Postharvest Biology and Technology 106 (2015) 44–52

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Detection of decay in fresh-cut lettuce using hyperspectral imaging and chlorophyll fluorescence imaging

Ivan Simko [a,*], Jose A. Jimenez-Berni [b], Robert T. Furbank [b]

[a] U.S. Department of Agriculture, Agricultural Research Service, U.S. Agricultural Research Station, 1636 E. Alisal St, Salinas, CA 93905, USA
[b] High Resolution Plant Phenomics Centre, Australian Plant Phenomics Facility, CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

ARTICLE INFO

Article history:
Received 2 February 2015
Received in revised form 4 April 2015
Accepted 17 April 2015
Available online 29 April 2015

Keywords:
Chlorophyll fluorescence imaging
Freezing damage
Fresh-cut lettuce salad
Hyperspectral imaging
Modified atmosphere packaging
Non-destructive phenotyping

ABSTRACT

Fresh-cut lettuce sold in modified atmosphere packaging (MAP) is a desirable, but highly perishable product. Decay of tissue can start a few days after processing and may be difficult to detect by quick visual observation. A system for early detection of decay and gradual evaluation of its progress is important both for lettuce processing industry and for breeding companies and institutions assessing quality of new cultivars and breeding lines. We have developed two lettuce decay indices (LEDI) that can be used to detect decay of leaf tissue. One of the indices ($LEDI_4$) is based on three wavelengths identified from hyperspectral imaging, while the second index ($LEDI_{CF}$) is based on chlorophyll fluorescence imaging. In addition to detecting lettuce decay, the indices identified tissue damaged by freezing temperatures. $LEDI_4$ and $LEDI_{CF}$ showed almost 97% accuracy in classifying tissue as being fresh or decayed when tested on red, dark green, green, light green, and yellow leaves. Specificity of the indices decreased when tested on fresh tissue with a very limited amount of chlorophyll that visually appeared to be almost white. Both indices detected lettuce decay without opening plastic MAP bags. The non-destructive nature of the methods thus allows rapid and repeated evaluation of samples over time and presents the opportunity for development of a commercial, high throughput scanner for evaluation of bagged, fresh-cut lettuce quality.

Published by Elsevier B.V.

1. Introduction

Lettuce (*Lactuca sativa* L.) is produced in many countries around the world making it commercially the most popular vegetable from the group of leafy vegetables (FAOSTAT, 2013). Lettuce leaves are usually consumed raw in sandwiches, salads, and side dishes. Since the introduction of fresh-cut lettuce in modified atmosphere packaging (MAP), consumer's preferences continue to shift toward packaged lettuce. However, lettuce is a highly perishable vegetable, and the cutting required in processing further shortens its shelf life (Bolin and Huxsoll, 1991). Due to substantial economic losses related to rapid deterioration, satisfactory shelf life of packaged fresh-cut lettuce is critical to the lettuce processing industry. Deterioration of fresh-cut leaves in MAP can start within a few days after processing (Hayes et al., 2014; Varoquaux et al., 1996), but can be difficult to observe visually. A rapid deterioration of processed lettuce may lead to decreased food safety because human enteric bacteria such as *Escherichia coli* and *Salmonella enterica* can survive better on damaged (Aruscavage et al., 2008; Simko et al., 2015) or deteriorated (M. Brandl and I. Simko, unpublished observations) leaves. Currently there is no standardized way of measuring salad deterioration in MAP. Visual observations of deterioration are subjective, time consuming, and may miss detection of the early stages of decay. Therefore, development of a method for automatic detection of lettuce decay is of great importance to the industry. Such a high throughput method could be used by the lettuce processing industry to quantitatively and objectively assess decay of fresh-cut lettuce, and by lettuce breeding companies to identify cultivars with improved shelf life.

Hyperspectral imaging accumulates data on light reflected by a sample from across electromagnetic spectrum, including regions that are not detectable by the human eye, e.g., ultraviolet, near-infrared, and infrared regions. Hyperspectral imaging is a well established technique in remote sensing of vegetation and plant biology (Jones and Vaughan, 2010) but has only recently begun to be used for non-destructive assessment of food quality and safety (Lorente et al., 2012; Wu and Sun, 2013). In leafy vegetables the technique was applied to detect decay in fresh-cut leaves of lamb's lettuce (*Valerianella locusta* L.) (Beghi et al., 2014) and to analyze quality of spinach (*Spinacia oleracea* L.) leaves (Diezma et al., 2013). Chlorophyll fluorescence detection using pulse amplitude modulated fluorometers and imaging fluorometers is

* Corresponding author. Tel.: +1 831 755 2862.
E-mail address: ivan.simko@ars.usda.gov (I. Simko).

http://dx.doi.org/10.1016/j.postharvbio.2015.04.007
0925-5214/Published by Elsevier B.V.

based on measuring the amount of light re-emitted by chlorophyll after excitation by red, blue or white light and has been used extensively in plant biology, particularly in photosynthesis research (Baker, 2008). This technique has also been used to investigate postharvest stress in fruits and vegetables (DeEll and Toivonen, 2003; Gorbe and Calatayud, 2012). In leafy vegetables fluorescence measurements have been applied to analyze changes occurring in stored leaves of lamb's lettuce (Ferrante and Maggiore, 2007), to detect optimal harvesting stages of lettuce (Pantazi et al., 2013), and to determine storage potential of lettuce (Schofield et al., 2005). Previously published analyses of leafy vegetables with hyperspectral imaging and chlorophyll fluorescence imaging were performed on green leaves. Packaged lettuce, however, includes leaves that range in color from light green to dark green and red. These products can also include basal parts of leaves and leaf ribs that appear almost white because they lack chlorophyll.

The objective of this project was to examine the feasibility of using hyperspectral imaging and chlorophyll fluorescence imaging for assessing deterioration of fresh-cut lettuce leaves ranging in color from dark red and dark green through green to light green, yellow, and almost white basal parts of leaves. Because visual manifestation of decayed tissue is similar in appearance to tissue damaged by freezing temperatures, both hyperspectral imaging and chlorophyll fluorescence imaging were also tested for their ability to assess freezing injury on lettuce leaves. Evaluating freezing injury is essential to lettuce industry, as both field-grown plants and harvested lettuce are highly sensitive to subzero temperatures.

2. Material and methods

2.1. Training set

Lettuce cultivars 'Eruption', 'Grand Rapids', 'Green Towers', 'La Brillante', 'Little Gem', 'Pavane', 'Red Leaf', 'Salinas', and 'Triple Threat' were grown in a growth chamber at 21 °C and 16 h photoperiod (500 μmol m^{-2} s^{-1}). Leaves of two months old plants were collected and cut into ca. 1–2 cm × 2 cm large pieces. Cut pieces were separated into five color groups based on their visual appearance: red (R), dark green (DG), green (G), light green/yellow (LG), and almost white (W). Material was packed into plastic bags and kept in the same condition as were used for plant growth. Bags were made from 63.5 μm thick polyethylene coextruded film with a manufacturer's determined O_2 transmission rating of 0.94 nmol s^{-1} m^{-2} Pa^{-1} (Printpack, Atlanta, GA, USA). After two weeks when material was visually rated as decayed, samples were removed from bags and hyperspectral imaging and chlorophyll fluorescence imaging were performed on twenty samples per color group. At the same time imaging analyses were performed on samples of freshly collected leaf tissue (20 samples per color group).

2.2. Evaluation set 1

The first evaluation set consisted of the same material as described in the training set, but processed at different times. Growing conditions, processing of samples, packing, and storage conditions were identical to those used for the training set. Twenty samples per color group and tissue quality category (fresh and decayed) were evaluated.

2.3. Evaluation set 2

Plants of the nine lettuce cultivars were grown as described for the training set. Disks 1.5 cm in diameter were cut from leaves of three weeks old plants with a cork borer. Disks were immediately placed in Petri dishes on a single layer of wet Whatman #1 filter paper. Each Petri dish contained nine disks, a single disk from each cultivar. A total of ten Petri dishes were prepared; half of them contained disks placed abaxial side up, and other half of them contained disks placed adaxial side up. Petri dishes were covered with lids and sealed with Parafilm M (Bemis, Neenah, WI, USA) to prevent drying of leaf disks. Disks were incubated at 21 °C in dark for 10 days. Hyperspectral and fluorescence scans were performed immediately after processing and at the end of incubation period. Because results of this evaluation were not notably different from those performed on evaluation set 1, data were combined and are reported as evaluation sets 1 and 2.

2.4. Evaluation set 3

Fresh looking plants (including roots) were purchased from a local supermarket. Products were labeled at the supermarket as green butterhead and red oak leaf lettuce. Plants were processed as described for the training set. Six bags (three from each type of lettuce) were kept in dark at 3 °C, the other six bags were kept in dark at 21 °C. Scans were performed on material in bags immediately after processing (day 0) and then at days 3 and 5 after processing. This evaluation was performed to test the feasibility of detecting decay within MAP bags, without removing tissue.

2.5. Evaluation set 4

The material and the storing conditions were the same as in evaluation set 3, but instead of plastic bags, leaf disks were placed into Petri dishes as described for evaluation set 2. Twenty disks, 10 from each lettuce type, were analyzed with spectral imaging and chlorophyll fluorescence imaging on day 0 (processing day), 3, 5, and 6.

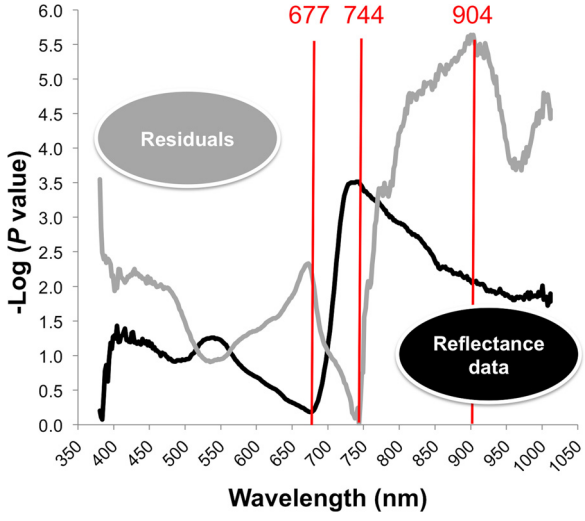

Fig. 1. Significance of difference between reflectance of fresh tissue and decayed tissue. Hyperspectral imaging was performed on 100 samples of both fresh tissue and decayed tissue from the training set (described in Section 2.1). Each of the two quality categories contained 20 samples from five color groups of lettuce: red, dark green, green, light green/yellow, and almost white. The black line shows *P*-values of Student's *t*-test calculated from reflectance data (maximum significance at 744 nm, minimum significance at 677 nm), while the grey line shows *P*-values of *t*-test calculated on residuals (maximum significance as 904 nm). Three labeled wavelengths were used for development of lettuce decay indices. For simplicity of plotting, *P*-values are presented as negative logarithms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)