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# Detection of postharvest quality loss in broccoli by means of non-colorimetric reflection spectroscopy and hyperspectral imaging

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ABSTRACT

Broccoli is a highly perishable vegetable. In academia, shelf life of broccoli is generally monitored using colorimetric devices based on diffuse reflection. However, actual quality and storability of a single broccoli curd after harvest remains uncertain before changes are noticed by the human eye. The aim of the present study was to evaluate broccoli quality loss during storage based on differences in reflectance spectra and to judge quality stages prior to color changes. Reflection spectra of broccoli harvested throughout one cultivation period in northern Germany (June–September) and stored at 16 °C and 95% relative humidity for four days were analyzed by principal component analysis (PCA). Apart from an overall increase of reflection in the visible range, changes of the spectral signature were limited to the red edge region. Quality and storability of broccoli could sufficiently be rated by the help of the normalized difference vegetation index (NDVI) and the inflection point of the red edge region, respectively. The inflection point was more sensitive to quality changes, while the NDVI was additionally influenced by differences in fertilizer application during cultivation. Analysis of hyperspectral images of broccoli curds showed an increase in both means and variance of Euclidean distances during storage. This applied to the overall VIS spectrum but was most prominent in the range of 530–650 nm. The variation could adequately be demonstrated by the help of composite images, when reflection intensities of three different wavelengths (530, 590, and 650 nm) were placed as RGB image channels. Means of Euclidean distances were highly correlated with means of the colorimetric parameter $b^*$ ($R^2 = 0.92$). Detected variation within the images was most likely based on different senescence status of single florets. However, classification procedures failed to sort the derived information into image classes because reflection intensity increased rather gradually. Further examinations at lower storage temperatures are required in order to clarify if early quality losses might be sooner detected by Euclidean distances in hyperspectral images compared to CIELAB-readings.



## 1. Introduction

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) is an immature inflorescence comprising a huge number of floral buds. It is a highly perishable vegetable. Although general models for the prediction of broccoli shelf life have been proposed (Ren et al., 2006; Schouten et al., 2009), the actual quality and storability of a single broccoli curd after harvest remains uncertain before changes are noticed by the human eye. In the rating of broccoli quality, instrumental color measurements based on reflection have been applied for more than three decades. Shewfelt et al. (1984) was the first to use colorimetry for broccoli but judged it less sensitive to color change than visual evaluation. Nowadays, it is generally agreed that color measurements are highly sensitive, fast and easy to conduct and are repeatable with high repetitious accuracy. Color measurements were applied in almost every investigation on broccoli in recent years, either to evaluate cultivation influences (Cogo et al., 2011; Hasperué et al., 2011, 2014; Zaicovski et al., 2008), suitability of varieties (Farnham and Björkman, 2011; Fernandez-Leon et al., 2012) or effectiveness of postharvest treatments (Aiamla-or et al., 2010; Fernández-León et al., 2013; Gómez-Lobato et al., 2012; Jin et al., 2015).

Color analysis is based on diffuse reflectance measurements. For broccoli both the CIE 1976 ($L^*$, $a^*$, $b^*$; abbreviated CIELAB), and the Hunterlab color-spaces are often applied. The CIELAB color

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definition from Commission Internationale de l'Eclairage (CIE) is based on color matching experiments performed with test panels (Ohta and Robertson, 2006a). The established RGB color matching functions (later converted into positive *XYZ* functions) represent the amounts of three reference stimuli derived from monochromatic lights of the wavelengths $\lambda_R$ = 700.0 nm, $\lambda_G$ = 546.1 nm, and $\lambda_B$ = 435.8 nm, needed to match monochromatic stimuli of the white color stimulus (Ohta and Robertson, 2006a). In conclusion, it should be noted that the origin of CIELAB lays in the color-receptive perception of human cones and that a series of calculations is needed to transform the original reflectance spectrum into values of the CIELAB.

Besides spot measurements, color parameters of broccoli were also derived from digital and hyperspectral images. Schouten et al. (2009) measured the color of the central floret of broccoli curds by RGB color image analysis and used the inverse red channel for color rating. Digital images were also used by Graeff et al. (2008) in order to evaluate the nitrogen status of broccoli plants on the field. The images were analyzed using CIELAB, and mean color parameters ($a^*$, $b^*$) were related to fertilizer applications. In addition to mean values of hue, chroma and lightness, Ishikawa and Hirata (2001) also used the proportion of yellow pixels to total pixels of the RGB color system as indicator of quality in digital images.

However, apart from colorimetry, reflection spectra of broccoli have only recently been analyzed in more detail. Using a hyper spectral imaging system, Hosaka et al. (2012) selected 10 areas per broccoli curd for analysis. The 2nd derivative of reflectance spectra from 380 to 1000 nm and the change in chlorophyll concentration were the input factors in an artificial neuronal network (Hosaka et al., 2012). In a preliminary study, Hernández-Hierro et al. (2014) related NIR wavelength segments to glucosinolate content in broccoli powders and then estimated its spatial distribution in broccoli slices by hyperspectral imaging. Senescence-induced changes of reflectance spectra have been reported in different fruits and vegetables without anthocyanin-synthesis, e.g. lemon (Merzlyak et al., 1999) and green asparagus (Sanchez et al., 2009). Concerning broccoli, there are approaches considering reflection spectra of broccoli on field scale in order to monitor water and nitrogen supply. Data were derived from a ground-based remote sensing system (El-Shikha et al., 2007) or from a satellite (Johnson and Trout, 2012). In this context, the normalized difference vegetation index (NDVI) is most commonly applied for spectral analyses. It is located at the transition area of electromagnetic wavelengths from the visible dark red to the NIR (Rouse et al., 1973). Accordingly, next to other indices, NDVI has been used on field scale in order to estimate crop coverage as well as water and nutrient status of broccoli (El-Shikha et al., 2007; Johnson and Trout, 2012).

The reported digital and hyperspectral image studies on broccoli, however, did rarely apply advanced image analysis taking into account the entire information included in images. Whenever single parts of broccoli images were investigated, it is supposed, that mean values of spectral reflectance were determined, instead of using image data on single pixel basis. Thus, the values were mainly treated similar to readings from single spot devices, being separated from spatial information. Concerning broccoli, this may be a knowledge gap: Due to the complex morphology, senescence changes within individual broccoli curds can be expected to occur inhomogeneously on the curd surface based on single floret buds (Kieffer et al., 1998; Tian et al., 1995). Hernández-Hierro et al. (2014) applied image analysis for determination of spatial glucosinolate distribution. However, they used a longitudinal slice of broccoli instead of the curd surface, where senescence changes normally occur.

Hence, the aim of this study was to evaluate differences in reflectance spectra of broccoli during storage and to judge quality stages independent of colorimetry. The reported color differences suggest that specific spectral regions are subject to transformation during storage. It was intended to analyze those changes in spot measurements of reflectance and, using operations of image analysis, in hyperspectral records of stored broccoli. Finally, we aimed to evaluate if methods of spectral analysis were able to rate quality changes of the curd prior to observed color changes. In this context, impacts of different levels of fertilization on reflection intensity and color changes of broccoli curds were also discussed.

## 2. Materials and methods

### 2.1. Harvest and storage of broccoli curds

For spot measurements of reflectance, a total number of 248 broccoli curds were harvested in 7 batches throughout the 2012 growing season (June–September). Broccoli was cultivated by one commercial grower and two research institutions at four different planting sites in Germany, which were located north of 52°00′N. Directly after harvest, broccoli were transported to the laboratory in closed insulated boxes cooled by an ice layer at the bottom of the box. Upon arrival in the lab, broccoli stems were cut 5 cm below the first branch and all leaves were removed prior to storage. To induce senescence, curds were subsequently stored at 16 °C and 95% relative humidity for 4 days. For hyperspectral imaging, broccoli heads were harvested in September 2013 and treated as described above. Hyperspectral images were recorded on day 1 and 4 of storage.

During cultivation, fertilizer was generally applied according to nutrient demand of broccoli, considering the respective soil properties. Amounts of fertilizer were lying between 3.7 and 10.4 g nitrogen, 0 and 5.1 g phosphorus and 0 and 5.4 g potassium per plant. For one broccoli batch, which was cultivated from May 4th to July 9th, 2012, fertilizer application was varied by the commercial grower resulting in different fertilizer doses of broccoli plants as indicated in Table 1.

### 2.2. Spectral measurements

Diffuse reflectance of broccoli was measured on individual spots of 2.5 $cm^2$ by using an OEM spectrometer (tec5, Oberursel, Germany) applying a MMS1 spectral sensor (Zeiss, Jena, Germany). Measurements were conducted underneath an opaque cover using a 50 W halogen lamp as light source (Fig. 1). Broccoli curds were placed in a distance of 10 cm to a collimator, which narrowed the derived reflection signal from the measuring spot. On each curd, measurements were randomly repeated six times. The measurement range was 400–1100 nm at a spectral resolution of 3.2 nm (interpolated to 2.5 nm). Wavelengths below 450 nm were not used for analysis due to a low signal-to-noise ratio.

Hyperspectral images were recorded according to Bauriegel et al. (2011) in the range of 400–1000 nm with a spectral resolution of 1.2 nm. The system comprised a spectrograph (ImSpector V10E, Spectral Imaging Ltd., Oulu, Finland) and a monochrome camera (Pixelfly qe, PCO AG, Kelheim, Germany). Curds were illuminated with a stabilized halogen lamp (150 W) and were recorded from bird's eye view. The minimum distance between curd and light source was 34 cm. Due to a recording and preprocessing time of 40 min per record, only 5 broccoli curds were recorded by hyperspectral images. The control of the camera system as well as black/white calibration was done using LabView 8.2 (National Instruments Corporation, Austin, TX, USA). Spatial resolution of images was 696 × 512 pixels.

In both measuring systems, white adjustment was done by the help of a spectralon standard (Zeutec, Rendsburg, Germany). As a