always below the detection limit.

[c] Data within a line followed by the same letter for the Process 1 and 2 at the same day are not significantly different according to Tukey's test. P value: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; N·S., not significant.

that, although the majority of the RAPD types belonged to the *Pseudomonas* genus, other bacteria were able to grow on PAB, since the comparison with the sequences within the GenBank database identified one *Rahnella victoriana* (Acc. No. KY939761). This species has been recently described from oak tissue affected by the acute oak decline (Brady et al., 2014) and might cause tissue decay of other plants.

The identification of the 21 *Pseudomonas* RAPD types was concluded with the sequencing of three additional genes: *gyrB*, *rpoB* and *rpoD* characterized by sequence length in the range 693–866, 1046–1132 and 654–822 bp. Although some sequences did not provide a certain species, the combination of the four gene sequences undoubtedly showed that the *Pseudomonas* group of P1 and P2 red chicory included 16 species (Table 2). In particular, the highest biodiversity in terms of number of RAPD types was registered for *P. fluorescens* (n = 3) followed by *P. endophytica*, *P. grimontii* and *P. poae* (n = 2 for each species). *P. fluorescens* is a saprophyte bacterium that colonize soil, water and plant surface environments (Ganeshan and Manoj Kumar, 2005). *P. poae* belongs to the group of fluorescent pseudomonads first described from the phyllosphere of grasses (Behrendt et al., 2003), *P. endophytica* is a recent novel species isolated from stem tissue of *Solanum tuberosum* (Ramírez -Bahena et al., 2015) and *P. grimontii* from natural mineral water (Baïda et al., 2002). None of the 16 species identified is reported as a human pathogen.

The distribution of *Pseudomonas* strains among the samples analysed showed that several RAPD types were found in both processes and over time. Table 3 shows only the RAPD types detected at the highest dilutions in plates and indicated a different evolution among the dominant bacteria of P1 and P2; e.g. at 15 d of refrigerated storage, cut red chicory was mainly characterized by the presence of *P. fluorescens*, *P. grimontii* and *P. psychrophila*, while the entire leaves were dominated by *P. extremaustralis*, *P. libanensis* and *P. trivialis*. Regarding the three species associated to the fresh cut chicories, the development of biofilms by *P. fluorescens* and *P. grimontii* has been object of recent investigations due to the food concerns of these microbial structures (Cunault et al., 2018). The highest presence of *P. fluorescens* in fresh cut products rather than entire leaves is not surprising, because the dominance of this species over the total bacterial population of fresh-cut leafy vegetables is well known (Myszka et al., 2017). *P. psychrophila* is characterized by a *trans*-unsaturated fatty acid profile necessary for the exposition to low temperatures (Yumoto et al., 2001) justifying its presence in refrigerated vegetables.

3.3. Physico-chemical parameters of red chicory

The appearance and quality of minimally processed vegetables may be negatively influenced by weight loss occurring during storage. This parameter is considered an indicator to infer impact of abiotic stress on fresh cut products (Hodges and Toivonen, 2008). Red chicory retained a high water content until the 15th day, since the total loss through time ranged between 1.49 g/100 g.f.w. for P2 at 9 d and 2.32 g/100 g.f.w. for P1 at 15 d (Table 4). Cut leaves were characterized by a higher weight loss than entire leaves. P2 leaves showed also a negligible weight loss until 12 days of storage. Water loss is mainly determined by the reduction of outer periderm or cuticle resistance to transpiration due to the cutting operation (Toivonen and DeEll, 2002), explaining why weight loss was registered at a higher extent in P1 samples. Nevertheless, the negligible weight loss difference between P1 and P2 might be due to the low water vapour permeability of the plastic bags used for packaging. Thus, dehydration is not a typical issue for fresh cut produce packed in sealed plastic films (Miceli and Miceli, 2014; Miceli et al., 2015).

TA was affected by cut; this parameter increased significantly for P1 samples ranging between 18.93 and 22.77 mg/100 g.f.w. during storage, while no significant changes were registered for P2 that showed barely 1.44 mg/100 g.f.w. of difference during the 15 d of monitoring (Table 4). The higher TA of cut leaves could be explained by the tissue breakdown. However, fresh cut products retain the capacity to respond physiologically and cushion the variations of acidity induced by the high levels of CO_2 which build up in the atmosphere inside the sealed bags (Olarte et al., 2009).

SSC, nitrates and ascorbic acid contents were neither affected by processing nor by storage, suggesting that packaging and low temperatures resulted in low respiration rates. In particular, SSC was in the range 5.2–5.8 °Brix for P1 samples and 5.4–5.6 °Brix for P2 samples. Regarding $N-NO_3$ and ascorbic acid contents, higher ranges were found for P2 rather than P1 process. Nitrate accumulation in leafy vegetables can be a significant cause of decreased nutritional quality. P1 and P2 showed an average initial content of 502.5 mg/kg f.w. of titrates has also stated by Santamaria (2006) who classified red chicory among vegetables with a middle content of nitrates (500–1000 mg/kg f.w.). The nutritional value of vegetables is often related to their ascorbic acid content. This compound is very labile providing a direct indication of product degradation. Our results indicated that cutting did not affect this parameter and that cold storage was effective in limiting ascorbic acid degradation in red chicory as known for other leafy vegetables (Lee and Kader, 2000).

The highest differences between P1 and P2 processes were revealed by the appearance analysis. Colour measurements showed major changes induced by cut (Table 4). At the end of storage, lightness (L*) was higher in P1 than in P2 leaves. The redness of leaf colour (a*) decreased during storage, but the reduction occurred more rapidly in P1 and, at the end of storage, the entire leaves (P2) were characterized by a higher a* value than P1 leaves. ΔE increased throughout the storage period, but the greatest colour differences were recorded for P1 that always displayed a higher ΔE than P2. Changes in chroma value were recorded during storage for leaves subjected to both processes, but the colour of P1 samples showed values slightly lower than P2 samples. Hue angle variation were affected more significantly by processing than storage. Colour is an important quality attribute of fresh cut leafy vegetables; it can provide indirect information on freshness and microbiological decay and influences consumer's choice and preferences. Colour changes during storage often determine marketability loss before chemical and nutritional alterations occur (Miceli and Miceli, 2014). Colour differences can be classified as very distinct (ΔE > 3; Adekunte et al., 2010) for both processing methods, but cutting determined a ΔE increase after 15 d of 27.1% against only 5.5% in P2.

Scores for overall visual quality (Fig. 1) decreased during storage with significantly higher values at 9 and 15 d for P2. Moreover, P2 leaves were characterized by an acceptability score above the limit of marketability at the end of the storage period. Overall appearance was significantly affected by processing and storage duration. Cefola et al. (2016) reported a significant decrease in scores during storage of two hybrids of fresh cut red chicory that reached the sensory acceptability limit after 13.5 days of storage (on average for the two hybrids). Similarly, in our trial P1 samples fell under the marketability limit between 12 and 15 days of storage at 4 °C, while P2 was characterized by a score above the limit of marketability at the end of the storage period.

4. Conclusions

In this study, red chicory was used as a vegetable system to compare two different transformation processes: a classic RTE production (P1); and a production with minimally processed entire

Table 2
Genetic identification of *Pseudomonas* RAPD types isolated from red chicory.

RAPD types	Identified by:											
	16S rRNA	Sequence length (bp)	Ac. No.	gyrB	Sequence length (bp)	Ac. No.	rpoB	Sequence length (bp)	Ac. No.	rpoD	Sequence length (bp)	Ac. No.
4G414	*P. endophytica*	1424	KY939743	*P. endophytica*	808	MG707234	*P. endophytica*	1071	MG707255	*P. endophytica*	720	MG707272
4G477	*P. viridiflava*	1438	KY939759	*P. viridiflava*	834	MG707235	n.d.			*P. viridiflava*	760	MG707273
4G483	*P. grimontii*	1431	KY939749	*P. grimontii*	798	MG707236	*P. grimontii*	1089	MG707256	*P. grimontii*	714	MG707274
4G504	*P. azotoformans*	1432	KY939740	*Pseudomonas* spp.	839	MG707237	*P. azotoformans*	1053	MG707257	*P. azotoformans*	731	MG707275
4G513	*P. brenneri*	1425	KY939742	*P. brenneri*	804	MG707238	*Pseudomonas* spp.	1069	MG707258	*P. brenneri*	708	MG707276
4G518	*P. marginalis*	1431	KY939753	*P. marginalis*	843	MG707239	*P. marginalis*	1051	MG707259	*P. marginalis*	779	MG707277
4G531	*P. yamanorum*	1431	KY939760	*P. yamanorum*	838	MG707240	*P. yamanorum*	1061	MG707260	*P. yamanorum*	822	MG707278
4G537	*P. baetica*	1423	KY939741	*P. baetica*	866	MG707241	*P. baetica*	1097	MG707261	*Pseudomonas* spp.	697	MG707279
4G558	*P. poae*	1431	KY939754	*P. poae*	855	MG707242	*P. poae*	1097	MG707262	*P. poae*	684	MG707280
4G619	*P. extremaustralis*	1446	KY939745	*Pseudomonas* spp.	839	MG707243	n.d.			*P. extremaustralis*	717	MG707281
4G628	*P. fluorescens*	1424	KY939748	*P. fluorescens*	845	MG707244	*P. fluorescens*	1091	MG707263	*Pseudomonas* spp.	750	MG707282
4G764	*P. endophytica*	1443	KY939744	*P. endophytica*	843	MG707245	*P. endophytica*	1083	MG707264	*P. endophytica*	703	MG707283
4G769	*P. grimontii*	1431	KY939750	*P. grimontii*	798	MG707246	*P. grimontii*	1132	MG707265	*P. grimontii*	713	MG707284
4G787	*P. libanensis*	1441	KY939752	*P. libanensis*	838	MG7072477	*Pseudomonas* spp.	1046	MG707266	*P. libanensis*	713	MG707285
4G793	*P. psychrophila*	1431	KY939756	*P. psychrophila*	838	MG707248	n.d.			*P. psychrophila*	822	MG707286
4G893	*P. helleri*	1422	KY939751	*Pseudomonas* spp.	693	MG707249	n.d.			*P. helleri*	654	MG707287
4G921	*P. trivialis*	1425	KY939758	*P. trivialis*	838	MG707250	*Pseudomonas* spp.	1087	MG707267	*P. trivialis*	713	MG707288
4G1010	*P. simiae*	1441	KY939757	*P. simiae*	798	MG707251	*P. simiae*	1093	MG707268	*P. simiae*	714	MG707289
4G1030	*P. poae*	1434	KY939755	*P. poae*	798	MG707252	*P. poae*	1052	MG707269	*P. poae*	714	MG707290
4G1034	*P. fluorescens*	1434	KY939746	*P. fluorescens*	841	MG707253	*P. fluorescens*	1094	MG707270	*P. fluorescens*	702	MG707291
4G1237	*P. fluorescens*	1424	KY939747	*P. fluorescens*	841	MG7072544	*P. fluorescens*	1128	MG707271	*P. fluorescens*	702	MG707292

n.d. not determined.

Table 3
Distribution of the dominant *Pseudomonas* RAPD types collected from cut and uncut red chicory samples during storage.

Species	Whole vegetables	Processed vegetables							
		Cut chicory (P1)				Entire leaves (P2)			
		0 d	9 d	12 d	15 d	0 d	9 d	12 d	15 d
P. azotoformans	4G504								
P. baetica							4G537		
P. brenneri						4G513			
P. endophytica		4G414							4G764
P. extremaustralis							4G619		
P. fluorescens			4G1034		4G1237			4G628	
P. grimontii		4G483			4G769				
P. helleri								4G893	
P. libanensis									4G787
P. marginalis		4G518							
P. poae			4G558				4G1030		
P. psychrophila					4G793				
P. simiae				4G1010					
P. trivialis									4G921
P. viridiflava						4G477			
P. yamanorum							4G531		

Table 4
Effect of processing and storage on weight loss, titratable acidity (TA), content of soluble solids (SSC), nitrate and ascorbic acid and leaf colour changes.

Processing	Storage	Weight loss	TA[a]		SSC	N-NO$_3$	Ascorbic Acid	L*		a*		b*		ΔE		Chroma		Hue angle	
	(d at 4 °C)	(g/100 g f.w.)	(mg/100 g f.w.)		(°Brix)	(mg/kg f.w.)	(mg/kg f.w.)												
Fresh cut (P1)	0		18.93	b	5.2	472.5	170.3	31.5	c	32.7	a	5.6	a			33.3	a	9.8	a
	9	1.85	ab	20.46	ab	5.8	577.5	183.8	35.0	ab	30.2	bc	4.2	b	5.49	b	30.5	bc	8.1	ab
	12	2.12	ab	22.48	a	5.5	478.1	184.5	33.2	bc	29.9	bc	4.0	bc	5.50	b	30.3	bc	7.7	ab
	15	2.32	a	22.77	a	5.6	474.3	190.5	34.9	ab	28.6	c	4.3	b	6.98	a	28.9	c	8.7	ab
Entire leaves (P2)	0		17.29	b	5.4	532.5	181.5	34.1	b	32.8	a	3.8	bc			33.1	a	6.8	b
	9	1.49	b	17.00	b	5.6	412.5	168.0	36.3	a	30.9	b	4.0	bc	4.05	c	31.2	b	7.4	b
	12	1.65	b	18.16	b	5.3	555.0	180.0	35.0	ab	30.8	b	3.2	c	4.02	c	31.0	b	6.0	b
	15	2.05	ab	18.73	b	5.6	567.5	198.0	35.2	ab	30.4	b	3.8	bc	4.28	bc	30.6	bc	7.2	b
Processing		*	***		ns	ns	ns	***		**		***		***		*		***	
Storage		*	*		ns	ns	ns	***		***		***		*		***		*	
Processing x Storage		ns	ns		ns	ns	ns	ns		ns		ns		ns		ns		ns	

Data within a column followed by the same letter are not significantly different according to LSD multiple range test. Probability: ns not significant; * significant at P < 0.05; ** significant at P < 0.01; *** significant at P < 0.001.

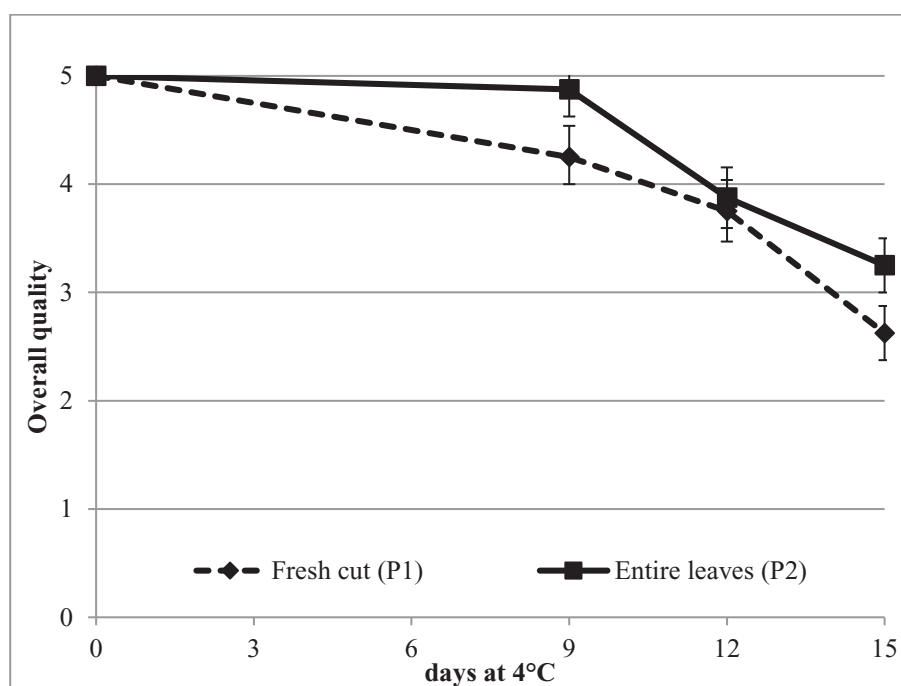[a] Tritatable acidity expressed as citric acid.

Fig. 1. Influence of processing and storage at 4 °C on overall quality of minimally processed red chicory (5: excellent or having a fresh appearance; 3: average - limit of marketability; 1: unmarketable).

leaves (P2). The integrated multidisciplinary (microbiological, chemical and physical) approach demonstrated the longer shelf life of minimally processed uncut leaves of red chicory compared with the fresh cut trial. P2 trial was characterized by a higher visual acceptability, lower weight loss and colour variations as well as lower increase of microbial parameters, especially *Pseudomonas* levels, than P1 trial. Thus, minimally processed uncut red chicory leaves retained their marketability until 15 d of refrigerated storage.

References

Abadias, M., Usall, J., Anguera, M., Solsona, C., Viñas, I., 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. Int. J. Food Microbiol. 123, 121–129.

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 Chem. 122, 500–507.

Baïda, N., Yazourh, A., Singer, E., Izard, D., 2002. *Pseudomonas grimontii* sp. nov. Int. J. Syst. Evol. Microbiol. 52, 1497–1503.

Bais, H.P., Ravishankar, G.A., 2001. *Cichorium intybus* L–cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects. J. Sci. Food Agric. 81, 467–484.

Behrendt, U., Ulrich, A., Schumann, P., 2003. Fluorescent pseudomonads associated with the phyllosphere of grasses; *Pseudomonas trivialis* sp. nov., *Pseudomonas poae* sp. nov. and *Pseudomonas congelans* sp. nov. Int. J. Syst. Evol. Microbiol. 53, 1461–1469.

Brady, C., Hunter, G., Kirk, S., Arnold, D., Denman, S., 2014. *Rahnella victoriana* sp. nov., *Rahnella bruchi* sp. nov., *Rahnella woolbedingensis* sp. nov., classification of *Rahnella* genomospecies 2 and 3 as *Rahnella variigena* sp. nov. and *Rahnella inusitata* sp. nov., respectively and emended description of the genus *Rahnella*. Syst. Appl. Microbiol. 37, 545–552.

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. J. Food Compos. Anal. 51, 76–84.

Cruciata, M., Gaglio, R., Scatassa, M.L., Sala, G., Cardamone, C., Palmeri, M., Moschetti, G., La Mantia, T., Settanni, L., 2018. Formation and characterization of early bacterial biofilms on different wood typologies applied in dairy production. Appl. Environ. Microbiol. 84, e02107–e02117.

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

Francis, G.A., Thomas, C., O'Beirne, D., 1999. The microbiological safety of minimally processed vegetables. Int. J. Food Sci. Tech 34, 1–22.

Gaglio, R., Francesca, N., Di Gerlando, R., Mahony, J., DeMartino, S., Stucchi, C., Moschetti, G., Settanni, L., 2017. Enteric bacteria of food ice and their survival in alcoholic beverages and soft drinks. Food Microbiol. 67, 17–22.

Ganeshan, G., Manoj Kumar, A., 2005. *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. J. Plant Interact. 1, 123–134.

Hodges, D.M., Toivonen, P.M., 2008. Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress. Postharvest Biol. Technol. 48, 155–162.

Jackson, C.R., Randolph, K.C., Osborn, S.L., Tyler, H.L., 2013. Culture dependent and independent analysis of bacterial communities associated with commercial salad leaf vegetables. BMC Microbiol. 13, 1–12.

Kennedy, J., Wall, P., 2007. Food safety challenges. In: Storrs, M., Devoluy, M.C., Cruveiller, P. (Eds.), Food Safety Handbook: Microbiological Challenges. Bio-Mérieux Education, France, pp. 8–19.

Lavelli, V., 2008. Antioxidant activity of minimally processed red chicory (*Cichorium intybus* L.) evaluated in xanthine oxidase-, myeloperoxidase-, and diaphorase-catalyzed reactions. J. Agric. Food Chem. 56, 7194–7200.

Lavelli, V., Pagliarini, E., Ambrosoli, R., Zanoni, B., 2009. Quality of minimally processed red chicory (*Cichorium intybus* L.) evaluated by anthocyanin content, radical scavenging activity, sensory descriptors and microbial indices. Int. J. Food Sci. Tech 44, 994–1001.

Lee, S.K., Kader, A.A., 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol. 20, 207–220.

Liao, C.-H., Sullivan, J., Grady, J., Wong, L.-J.C., 1997. Biochemical characterization of pectate lyases produced by fluorescent pseudomonads associated with spoilage of fresh fruits and vegetables. J. Appl. Microbiol. 83, 10–16.

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 Sci. Technol 69, 474–481.

Miceli, A., Miceli, C., 2014. Effect of nitrogen fertilization on the quality of Swiss chard at harvest and during storage as minimally processed produce. J. Food Qual. 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. Carpath. J. Food Sci. Technol. 7, 70–74.

Mulet, M., Lalucat, J., García-Valdés, E., 2010. DNA sequence-based analysis of the *Pseudomonas* species. Environ. Microbiol. 12, 1513–1530.

Myszka, K., Schmidt, M.T., Majcher, M., Juzwa, W., Czaczyk, K., 2017. β-Caryophyllene rich pepper essential oils suppress spoilage activity of *Pseudomonas fluorescens* KM06 in fresh-cut lettuce. LWT - Food Sci. Technol 83, 118–126.

Nguyen-the, C., Prunier, J.P., 1989. Involvement of pseudomonads in deterioration or 'ready-to-use'salads. Int. J. Food Sci. Tech. 24, 47–58.

Olarte, C., Sanz, S., Echàvarri, J.F., Ayala, F., 2009. Effect of plastic permeability and exposure to light during storage on the quality of minimally processed broccoli and cauliflower. LWT Food Sci. Technol 42, 402–411.

Park, S., Szonyi, B., Gautam, R., Nightingale, K., Anciso, J., Ivanek, R., 2012. Risk factors for microbial contamination in fruits and vegetables at the preharvest level. J. Food Prot 75, 2055–2081.

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 Food Sci. Technol. 28, 77–86.

Ramírez -Bahena, M.H., Cuesta, M.J., Tejedor, C., Igual, J.M., Fernández-Pascual, M., Peix, A., 2015. *Pseudomonas endophytica* sp. nov., isolated from stem tissue of *Solanum tuberosum* L. Spain. Int. J. Syst. Evol. Microbiol. 65, 2110–2117.

Saltveit, M.E., 1997. Physical and physiological changes in minimally processed fruits and vegetables. In: Tomas-Barberan, F.A., Robins, R.J. (Eds.), Phytochemistry of Fruit and Vegetables. Oxford University Press, London, pp. 205–220.

Santamaria, P., 2006. Nitrate in vegetables: toxicity, content, intake and EC regulation. J. Sci. Food Agr 86, 10–17.

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

Verhoeff-Bakkenes, L., Jansen, H.A., in 't Veld, P.H., Beumer, R.R., Zwietering, M.H., van Leusden, F.M., 2011. Consumption of raw vegetables and fruits: a risk factor for *Campylobacter* infections. Int. J. Food Microbiol. 144, 406–412.

Weisburg, W., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703.

Wulfkuehler, S., Dietz, J., Schmidt, H., Weiss, A., Carle, R., 2015. Quality of fresh-cut radicchio cv. Rosso di Chioggia (*Cichorium intybus* L. var. *foliosum* Hegi) as affected by water jet cutting and different washing procedures. Eur. Food Res. Technol 240, 159–172.

Yumoto, I., Kusano, T., Shingyo, T., Nodasaka, Y., Matsuyama, H., Okuyama, H., 2001. Assignment of *Pseudomonas* sp. strain E-3 to *Pseudomonas psychrophila* sp. nov., a new facultatively psychrophilic bacterium. Extremophiles 5, 343–349.

Zagory, D., 1999. Effects of post-processing handling and packaging on microbial populations. Postharvest Biol. Technol. 15, 313–321.