Postharvest Biology and Technology 124 (2017) 91–99

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Research paper

Bacterial population dynamics and sensorial quality loss in modified atmosphere packed fresh-cut iceberg lettuce

M.J.M. Paillart[a,c,*], J.M.B.M. van der Vossen[b,c], E. Levin[b,c], E. Lommen[b,c], E.C. Otma[a,c], J.C.M.A. Snels[a,c], E.J. Woltering[a,c]

[a] Wageningen Food & Biobased Research, PO Box 17, 6700AA Wageningen, The Netherlands
[b] Netherlands Organisation for Applied Scientific Research – TNO, Postbus 360, 3700AJ Zeist, The Netherlands
[c] TI Food & Nutrition, PO Box 557, 6700AN Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 15 June 2016
Received in revised form 7 October 2016
Accepted 11 October 2016
Available online xxx

Keywords:
Sensorial quality
Fresh-cut lettuce
Leuconostoc spp
Bacterial population dynamics
Shelf life
Off-odour

ABSTRACT

The end of shelf life of fresh-cut iceberg lettuce packed in modified atmosphere (MA) is determined by its visual quality and by its microbial load. The total microbial load should not exceed 6–7 log CFU g^{-1} mesophilic counting and be free of human pathogens. Not much is known about specific interactions between the levels of specific spoilage micro-organisms and the sensorial quality attributes of the fresh-cut product. We studied the changes in sensorial quality attributes and the growth and population dynamics of microorganisms in three independent batches of MA packed fresh-cut iceberg lettuce stored at 7 °C.

The sensorial quality of the fresh-cut product was daily evaluated by visual determination of color, shape (indication of crispness) and discoloration (browning); together these attributes were translated into an Overall Visual Quality (OVQ) value. In addition, off-odour and sourness production were scored immediately after opening of the bags. Samples of the fresh-cut product were taken to determine the total microbial load and to investigate the bacterial species composition using mass sequencing analysis.

The decrease of OVQ over time showed a down sigmoid curve, reaching the limit of consumer acceptance after about eight days. Total bacterial counts increased from about 5 log at day 1 to about 8 log colony forming units (CFU) per gram at day 7. Initially, *Pseudomonas* species were dominant but when the package became anaerobic, lactic acid bacteria (LAB), in particular *Leuconostoc* spp. and *Lactococcus* spp. became more abundant and dominated the bacterial population. Especially the rapid growth of *Leuconostoc* species correlated with development of a sour off-odour, represented by the accumulation of acetic and lactic acids in the product and the decrease in OVQ. Results indicate that metabolites produced by LAB are responsible for off-odour production and loss of sensorial quality.

Based on this knowledge, new strategies for shelf-life improvement may be designed to prevent the dominance of LAB in fresh cut lettuce or make shelf-life better predictable based on initial counts of LAB, that may assist the industry and retail to reduce food waste.

1. Introduction

Fresh-cut vegetables have become increasingly popular over the last two decades on the West –European food market. With an annual growth volume of about 4%, fresh-cut produce has become the most profitable product in the fruit and vegetables segment (Van Rijswick, 2011). Due to the relatively short shelf-life of fresh-cut products that do not exceed 6–9 d for fresh-cut fruit (Gil et al., 2006), retailers are faced with high food waste volume in this market segment. In 2008, food wastage of fresh-cut vegetable represented 4.4% of all food waste (at retailer level) and lettuce/mixed salad accounted for 24.5% of the total fresh-cut segment (Ventour, 2008). Food wastage is mainly due to unsold product when it reaches the end of the "use by date" printed on the package. The "use by date" is required by the food industry to notify the consumer about the quality and safety of the product.

* Corresponding author at: Wageningen Food & Biobased Research, PO Box 17, 6700 AA Wageningen, The Netherlands.
E-mail addresses: Maxence.paillart@wur.nl (M.J.M. Paillart), Jos.vandervossen@tno.nl (J.M.B.M. van der Vossen), Evgeni.levin@tno.nl (E. Levin), Eveline.lommen@tno.nl (E. Lommen), Els.otma@wur.nl (E.C. Otma), Joost.snels@wur.nl (J.C.M.A. Snels), Ernst.woltering@wur.nl (E.J. Woltering).

http://dx.doi.org/10.1016/j.postharvbio.2016.10.008

The "use-by" date is an arbitrary measure based on expected quality decay of the product but does not always reflect the real sensorial quality of the product. It is common to observe fresh-cut produce reaching the "use-by" date is still perfectly acceptable according to its sensorial or microbiological quality attributes. A better understanding of the factors affecting the "quality" of the fresh-cut produce will permit to correctly predict the shelf life performance of fresh-cut produce and to reduce the volume of food wastage.

Fresh-cut preparation induces substantial wounding that significantly reduces the storability (shelf life) of the produce. The shelf life of fresh-cut product such as fresh-cut iceberg lettuce is defined by food producers according to two main criteria: the produce's sensorial quality and its microbiological load. The sensorial quality should be above the consumer's acceptance limit till consumption date. At purchase, consumers base their choice mainly on the overall visual quality (OVQ), being a combination of quality features that can be judged through the packaging (James and Ngarmsak, 2010). The OVQ is mainly defined by the colour and the shape, the latter being an indication of freshness or crispness of the product. Once the consumer opens the bag for consumption, the presence of off-odour and sourness also play an important role in the product quality judgement. Another criterion is based on the microbiological load. Although the European food authority EFSA does not define the maximum levels of total count in fresh-cut products, French regulation has fixed the maximum acceptable contamination value at the end of the microbiological shelf life to 5×10^7 cfu g^{-1} (Corbo et al., 2006). Ragaert et al. (2007) reported that sensorial quality decays were observed when psychrotophic microbial load exceeded 8 log cfu g^{-1}. On the other hand, spoilage of the fresh-cut product was observed when lactic acid bacteria load exceeded 6 log cfu g^{-1} (García-Gimeno and Zurera-Cosano, 1997). Apart from microbial and sensory spoilage, the fresh-cut vegetables or fruit have to be free of human pathogens during their complete shelf life period.

Sensorial quality decay and microbiological growth in fresh-cut produce have been extensively studied in isolation, but their interactions have only been studied in a few cases. Some studies (Pothakos et al., 2012; Vihavainen et al., 2008) report correlations between sensorial quality decay and total microbial load but the possible mechanistic interactions between particular bacterial species and quality decay have not been investigated in detail. We investigated simultaneously sensorial quality losses and bacterial population dynamics of fresh-cut iceberg lettuce stored for 9 d at 7 °C. A correlation analysis was applied on all attributes to determine relationships between the different aspects of quality loss and microbial growth.

2. Materials and methods

2.1. Plant material, processing and packing

The experiments were carried out with three independent production batches within a period of five weeks in autumn 2012. For each production batch, iceberg lettuce (*Lactuca sativa* cv. Mirette – Rijk Zwaan) cultivated in the Netherland (Noord-Holland province) in open field was used. Heads were harvested when reaching the standard commercial maturity stage and processed within 3 d after harvest. Prior to processing, the product was stored for maximum two days at a temperature of about 2 °C.

The fresh-cut samples were prepared at a commercial processing plant. Lettuce heads were de-cored and sliced in strips of 13 mm and washed in three successive water bath (4 °C) free of sanitizer. Two hundred grams of fresh-cut iceberg lettuce was packed in bi-oriented poly-propylene bags with antifog coating (OTR 1600 cm^3 O_2 m^{-2} day^{-1} bar^{-1} at 23 °C and 0% RH; bag dimension 19×21 cm). The head space of the bag was flushed with 5–6 kPa oxygen and 12 kPa carbon dioxide. Bags were stored at 7 °C in darkness till evaluation. The storage temperature was selected to match the average temperature measured during retail display in several supermarkets (data not shown).

During a period of 9 or 10 d (depending on the experiment), samples (three replications for each measurement) were daily analysed for headspace gas composition, sensorial attributes, acetic and lactic acid contents and microbiological load and species composition.

2.2. Headspace composition and sensory evaluation

On each sampling date, the gas composition in the package headspace was measured just before opening the bags. Oxygen and carbon dioxide contents were analysed with PB I Checkmate 2 (Dansensor A/S, Ringsted, Denmark) via a septum glued directly on the bag.

Sensorial quality attributes were evaluated over a period of 9 or 10 d (depending on the experiment) by a trained panel of three persons. The sensorial quality was evaluated according to the score scale developed by Kader et al. (1973), also called the Karlsruher scale. The different sensorial parameters (such as OVQ, colour, shape and off-odour) were scored according to this scale. The method scores the attributes on a 9 points scale (1: Very bad, 2: Bad, 3: Poor, 4: Borderline, 5: Mediocre, 6: Satisfactory, 7: Good, 8: Very good, 9: Excellent); only full scores were given. Before the evaluation, panellists agreed to fix the consumer acceptance limit between scores 3 and 4.

The sensorial evaluation was performed in two steps. Firstly, the OVQ was evaluated (overall colour and shape of the leaf strips), while the fresh-cut lettuce was still in the package. The OVQ scores indicate the quality as judged by the consumer at purchase and is mainly dependent on colour and shape preservation. Secondly, the package was opened and the odour was immediately scored using the Karlsruher scale. In addition to the overall odour scoring, the sourness development was scored separately on a scale from 1 to 5 (1: repulsive and persistent acid smell, 2: strong and unpleasant acid smell, 3: typical acid smell at opening and still present after 30 s – still acceptable, 4: light sourness at opening, disappearing within 30 s, 5: no acid smell). Thereafter, the product was spread out over a blue background and the amount of leaf strips showing typical discoloration (pink, brown and darker green) was scored. The discolored leaves, consisting essentially in brown discoloration, were sorted according to impairments to their original color. The percentage of leaves presenting significant discoloration was calculated by dividing the amount of discolored leaves by the total amount of leaves present in the bag. All the sensorial scores served as input for the correlation analysis.

2.3. Determination of acetic acid and lactic acid

Acetic and lactic acid contents were measured on samples collected on day 1, 3, 6 and 9. Samples of 18 g of fresh-cut product were homogenized in 30 mL of cold sulfuric acid (1 M) with ultra-Turrax (IKA, Staufen, Germany). After centrifugation (30 min at 3220 g and 4 °C), the supernatant was filtered through 0.45 μm-filter and stored at −21 °C till analysis with HPLC.

The HPLC analysis was carried out with a Waters HPLC instrument system (Waters Pharmaceutical Division, Milford, MA, USA), equipped with a Waters 600E Pump-Controller, an isocratic pump (Waters 610 Fluid Unit), coupled with a Waters 486 Tunable Absorbance Detector, and a Waters 717 Autosampler. The system was operating by EmpowerTM 2 Chromatography Data Software. The organic acids were separated on a Guard column (Nova-Pak C18, particle size 4 μm) followed by a Shodex-Ionpak