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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 3×3 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 $h^\circ = \arctan(b^*/a^*)$ and $C^* = (a^{*2} + b^{*2})^{1/2}$. Total colour difference (ΔE) was calculated as