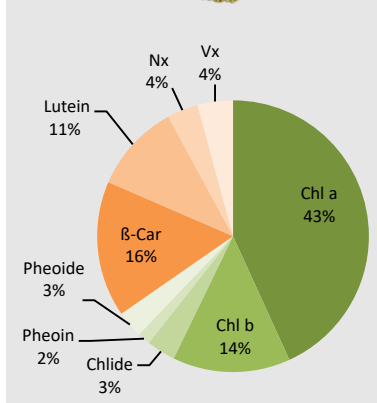


# Storability of broccoli – investigations of optical monitoring, chlorophyll degradation and predetermination in the field

- Dissertation -  
Theresa Kabakeris



**Bornimer Agrartechnische Berichte**

Heft 98

Berlin 2016 / Potsdam-Bornim 2017

# **Storability of broccoli – investigations of optical monitoring, chlorophyll degradation and predetermination in the field**

**Dissertation  
Theresa Kabakeris**

---

---

Bornimer Agrartechnische Berichte  
Heft 98

Potsdam-Bornim 2018

**Herausgeber:**

Leibniz-Institut für Agrartechnik und Bioökonomie e.V.  
Max-Eyth-Allee 100  
14469 Potsdam-Bornim  
☎ (0331)-5699-0  
Fax.: (0331)-5699-849  
E-mail: atb@atb-potsdam.de  
Internet: <http://www.atb-potsdam.de>

Januar 2018

**Redaktion:**

Theresa Kabakeris

**Typografische Gestaltung:**

Theresa Kabakeris

Berlin, Humboldt-Universität zu Berlin, Dissertation 2016

Herausgegeben vom Leibniz-Institut für Agrartechnik und Bioökonomie e.V. (ATB) mit Förderung durch das Bundesministerium für Ernährung und Landwirtschaft (BMEL) und das Ministerium für Wissenschaft, Forschung und Kultur des Landes Brandenburg (MWFK).

Für den Inhalt der Beiträge zeichnen die Autoren verantwortlich.

Eine Weiterveröffentlichung von Teilen ist unter Quellenangabe und mit Zustimmung des Leibniz-Instituts für Agrartechnik und Bioökonomie e.V. möglich.

ISSN 0947-7314

© Leibniz-Institut für Agrartechnik und Bioökonomie e.V., 2018



## Table of contents

<b>1. Problem statement</b> .....	<b>1</b>
<b>2. Hypotheses and objectives</b> .....	<b>2</b>
<b>3. Literature review</b> .....	<b>4</b>
<b>3.1. Broccoli quality as a function of physiology</b> .....	<b>4</b>
3.1.1. Temperature .....	4
3.1.2. Water .....	7
3.1.3. Radiation .....	9
3.1.4. Fertilization .....	11
3.1.5. Generative development .....	11
<b>3.2. Determination and prediction of broccoli quality</b> .....	<b>13</b>
3.2.1. Reflection measurements .....	14
3.2.1.1. Calculation of CIELAB values .....	14
3.2.1.2. Color modelling .....	16
3.2.1.3. Image analysis .....	17
3.2.2. Chlorophyll fluorescence .....	18
3.2.3. Chlorophyll degradation .....	19
<b>4. Methods and materials</b> .....	<b>22</b>
<b>4.1. Plant material</b> .....	<b>22</b>
4.1.1. Broccoli harvest batches .....	22
4.1.2. Experimental sites .....	24
4.1.3. Storage conditions .....	25
<b>4.2. Cultivation factors</b> .....	<b>26</b>
4.2.1. Fertilization .....	26
4.2.2. Plastic film cover .....	27
4.2.3. Net cover .....	30
<b>4.3. Maturity stages</b> .....	<b>30</b>
4.3.1. Bud size determination .....	30
4.3.2. Curd size determination .....	31
<b>4.4. Reflection measurements</b> .....	<b>31</b>
4.4.1. Color determination .....	31
4.4.2. Spot measurements of reflection .....	32
4.4.3. Hyperspectral images .....	32

<b>4.5. Chlorophyll fluorescence measurements.....</b>	<b>32</b>
4.5.1. Fluorescence imaging .....	33
4.5.2. Fluorescence spot measurements.....	33
<b>4.6. Pigment analysis.....</b>	<b>33</b>
<b>4.7. Data processing .....</b>	<b>34</b>
4.7.1. Rating of storability .....	34
4.7.2. Pre-treatment of reflection spectra .....	35
4.7.3. Analysis of reflection spectra .....	36
4.7.4. Identification of external impacts on quality .....	36
<b>5. Results .....</b>	<b>38</b>
<b>5.1. Optical methods for quality rating.....</b>	<b>38</b>
5.1.1. Color determination (CIELAB) .....	42
5.1.2. Reflection spectra .....	46
5.1.3. Hyperspectral imaging.....	51
5.1.4. Chlorophyll fluorescence .....	56
<b>5.2. External influences .....</b>	<b>60</b>
5.2.1. Cultivation factors .....	60
5.2.2. Climatic factors .....	65
<b>5.3. Physiology of broccoli curds.....</b>	<b>69</b>
5.3.1. Curd development .....	69
5.3.2. Pigment composition .....	72
5.3.2.1. <i>Chlorophyll degradation</i> .....	72
5.3.2.2. <i>Carotenoids during storage</i> .....	77
<b>6. Discussion.....</b>	<b>81</b>
<b>6.1. Sensitivity of non-destructive indexes postharvest .....</b>	<b>81</b>
6.1.1. Color measurements .....	81
6.1.2. Further spectral analysis, including imagery.....	82
6.1.3. Chlorophyll fluorescence measurements.....	84
6.1.4. Prediction potential .....	85
<b>6.2. Predetermination of the broccoli curd .....</b>	<b>86</b>
6.2.1. Curd morphology and development.....	86
6.2.2. Patterns of photosynthetic pigments.....	88
<b>6.3. External factors on the field affecting storability.....</b>	<b>91</b>
6.3.1. Radiation and temperature during floral initiation .....	92

6.3.2. Light quality during curd growth .....	93
6.3.3. Plant nutrition.....	95
6.3.4. Water supply preceding harvest .....	96
<b>7. Conclusions.....</b>	<b>98</b>
<b>8. References.....</b>	<b>103</b>

## List of figures

Fig. 1: World production of cauliflower and broccoli (tonnes) (FAOSTAT 2014). .....	1
Fig. 2: Brassica oleracea cv. 'Green Harmony' F1 grown under three different day/night temperature regimes during reproductive development. (A) At 16 °C/12 °C (B) At 22 °C/17 °C (C) At 28 °C/22 °C (taken from Duclos & Björkman 2008). .....	5
Fig. 3: Water loss of harvested broccoli held in air at 20 °C after 2 hours (left) and 26 hours (right), respectively. Magnetic resonance images showing signals related to the intensity of the water activity (taken from Coupe et al. 2003). .....	9
Fig. 4: Transition of broccoli to the floral stage. Development of flower bud primordia (A + arrows in B) and arrangement of branch primordia following a phyllotactic spiral of 5 (left) and 8 (right) (B). Scanning electron micrographs, bar = 100 µm (taken from Kieffer et al. 1998). .....	13
Fig. 5: Color matching functions of the CIE 1931 standard colorimetric observer (Minolta 1996). .....	15
Fig. 6: Schematic diagram of early chlorophyll degradation modified after Heaton and Marangoni (1996). .....	20
Fig. 7: Broccoli fully meeting marketing standards of UNECE (2010) harvested at September 26 <sup>th</sup> , 2013 (A) and broccoli of non-marketable quality harvested on July 18 <sup>th</sup> , 2012 (B). .....	24
Fig. 8: Location of trial fields in northern Germany. Colors of symbols indicate institution affiliation: green – Behr AG, red – Leibniz Institute of Vegetable and Ornamental Crops, blue – Leibniz Universität Hannover. .....	25
Fig. 9: Storage of broccoli in a single chamber (A) of the storage room (B). .....	25
Fig. 10: Plastic film covers placed on broccoli for variation of light quality (A) and fully transparent covers without light variation (B). .....	27
Fig. 11: Light transmitted (%) by blue (A) and green (B) plastic film cover for each wavelength (LEE filters, Andover, UK). .....	28
Fig. 12: Climatic conditions under plastic film boxes placed on broccoli plants during curd growth. Temperature (A) and humidity (C) of a batch harvested on July 18 <sup>th</sup> was compared to conditions recorded by a weather station. Temperature (B) and humidity (D) of a batch harvested on September 11 <sup>th</sup> was compared to conditions recorded in the plant stand without plastic film cover. .....	29
Fig. 13: Microscopic image of a broccoli curd harvested on August 4 <sup>th</sup> , 2011 (A) and analysis of bud diameters (B). The white bar in (A) represents 1 mm length in the focal plane. .....	31
Fig. 14: Schematic diagram of spot reflection measurements of broccoli curds. ....	32
Fig. 15: Example of SQL data base query: Hours with temperatures higher than 25 °C, occurring during a period of 16 to 24 days before harvest, were counted in the existing climate data (c.) for each harvest batch (u.). .....	37
Fig. 16: Broccoli harvested on June 8 <sup>th</sup> , 2012 at harvest day (A) and after 3 days of storage (B) at 16 °C and high RH (~95 %). .....	38



- Fig. 17: Correlation matrix of non-destructive optical parameters representing the output of CIELAB (hue angle), of reflection spectra (Red edge inflection point) and fluorescence imaging (Fv/Fm). Broccoli was harvested on June 8<sup>th</sup>, 2012 and stored for 4 days at 16 °C and high RH (~95 %). Asterisks indicate significant Pearson correlations..... 39
- Fig. 18: Correlation matrix of non-destructive optical parameters representing the output of CIELAB (hue angle), of reflection spectra (Red edge inflection point) and fluorescence imaging (Fv/Fm). Broccoli harvested on June 25<sup>th</sup>, 2012 and stored for 4 days at 16 °C and high RH (~95 %). Asterisk indicates significant Pearson correlations. .... 41
- Fig. 19: Hue angle (mean ± confidence interval) of seven broccoli batches harvested throughout the 2012 growing season. Storage took place at 16 °C and high RH (~95 %). Different letters indicate significant differences within one broccoli batch. Hue angle: 90° = yellow, 180° = bluish-green..... 43
- Fig. 20: Parameter b\* (mean ± confidence interval) of seven broccoli batches harvested throughout the 2012 growing season. Storage took place at 16 °C and high RH (~95 %). Different letters indicate significant differences within one batch. .... 44
- Fig. 21: Hue angle (mean ± confidence interval) of broccoli batches on 4 days of storage at 16 °C and high RH (~95 %). Batches were harvested throughout the 2012 growing season. Different letters indicate significant differences between broccoli batches on single storage days. On day 1, only the broccoli harvested on July 18<sup>th</sup> formed an independent statistical subgroup..... 45
- Fig. 22: b\* (mean ± confidence interval) of broccoli batches on 4 days of storage at 16°C and high RH (~95%). Batches were harvested throughout the 2012 growing season. Different letters indicate significant differences between broccoli batches on single storage days. On day 1, only the broccoli harvested on July 18<sup>th</sup> formed an independent statistical subgroup..... 46
- Fig. 23: Normalized reflection spectra of one broccoli curd on harvest day (June 8<sup>th</sup>, 2012) to day 4 of storage at 16 °C and high RH (~95 %). Line patterns: harvest day – solid, storage day 1 – dash dot dash (hidden), day 2 – long dash, day 3 – medium dash, day 4 – short dash. .... 47
- Fig. 24: Principal components of the normalized reflection spectra of broccoli curds (A) and of their 1<sup>st</sup> (B) and 2<sup>nd</sup> (C) derivative (N=4305). Values of the 2<sup>nd</sup> derivative were spline-interpolated. Only PCs explaining ≥ 1 % of variance were plotted. Broccoli was harvested throughout the season 2012 (June-September) and stored for 4 days at 16 °C and high RH (~95 %). .... 48
- Fig. 25: Red edge inflection point of spectra in the range 680-730 nm (mean ± confidence interval) of seven broccoli batches harvested throughout the 2012 growing season. Storage was done at 16 °C and high RH (~95 %). Different letters indicate significant differences within one batch. .... 49
- Fig. 26: NDVI (mean ± confidence interval), calculated as  $[(R780 - R675) / (R780 + R675)]$ , where R675 is the reflection intensity at  $\lambda = 675$  nm. Seven broccoli batches were harvested throughout the 2012 growing season and stored at

**VI Storability of broccoli – investigations of optical monitoring, chlorophyll degradation and predetermination in the field**

---

16 °C and high RH (~95 %). Different letters indicate significant differences within one harvest batch..... 50

Fig. 27: Reflection spectra of classes (mean ± stdev) derived by the Fuzzy-c-means classification of bands 262-288 of hyperspectral images (~ wavelengths 700 - 730 nm) of one broccoli curd harvested on September 9<sup>th</sup>, 2013 after 1 and 4 days of storage at 16 °C and high RH (~95 %). ..... 51

Fig. 28: Result of Fuzzy-c-means classification (target: 3 classes) of principal components 1-3 after equal area normalization. Broccoli harvested on September 9<sup>th</sup>, 2013 after 1 (A) and 4 (B) days of storage at 16 °C and high RH (~95 %). ..... 52

Fig. 29: Reflection intensities of two broccoli curds after equal area normalization at wavelengths of 530, 590 and 650 nm on day 1 (A) and day 4 (B) of storage at 16 °C and high RH (~95 %). ..... 54

Fig. 30: Array of reflection data of normalized hyperspectral images at wavelengths 530 nm, 650 nm and 590 nm. Pixel-wise reflection intensity of one broccoli curd after 1 (pink) and 4 (black) days of storage at 16 °C and high RH (~95 %). Image data were randomly reduced to 1 %. ..... 55

Fig. 31: Relationship between means of the CIELAB parameter b\* (n = 6 measurements / broccoli) and mean Euclidean distances of hyperspectral images of broccoli on day 1 and 4 of storage at 16 °C and high RH (~95 %). 56

Fig. 32: Maximum photochemical efficiency (Fv/Fm) (mean ± confidence interval) of seven broccoli batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). Different letters indicate significant differences within one batch between different storage days. .... 57

Fig. 33: Actual photon yield of photosynthetic electron transport  $\Phi_{PSII}$  (mean ± confidence interval) in six broccoli batches harvested throughout the 2012 growing season. The frame for August 30<sup>th</sup> was intentionally left blank (missing values). Storage was conducted at 16 °C and high RH (~95 %). Different letters indicate significant differences within one batch between different storage days. .... 58

Fig. 34: Maximum photochemical efficiency (Fv/Fm) of five broccoli batches during 4 days of storage at 16 °C and high RH (~95 %). Batches were harvested throughout the 2012 growing season. Different letters indicate significant differences between broccoli batches on the respective storage days. .... 59

Fig. 35: Maximum photosynthetic capacity (Fv/Fm) of one broccoli curd harvested on June 8<sup>th</sup>, 2012 on days 1, 2, 3 and 4 of storage at 16 °C and high RH (~95 %). False color scale ranges from 0.55 (red) to 0.75 (blue). White arrow points at growing spot of lower Fv/Fm values. .... 59

Fig. 36: Non-destructive quality indexes of two broccoli batches grown under different covers from button stage onwards or uncovered ( $\triangleq$ “none”) after 4 days of storage at 16 °C and high RH (~95 %). Different letters indicate significant differences between cover types on storage day 4. Some error bars are hidden by symbols. .... 61

- Fig. 37: Lightness  $L^*$  and NDVI  $(R780 - R675) / (R780 + R675)$  of broccoli harvested on July 9<sup>th</sup> 2012, during 4 days of storage at 16 °C and high RH (~95 %). Non-overlapping confidence limits are significantly different ( $\alpha = 0.05$ ). ..... 62
- Fig. 38: NDVI  $(R780 - R675) / (R780 + R675)$  of seven broccoli batches on day 1 and 4 of storage corresponding to amounts of nitrogen fertilizer (g/plant) applied during cultivation. Batches were harvested throughout the 2012 growing season and stored at 16 °C and high RH (~95 %) for 4 days. Fertilization in batch harvested on July 9<sup>th</sup> was varied. .... 63
- Fig. 39: Lightness  $L^*$  of seven broccoli batches on day 1 and 4 of storage, corresponding to amounts of nitrogen fertilizer (g/plant) applied during cultivation. Batches were harvested throughout the 2012 growing season and stored at 16 °C and high RH (~95 %) for 4 days. Fertilization in batch harvested on July 9<sup>th</sup> was varied. .... 64
- Fig. 40: Time required for curd development (planting to harvest) and corresponding sum of temperatures during the growing season, lasting from day of year (DOY) 160 = June 8<sup>th</sup> to DOY 269 = September 26<sup>th</sup>. .... 65
- Fig. 41: The hue angle of broccoli curds related to the number of hours exceeding 25 °C during the cultivation period of 16 to 24 days before harvest. Broccoli batches were harvested in 2012 and 2013 and stored for four days at 16 °C and high RH (~95 %). Hue angle: 90° = yellow, 180° = bluish-green. Lines: solid = regression; dashed = 95 % prediction limits. .... 66
- Fig. 42: The hue angle of broccoli curds related to the number of hours with radiation exceeding 600 W/m<sup>2</sup> during the cultivation period of 16 to 24 days before harvest. Broccoli batches were harvested in 2012 and 2013 and stored for four days at 16 °C and high RH (~95 %). Hue angle: 90° = yellow, 180° = bluish-green. Lines: solid = regression; dashed = 95 % prediction limits. .... 67
- Fig. 43:  $b^*$  values of broccoli curds related to the sum of water (precipitation + irrigation) received during the fortnight preceding harvest. Broccoli batches were harvested in 2012 and 2013 and stored for four days at 16 °C and high RH (~95 %). No irrigation data was available for broccoli harvested on June 25<sup>th</sup>, 2012. Lines: solid = regression; dashed = 95 % prediction limits. .... 68
- Fig. 44: The diameters of single buds of broccoli on different regions of the curd. Broccoli was harvested from different field allotments on August 4<sup>th</sup>, 2011.... 70
- Fig. 45: The curd and bud diameters of broccoli harvested on August 4<sup>th</sup>, 2011, plotted against hue angle after 8 days of storage at 10 °C and high RH (~95 %). Asterisks indicate significant Pearson correlations. .... 71
- Fig. 46: Curd diameter of seven broccoli batches harvested throughout the 2012 growing season and plotted against hue angle after 4 days of storage at 16 °C and high RH (~95 %). Asterisks indicate significant Pearson correlations. .... 72
- Fig. 47: Concentrations of chlorophyll a and b in five broccoli batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). Mean  $\pm$  Confidence interval (n = 2-3, repeat determination). Different letters indicate significant differences within one harvest batch. .... 73

**VIII Storability of broccoli – investigations of optical monitoring, chlorophyll degradation and predetermination in the field**

---

Fig. 48: Chlorophyll a/b ratio based on molar terms. Concentrations determined in broccoli curds from four batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). ..... 74

Fig. 49: Chlorophyll derivatives detected in broccoli from five batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). Each dot represents the concentration in florets of a single broccoli. .... 75

Fig. 50: Chlorophyll a divided by the sum of green chlorophyll derivatives in molar terms. Pigments were detected in broccoli curds harvested on August 30<sup>th</sup> and September 11<sup>th</sup>, 2012. Storage was conducted at 16 °C and high RH (~95 %). .... 76

Fig. 51: Quotient of Pheoin divided by chlorophyll a in molar terms. Concentrations determined in broccoli curds of five batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). ..... 77

Fig. 52: Levels of carotenoids in five broccoli batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). Mean ± 95 % confidence interval (n = 2-3, repeat determination). Overlapping confidence intervals are not significantly different ( $\alpha = 0.05$ ). ..... 78

Fig. 53: Sum of chlorophylls (mean chlorophyll a + b, Chlide, Pheoin, Pheoide) and carotenoids (mean  $\beta$ -Car, Nx, Vx, Lut) detected in broccoli from five batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). ..... 79

Fig. 54: The proportion of pigments (molar terms) in one broccoli curd harvested on June 8<sup>th</sup>, 2012 on harvest day (A) and on day 3 of storage at 16 °C and 95 % RH (B). ..... 80

## List of tables

Table 1: Overview of broccoli harvest batches used for various investigation purposes. ....	23
Table 2: Fertilizer application amounts on broccoli cultivated from May 4 <sup>th</sup> to July 9 <sup>th</sup> , 2012 (g/plant).....	26
Table 3: Pearson correlation coefficients of non-destructive optical parameters representing the output of the CIELAB (hue), reflection spectra (Red edge) and fluorescence imaging (Fv/Fm) measured over 4 days of storage at 16 °C and high RH (~95 %) on two broccoli harvest batches. Asterisks indicate significance. ...	40
Table 4: CIELAB values of a single broccoli harvested on August 4 <sup>th</sup> , 2011 and stored for 8 days at 10 °C and high RH (~95 %). ....	42
Table 5: Percentage variance of principle components of diffuse reflection spectra (and 1 <sup>st</sup> and 2 <sup>nd</sup> derivation of spectra) recorded on broccoli curds. Broccoli was harvested throughout the season 2012 and stored for 4 days at 16 °C and high RH (~95 %). ....	48
Table 6: Statistics regarding Euclidean distances within hyperspectral images of broccoli (n = 5) during storage at 16 °C and high RH 95 %.....	53
Table 7: Levels of significance (analysis of variance) for the main and interactive effects of storage and cultivation factors on non-destructive quality indexes. ....	60

## **Abstract (German)**

Brokkoli (*Brassica oleracea* var. *italica*) besitzt nach der Ernte nur eine geringe Haltbarkeit. Das Ziel der vorliegenden Arbeit war es, eine Vorhersage der Haltbarkeit von Brokkoli zum baldmöglichsten Zeitpunkt nach der Ernte zu erreichen.

Hierfür wurde zum einen untersucht, inwiefern sich nicht-destruktive, optische Messungen für eine Haltbarkeitsvorhersage eignen. Die optischen Messungen gehörten teilweise zu gängigen Methoden der Qualitätsbeurteilung in der wissenschaftlichen Analytik (Farbbestimmung, Chlorophyllfluoreszenzanalyse). Zusätzlich wurden auch Auswerteverfahren von Messungen diffuser Reflexion angewandt, die sowohl als punktuelle Messung als auch in Form von Hyperspektralbildern durchgeführt wurden. Als weitere mögliche Indikatoren für die Haltbarkeitsvorhersage nach der Ernte wurde der generative Zustand von Brokkoli mittels Messungen von Knospendurchmessern in mikroskopischen Aufnahmen sowie von Durchmessern ganzer Köpfe bestimmt.

Außerdem wurden die Konzentrationen von Chlorophyll *a* und *b*, von dessen grünen Abbauprodukten Chlorophyllid *a* (Chlide), Pheophytin *a* (Pheoin), Pheophorbid *a* (Pheoide) sowie von den Carotinoiden  $\beta$ -Carotin ( $\beta$ -Car), Lutein (Lut), Neoxanthin (Nx) und Violaxanthin (Vx) in den Blütenknospen bestimmt. Der Hintergrund hierfür war die Prüfung einer Indikatorfunktion von Chlorophyllabbauprodukten für die weitere Haltbarkeit von Brokkoli sowie die Untersuchung des qualitätsmindernden Farbwechsels.

Des Weiteren wurde die Bedeutung von Einflüssen während des Anbaus auf die Haltbarkeit von Brokkoli untersucht. Hierfür wurden verschiedene Pflanzenabdeckungen eingesetzt, die die Quantität und Qualität der eingestrahlten Globalstrahlung veränderten. Zusätzlich wurden die Düngegaben in einem Anbausatz gezielt variiert. Basierend auf Abfragen in einer erstellten Klimadatenbank wurde der Einfluss klimatischer Ereignisse während verschiedener Wachstumsphasen auf die Haltbarkeit eruiert.

Sensibel gegenüber Veränderungen von Brokkoli während der Lagerung zeigten sich die photosynthetische Leistungsfähigkeit der Brokkoliköpfe ( $F_v/F_m$ ,  $\Phi_{PSII}$ ), der CIELAB-Skalenwert der blau-gelben Farbwahrnehmung ( $b^*$ ) sowie der Wendepunkt des Reflektionsspektrums im Übergangsbereich von sichtbarer dunkelroter zu infraroter elektromagnetischer Strahlung (REIP). Allerdings war auf der Basis der Sensibilität der Parameter keine Vorhersage des schlussendlichen Wertenniveaus nach vier Tagen Lagerung bei 16 °C und hoher relativer Luftfeuchte (RH) (~ 95 %) möglich. Qualitätsindices, die sich ab dem gleichen Lagertag in verschiedenen Erntesätzen veränderten, zeigten nach vier Tagen wiederum eine unterschiedliche Höhe. Zudem spiegelte die Höhe der Werte der photosynthetischen Leistungsfähigkeit ( $F_v/F_m$ ,  $\Phi_{PSII}$ ) die Qualität der verschiedenen Erntesätze im Vergleich nur unzureichend wider.

Unterschiede innerhalb von Brokkoliköpfen waren mithilfe von Euklidischen Distanzen in Hyperspektraldaten frühzeitig detektierbar und konnten durch den Einsatz von drei Wellenlängen im sichtbaren Bereich als RGB-Bild dargestellt werden. Obwohl die Unterschiede in den Bilddaten erkennbar auf der konsekutiven Seneszenz der einzelnen Blütenknospen beruhten, stellten deren Durchmesser keine Indikatoren für die weitere

Haltbarkeit dar. Dagegen waren die Durchmesser ganzer Brokkoliköpfe innerhalb einzelner Erntesätze für die Haltbarkeit ausschlaggebend.

Das Gelbwerden der Brokkoli beruhte auf dem Abbau von Chlorophyll, das die vorhandenen Carotinoide demaskierte. Es kam zu keiner Anreicherung von Chlorophyllabbauprodukten während der Lagerung, allerdings war ein Erntesatz mit sehr guter Haltbarkeit durch einen starken und kontinuierlichen Anstieg des Chlorophyll *a/b* Verhältnisses gekennzeichnet.

Unterschiedliche N-Düngegaben korrespondierten sowohl mit dem Helligkeitswert  $L^*$  des CIELAB Farbraums ( $R^2 = 0,70$ ) als auch mit dem Vegetationsindex NDVI ( $R^2 = 0,73$ ) von Brokkoliköpfen nach der Ernte. Dies stand jedoch nicht im Zusammenhang mit der weiteren Haltbarkeit. Während des Anbaus hatten sowohl eine temporäre Einschränkung der Lichtqualität durch Abdeckung mit lichtselektiven Folien ( $\lambda = 400-500 \text{ nm}$ ) als auch eine erhöhte Anzahl von Stunden mit starker Sonneneinstrahlung ( $> 600 \text{ W m}^{-2}$ ) negative Auswirkungen auf die Haltbarkeit ( $R^2 = 0,47$ ). Teilweise korrespondierten hohe Einstrahlungswerte mit Temperaturen über  $25 \text{ °C}$  ( $R^2 = 0,29$ ). Maßgebliche Auswirkungen auf die Haltbarkeit von Brokkoli zeigte die Wasserverfügbarkeit in den letzten zwei Wochen vor der Ernte ( $R^2 = 0,71$ ).

Die Vorhersage der Haltbarkeit von Brokkoli kann daher mit hoher Wahrscheinlichkeit auf der Basis von Einflüssen aus dem Anbau durchgeführt werden. Zur schnellen nicht-destruktiven Beurteilung des aktuellen Qualitätszustandes eigneten sich insbesondere der Parameter  $b^*$  sowie der spektrale Index REIP. Mithilfe geeigneter Monitoringsysteme während des Anbaus (Datenbank aus Klima- und Kulturdaten) und während der Lagerung (reflektionsbasierte Handgeräte) ist daher mit einer besseren Bestimmung sowie Vorhersage der Haltbarkeit zu rechnen.

## **Abstract**

Broccoli (*Brassica oleracea* var. *italica*) is a highly perishable crop. The aim of the work presented here is to find ways to predict storability of broccoli shortly after harvest. Several methods are examined regarding their suitability for predicting broccoli storability.

The non-destructive, optical measuring devices used in this work represent some of the common methods used for scientific quality assessment (color determination, chlorophyll fluorescence analysis). In addition, spectral data from diffuse reflection measurements, from both spot measurements and hyperspectral images, was evaluated. The generative development of broccoli curds was studied using diameters both of single buds and of whole curds, and was related to subsequent storability.

The concentrations of the chlorophylls *a* and *b*, green chlorophyll catabolites chlorophyllide *a* (Chlide), pheophytin *a* (Pheoin), pheophorbide *a* (Pheoide) and carotenoids  $\beta$ -carotene ( $\beta$ -Car), lutein (Lut), neoxanthin (Nx) and violaxanthin (Vx) were determined in floral buds of broccoli through the use of high pressure liquid chromatography (HPLC). The goal was to determine if chlorophyll catabolites may function as indicators of broccoli storability and, furthermore, to relate the concentrations of photosynthetic pigments to the color change of broccoli during storage.

In order to investigate effects from the field on the subsequent storability of broccoli, cultivation methods were altered. Plants were partly covered with covers of different materials, which affected the quantity and quality of incident global radiation. The application of fertilizers was specifically varied in one broccoli batch. Additionally, the influence of climatic events on the shelf life of broccoli was analyzed by querying an established database.

The non-destructive indexes that proved to be most sensitive to changes during storage were the levels of photosynthetic efficiency ( $F_v/F_m$ ,  $\Phi_{PSII}$ ), the CIELAB color space coordinate on a blue to yellow scale ( $b^*$ ) and the inflection point of reflection spectra in the transition region from dark red to infrared electromagnetic wavelength range (REIP). However, based on parameter sensitivity, the prediction of value levels after four days of storage at 16 °C and high relative humidity (RH) (~ 95 %) was unfeasible. Quality indexes that changed on the same storage day did not necessarily end up at the same value level. Additionally, values of both  $F_v/F_m$  and  $\Phi_{PSII}$  did not adequately reflect the quality status of broccoli when different harvest batches were compared.

In hyperspectral images, early quality changes were effectively rated based on Euclidean distances within broccoli curds and were displayed using reflection intensities at three wavelengths within the visible electromagnetic spectrum as RGB channels. The observed variation within broccoli curds was related to differences in the degree of senescence of single floral buds. However, the diameters of floral buds did not correspond to a rating of storability. In contrast, curd diameters within individual harvest batches were related to subsequent yellowing.



The yellowing of broccoli was based on the decomposition of chlorophyll, which unmasked the carotenoids present. No accumulation of chlorophyll catabolites was found during storage. A trend towards rapidly increasing chlorophyll *a/b* ratios was observed in a broccoli batch with particularly high storability.

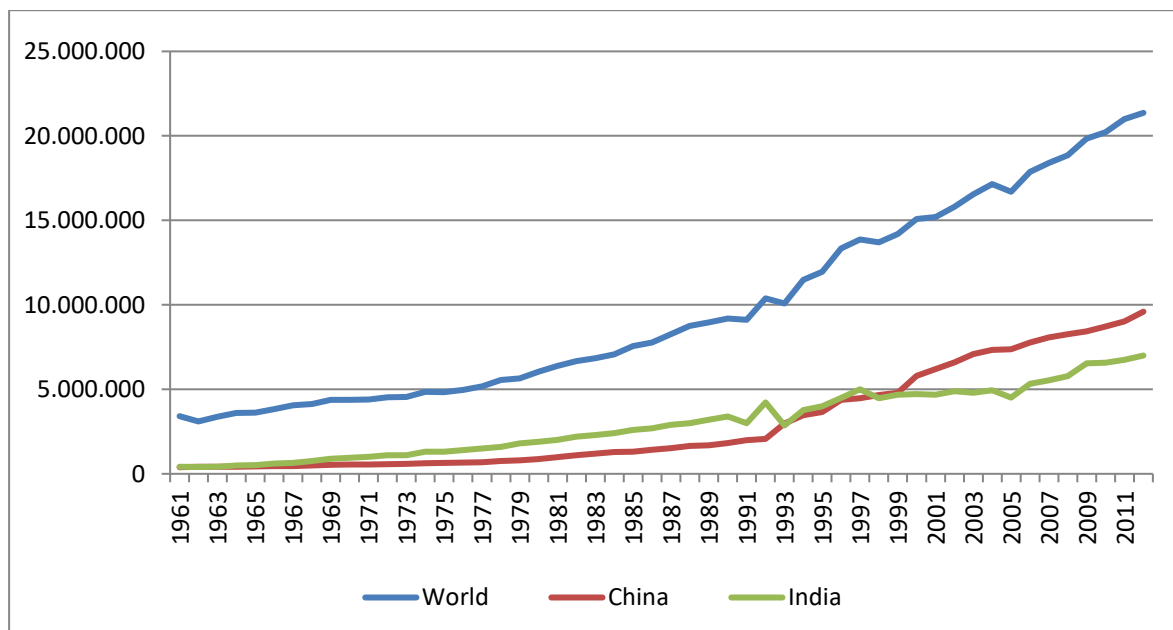
Different N-fertilization of broccoli curds closely corresponded both with values of initial lightness  $L^*$  of the CIELAB color space ( $R^2 = 0.70$ ) and normalized difference vegetation index NDVI ( $R^2 = 0.73$ ). Yet, N-fertilization had no impact on further storability. A negative impact on storability was observed when the spectrum of incident radiation on the field was temporarily reduced by using plastic covers with limited transmittance ( $\lambda = 400-500$  nm). In a similar way, an increased number of hours with high solar radiation intensity ( $> 600 \text{ W m}^{-2}$ ) during cultivation had a negative impact on broccoli storability ( $R^2 = 0.47$ ). The impact of high irradiation partly corresponded with temperatures higher than  $25 \text{ }^\circ\text{C}$  ( $R^2 = 0.29$ ). A strong predetermination of curd storability was observed based on the water availability for broccoli plants in the last two weeks before harvest ( $R^2 = 0.71$ ).

A successful prediction of broccoli storability is therefore most likely to be conducted by looking at effects during cultivation. For early non-destructive evaluation of the current quality status of broccoli, both the parameter  $b^*$  and the spectral index REIP were highly suitable. Therefore, through the help of monitoring systems throughout cultivation (database of climate and cultivation incidents) in combination with reflection-based, hand-held devices during storage, the prediction and rating of broccoli storability can certainly be improved.



## 1. Problem statement

Broccoli (*Brassica oleracea* var. *italica*) production has been expanding since the 20<sup>th</sup> century worldwide, with China and India as the largest producers (Fig. 1). As broccoli is known to be a highly perishable commodity, numerous postharvest treatments for the maintenance of postharvest quality have been proposed in horticultural science. Lately, the use of 1-MCP, controlled atmosphere storage and illumination with light of different wavelengths postharvest have been highly promoted (Büchert et al. 2011b; Zhan et al. 2012; Fernández-León et al. 2013b; Jin et al. 2015). Nevertheless, market prices for broccoli, although they fluctuate over the season, are generally low (Hormes 2008; Atallah & Gomez 2013). Consequently, producer investments in elaborate postharvest equipment do not necessarily pay for themselves. Most likely, postharvest treatments which have been proved to be effective for many years - rapid cooling after harvest, optimized storage conditions near 0 °C as well as high RH and, if possible, modified CO<sub>2</sub> and O<sub>2</sub> (Klieber & Wills 1991) will still remain the most practicable effective treatments in postharvest management.



**Fig. 1: World production of cauliflower and broccoli (tonnes) (FAOSTAT 2014).**

However, producers notice a considerable variation in the shelf life of broccoli within and between batches that is not explained by different handling or storage conditions (Pink et al. 2000; Wurr et al. 2002; Pliakoni et al. 2015). Because of this variation, calculating the maximum storage days for freshly harvested broccoli batches is rarely possible. If shelf life duration was predictable at an early stage of the value chain, preferably at harvest, producers would have more opportunities to control storage capacities and to defer certain harvest batches when market prices are low. Alternatively, they could also pass this advantage on to the market and possibly achieve higher product prices. In both cases, the position of broccoli growers in the market would be strengthened and storage capacities could be managed in a more rational way.

## 2. Hypotheses and objectives

The present work was conducted in the framework of a joint research project dealing with the scheduling of *Brassica* production, taking into account the whole value chain (Herppich et al. 2013). The focus in this part was on postharvest scheduling strategies, where the objective was to find possibilities for shelf life prediction as early as possible after harvest. The aim was to enable producers to anticipate the quality and storability of broccoli batches and thus give them more flexibility and market power.

One possibility to monitor product quality during storage is the output of knowledge-driven, non-destructive devices. In research, color measurements based on reflection have been widely used for the monitoring of as well as for the creation of prediction models of broccoli quality in storage. They have been conducted in almost every publication dealing with broccoli in recent years, evaluating cultivation influences (Zaicovski et al. 2008; Cogo et al. 2011; Hasperué et al. 2011; Hasperué et al. 2014), the suitability of various varieties (Farnham & Björkman 2011; Fernandez-Leon et al. 2012) as well as the effectiveness of postharvest treatments (Aiamla-or et al. 2010; Gómez-Lobato et al. 2012c; Fernández-León et al. 2013a; Jin et al. 2015). Additionally, chlorophyll fluorescence has proved to provide valuable information about the quality characteristics of the broccoli curd. If the output from any knowledge-driven, non-destructive device indicated a certain storage quality prior to visual perception, growers might use those data to make their decision about keeping or selling specific batches. In this sense, an evaluation of optical measuring techniques with regard to information that growers might obtain from them is needed.

Optical methods applied to the broccoli curd are based on its chlorophyll content and its ability to conduct photosynthesis, as it is a green floral part of the plant. Visible quality loss in broccoli is therefore inseparable from the degradation of chlorophyll, especially in the sepals which cover the buds. Recently, pathways of chlorophyll catabolism have garnered significant attention. There has been, on the one hand, an interest in substantial knowledge regarding possible pathway patterns and the enzymes involved in chlorophyll degradation (Büchert et al. 2011a; Saga & Tamiaki 2012; Hörtensteiner 2013). On the other hand, gene expression and chlorophyll degrading enzymes have already been used to demonstrate effects of postharvest treatments on broccoli (Büchert et al. 2011b; Aiamla-or et al. 2012). However, it remains unclear if actual levels of early stage chlorophyll catabolites might indicate the postharvest quality of broccoli. If so, they could be used to predict the shelf life of broccoli and potentially other green vegetables as well.

Last but not least, factors determining broccoli quality during its cultivation on the field are of substantial interest to the grower. In comparison to postharvest treatments, climate and cultivation factors have been studied to a much lesser extent. This might be due to the high work load incurred when both factors of cultivation as well as postharvest measurements are examined. However, it is the cultivation factors that are expected to have the most significant influence on vegetable quality (Weston & Barth 1997). In experiments with field-grown broccoli, the results are often influenced to a greater extent

by weather conditions than by the trial design itself (Toivonen et al. 1994; Leja et al. 2002). It is presumed that the impact of weather conditions on vegetable production may increase in the future, affecting postharvest quality as well (Moretti et al. 2010). Consequently, knowledge regarding environmental factors influencing quality is of eminent importance. Effective strategies are needed to evaluate climate influences on broccoli quality and storability, and to transfer those principles into cultivation practice.

In summary, the present work purses the following objectives in order to attend to the challenges of postharvest scheduling in broccoli production:

- Identification of a quality rating method that can give clear evidence regarding the (further) storability of broccoli
- Rating of the relative influence of pre-harvest conditions on the post-harvest life of broccoli
- Identification of the early senescence indicators in curd development and the chlorophyll catabolism of broccoli curds

The following chapter contains a review of all relevant approaches in the addressed fields. In the first subchapter, the major environmental influences during the whole life cycle of the broccoli plant and its edible curd are introduced. The development of the broccoli curd and the role curd maturity plays in the predetermination of storability is referred to. Next, the reported possibilities for the measurement and prediction of broccoli storability are reviewed. The last part of the literature overview deals with chlorophyll degradation processes in broccoli.

### 3. Literature review

#### 3.1. Broccoli quality as a function of physiology

The growth, development and quality of broccoli plants naturally depend on the environment during cultivation, from seeding or transplanting to floral initiation and finally to the growth of the floral organ, the broccoli curd. After harvest, many physiological processes take place, such as an increase in respiratory rate, ethylene production and sensitivity, as well as losses in chlorophylls, sugars, organic acids, and proteins (King & Morris 1994a; King & Morris 1994b; Tian et al. 1995; Zaicovski et al. 2008).

In relation to storage, the involvement of many genes has been discussed, among them *BoCLH1* (Chen et al. 2008a) and *BoPPH*, *BoPaO* and *BoLOX1* (Gómez-Lobato et al. 2012a, b; Gómez-Lobato et al. 2012c; Hasperué et al. 2013). A detailed overview of possible candidates is presented in Chen et al. (2008b). Differences in shelf life in terms of chlorophyll loss have been observed among cultivars (Toivonen & Sweeney 1998), which is a clear indicator of genetic determination. In order to analyze the expression of different genes and their possible connection to storability under varying radiation and temperature regime, further research is needed.

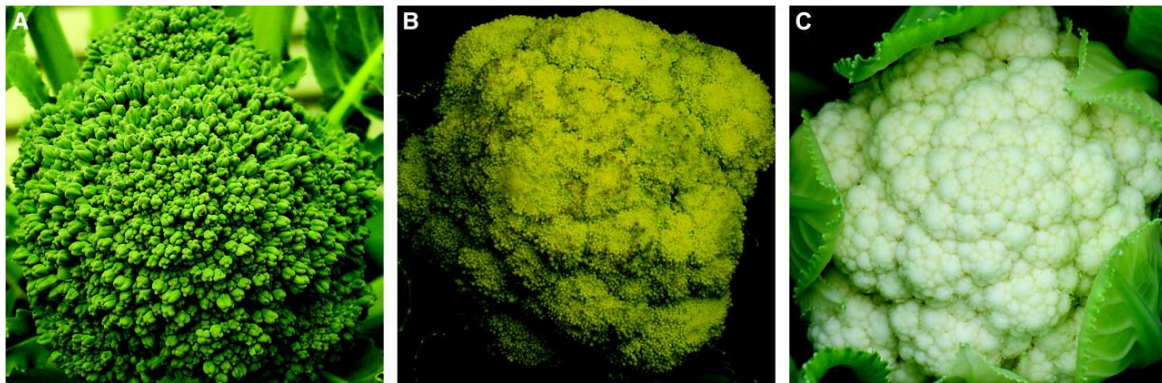
In the following subchapters, the most important influences on broccoli production and storage are reviewed in relation to their impact on quality and storability.

##### 3.1.1. Temperature

Regarding studies on the influence of preharvest factors on broccoli, temperature is the most frequently regarded factor. The developmental stages in which broccoli plants are exposed to certain temperatures are of great importance. It has been known for a long time that broccoli floral development is mainly dependent on temperature and is probably inhibited by temperatures above 22 °C (Gauss & Taylor 1969). Many examinations concerning temperature influences on the growth and development rate were conducted by numerous authors during the 1980's and 90's in order to predict head maturity stage and the exact temperatures leading to different development stages (for a detailed overview, see Tan 1999). The aim was to control broccoli production towards predictable harvest dates, which is one of the most relevant agronomic factors (Dixon 2007; p.107). This issue is still of concern in more recent research (Kałużewicz et al. 2012; Lindemann-Zutz et al. 2016).

The most obvious proof for the considerable influence of temperature on development of *Brassica oleracea* species is revealed in the investigations of Labate et al. (2006), followed by Duclos and Björkman (2008). In order to identify genes responsible for the required cessation of generative development, a *B.oleracea* F<sub>1</sub> hybrid cultivar 'Green Harmony' with a great range of phenotypic variation was grown under different day/night temperature regimes. The growth type changed significantly towards a cauliflower-like instead of a broccoli-like appearance when the temperature level was rising (Fig. 2). In

conclusion, moderate temperatures may allow floral development to proceed further than it would at warm temperatures, as cauliflower floral development already ceases at the meristem stage (Carr & Irish 1997). It has been observed, however, that different temperatures during the vernalization phase regularly cause different phenotypic disorders in cauliflower (Fujime & Okuda 1996). In this sense, phenotypic variability can be interpreted as mild form of riceyness and bractiness as in Fig. 2, B and C, respectively (Hasan 2014, personal communication).



**Fig. 2: Brassica oleracea cv. 'Green Harmony' F1 grown under three different day/night temperature regimes during reproductive development. (A) At 16 °C/12 °C (B) At 22 °C/17 °C (C) At 28 °C/22 °C (taken from Duclos & Björkman 2008).**

When broccoli plants are exposed to heat (35 °C) during the early generative state, the development of single buds is inhibited, so that symptoms like non-uniform bud and head surface shape, as well as puffy or enlarged sepals appear (Björkman & Pearson 1998). According to Heather et al. (1992) broccoli is in its most sensitive stage when heads measure 5-10 mm in diameter, which corresponds to the time around 3 weeks before harvest, depending on the cultivar. Björkman and Pearson (1998) investigated this phenomenon in more detail, assuming that the detrimental effect of heat is highest at the very beginning of reproductive development when the meristems measure about 0.3 mm. The effect decreased with advancing development stages and increased with the length of heat exposure. In contrast, a negative correlation was shown between the physiological disorder of broccoli called brown bead and the average maximum temperature during the first week following button stage, when curds measured 2.5 cm (Jenni et al. 2001). Fujime and Okuda (1996) also studied quality attributes related to flower development in broccoli and cauliflower, but only the latter were subject to investigations of temperature influences, resulting in fuzzy or ricy curds. Similarly, a model of temperature regimes in combination with susceptible curd diameter stages explained 66 and 71 percent of the variation in the bracting and riciness of cauliflower, respectively (Grevsen et al. 2003).

The head color of the broccoli cultivar 'Southern comet' became unacceptable if the average minimum temperatures of the growing season exceeds 17 °C and the acceptability of 'Citation' head color and compactness decreases with increasing mean maximum temperatures (Dufault 1996). According to Kałużewicz et al. (2009) the number of hours during cultivation when temperatures exceed 20 °C is directly related to lower yields and, when they occur in the harvest phase, the number of hours contributes to the

loosening of heads. Temperatures of 5-15 °C during the harvest period led to the maximum number of marketable curds (Kałużewicz et al. 2009).

These investigations concentrated solely on the rating of quality at harvest, but did not consider the shelf life aspects of broccoli. The only investigations concerned with preharvest temperature influences on the postharvest quality of broccoli were part of the project "Long Life Broccoli", in which results indicated that bud yellowing after harvest is accelerated by higher temperatures 7 days prior to broccoli maturity (Anonymous 2001).

The studies in publications dealing with temperature effects on shelf life began their measurements on the day of harvest without taking preharvest factors into consideration: A three-hour delay in cooling after harvest has been observed to cause significant losses in market life of fresh 'Green Duke' broccoli, which was indicated by a decrease in hue angle on day 3 and a much lighter color on day 7 of storage compared to broccoli top iced within 0.5 h after harvest (Brennan & Shewfelt 1989). Similarly, Forney and Rij (1991) conclude that broccoli that are kept cool (iced after harvest and kept at 3 °C from then on) have better ratings of color, turgidity and general appearance after seven days of storage than broccoli stored at 20 °C overnight after harvest before being cooled.

During storage, higher temperatures have a detrimental effect on broccoli quality, resulting in higher respiration, decomposition of the chloroplast membrane and the subsequent loss of green color (King & Morris 1994a; King & Morris 1994b). Investigations of temperature influences on the yellowing of broccoli purchased at a local market showed complete color retention during 30 days when the broccoli was stored at 0 °C, while yellowing was observed within 6 days when broccoli was stored at 10 °C, following an Arrhenius-type function (Ren et al. 2006). In the model of Schouten et al. (2009), effects of temperature on color changes during the storage of freshly harvested broccoli were investigated in relation to different gas atmospheres. Color behavior in the 5 °C storage did not differ much over 20 days of storage, but broccoli stored at 18 °C yellowed rapidly during 10 days of storage, likewise following an Arrhenius-type function. Storage of freshly harvested broccoli at different temperatures from 0 °C to 20 °C showed a range of 2 to more than 35 days before yellowing began, emphasizing the importance of storage temperature influence on shelf life (Cantwell & Kasmire 2002). The longest storage life was reported by Klieber and Wills (1991): 'Shogun' broccoli was stored for 8 weeks at 0 °C without showing any color changes, though its marketability was critically reduced then because of rotting.

In contrast to the degradation of broccoli stored at higher temperatures, the temporary application of hot water and even hot air treatments over a certain period of time has been found to have positive effects on shelf life. Tian et al. (1996) observed that a range of different durations and temperatures of hot water treatment all reduced hue angle decrease during storage. Some applications, for instance the combination of 7.5 min at 47 °C or 10 sec at 55 °C, did not show any heat damage in terms of water soaking and subsequent tissue infections. Even without water, higher temperatures may have beneficial effects on storability: Hot air treatment (45 °C - 50 °C) of harvested broccoli at



different time intervals caused higher levels of chlorophylls during storage at 15 and 20 °C, respectively (Funamoto et al. 2002; Costa et al. 2005b). This phenomenon, a great contrast to the widely approved cold storage conditions, is explained by the reduced activity of the chlorophyll degrading enzymes chlorophyllase and peroxidase (Funamoto et al. 2002).

### 3.1.2. Water

Water plays a major role in broccoli production. A 14 % reduction in watering may lead to a 20 % reduction in broccoli yield (El-Shikha et al. 2007). Concerning growth parameters, more available water enhances germination rates of direct-seeded broccoli, but also reduces the subsequent growth of broccoli seedlings (Li et al. 2011). Broccoli plant water holding (g/plant) shows significant correlations to the uptake and amount of nitrogen as well as to head yield (Li et al. 2011). Consequently, head weights, diameter and stem turgor are reduced when water stress is applied during the growing phase (Wurr et al. 2002). In contrast, stem turgor is sustained to a significant extent during storage when water stress is only applied prior to harvest (Wurr et al. 2002).

A few reports suggest that the low water availability of broccoli plants during growth has a positive effect on broccoli shelf life in terms of color preservation (Wurr et al. 2002; Zaicovski et al. 2008; Cogo et al. 2011). Concerning the effective date of this phenomenon, the authors' ratings differ: On the one hand, high water stress with a water soil pressure of 0.4 MPa<sup>1</sup> during the whole cultivation period has an overall positive effect on green color retention (Zaicovski et al. 2008; Cogo et al. 2011). Similar observations have been made when water stress (as compost water potential of –0.2 to –0.6 MPa) was applied at the end of cultivation, 60 to 75 days after planting (Wurr et al. 2002). On the other hand, water stress during earlier periods of cultivation has been found to have a negative influence on shelf life: time from harvest to unacceptable color is decreased when similar stress levels are induced two and three weeks following head initiation (Wurr et al. 2002). In contrast, irrigated broccoli has been observed to have a higher greenness level at harvest in terms of –a\* values, compared to non-irrigated broccoli (Babik & Elkner 2000).

Zaicovski et al. (2008) associate the described effect of color retention in broccoli grown under water stress with higher amounts of the cytokinins zeatin and zeatinribose. Cytokinins are known to play a crucial role in broccoli shelf life, preserving color and chlorophyll content (Shewfelt et al. 1983; Costa et al. 2005a).

Occurrence of brown bead is negatively correlated with the sum of rainfall and irrigation from button stage (2.5 mm) to maturity which might indicate that the phenomenon is caused more by drought than by water excess (Jenni et al. 2001).

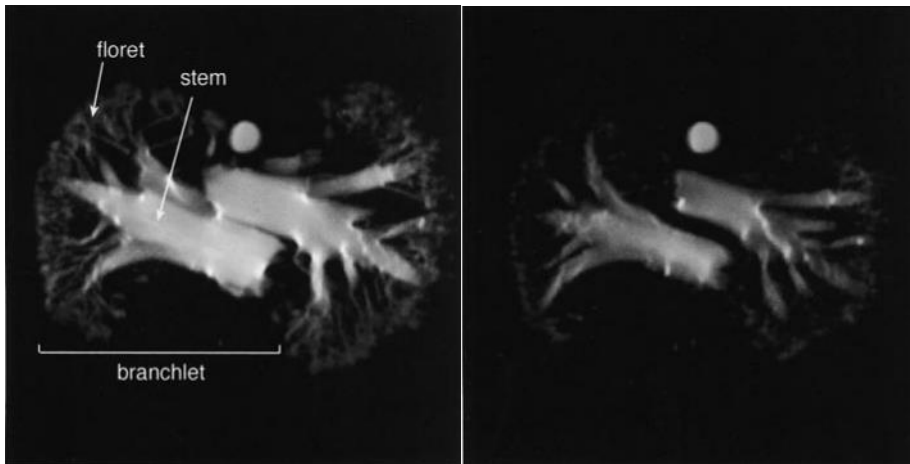
---

<sup>1</sup> It is assumed that absolute amounts are used by the authors instead of negative numbers (supplementary note)

There are conflicting reports about the relation of water stress and glucosinolate contents in broccoli, a compound group at the focus of numerous investigations predominantly because of its considerable health benefits, but also its potential risks, which are the subject of discussion recently (Latté et al. 2011; Manchali et al. 2012). Vallejo et al. (2003) report that glucosinolate contents of eight broccoli cultivars grown in Murcia, Spain, from April to July were significantly higher than contents in early spring, which was explained by the very low amounts of precipitation (5.4 mm) in the later season. On the other hand, Robbins et al. (2005) report that water stress (80 % compared to 100 % of evapotranspiration losses) reduced glucosinolate levels as well as phenolic compounds in 'Majestic' and 'Legacy' broccoli. Investigations of glucosinolate induction in broccoli following three water treatments show neither differences in subsequent herbivory nor in enhanced glucosinolate levels when plants were grown under well watered and water-logged conditions, respectively (Khan et al. 2010, 2011). Fortier et al. (2010) report that the absence of irrigation increases concentrations of polyphenolics in broccoli.

After harvest, the loss of water proceeds very fast. Water losses of horticultural products generally depend on the amount of water vapor in the surrounding atmosphere. Differences in water vapor pressure between the interior of stored fruits or vegetables and the surrounding atmosphere can be used to estimate the movement of water between the product and its environment (Kays 1991, p.432). In harvested broccoli, there is usually no replacement of water through uptake, even though some researchers have promoted the uptake of water from cups during experimentation (Büchert et al. 2011c). As a result of water loss, the whole broccoli curd shrinks, with flower buds being the most sensitive parts (Fig. 3). It is assumed that proteolysis is one of the major processes during broccoli senescence and that the expression of protease genes can therefore indicate the current senescence status (Coupe et al. 2003). Because of the expression of dehydration-responsive cysteine proteases, quality losses during postharvest are possibly triggered by water stress (Coupe et al. 2003).

Investigations from Klieber and Wills (1991) conclude that small differences in RH during storage can have a great effect on shelf life: The shelf life of 'Shogun' broccoli stored at 0 °C and 100 % RH was twice as long (8 weeks) then that stored in 97 % or 94 % RH. The limiting factor in this case was seen in wilting or shriveling, following the quality rating scale of Wang (1979, see also chapter 3.2).



**Fig. 3: Water loss of harvested broccoli held in air at 20 °C after 2 hours (left) and 26 hours (right), respectively. Magnetic resonance images showing signals related to the intensity of the water activity (taken from Coupe et al. 2003).**

In order to prevent the loss of water, broccoli is in general wrapped with different packaging material. Thus, the water vapor inside the package is increased, which reduces the water vapor deficit. As a result of accelerated respiration, the composition of atmospheric gases is modified towards higher CO<sub>2</sub> and lower O<sub>2</sub> contents (Zhuang et al. 1994; Serrano et al. 2006). It is difficult to rate the effect of water loss on broccoli independent of the impact of modified atmosphere since, in most cases, increased RH is achieved through wrapping of the curds. Combinations of different temperature levels and modified atmosphere treatments can lead to variation in color changes (Ren et al. 2006). In examination of Ren et al. (2006), unpacked broccoli retained its green color for a longer period than packed broccoli at both 0 °C and 5 °C, while packaging proved to be favorable at the 10 °C level. In contrast, Serrano et al. (2006) observed that unwrapped broccoli curds lost weight, green color and phenolic compounds more quickly compared to broccoli wrapped with different materials during storage at 1 °C and 90 % RH.

However, it is not clear if water loss and bud yellowing are related in the sense that they occur as a result of the same physiological process. Some investigations indicate the opposite, as results show variation in weight loss and yellowing. Toivonen and Sweeney (1998) report that differences in the chlorophyll content retention of different cultivars were not necessarily related to differences in water loss. The chlorophyll content of the cultivar 'Greenbelt' remained stable over 4 days at 13 °C, whereas the higher chlorophyll content of 'Emperor' declined during storage.

### 3.1.3. Radiation

Radiation and daylength have been considered in several studies related to the vernalization requirements of broccoli. Floral development is accelerated by a longer photoperiod at two different temperatures (17 °C and 19 °C), depending on cultivar (Fujime et al. 1988). Similarly, vernalization as the time from sowing to button stage (10 mm) was accelerated by increasing the photoperiod, which has been interpreted as a response to the higher energy amounts received, rather than to a photoperiodic effect

(Gauss & Taylor 1969). As part of a model for the maturity prediction of broccoli, solar radiation accounts for nearly 18 percent of the variation of total growth duration, while temperature as the main influencing factor explains nearly 75 % (Marshall & Thompson 1987). Considering of the sum of solar radiation improved the prediction model from Grevsen (1998) as well, although the improvement in this case is similar to the addition of the effect of the planting month, which can be much more easily achieved. In the work of Tan (2000), the influence of solar radiation on vernalization is seen to be negligible, accounting only for 2 to 6 percent of the variation over the time of plant emergence to floral initiation and showing no trend in earlier floral initiation.

According to Francescangeli et al. (2007), when broccoli is grown under shade, the growth cycle is extended up to 15 days. Cultivation time is therefore more sensitive to shade than the parameter spear yield, which was only reduced when 70 % shade was applied to the plants (Francescangeli et al. 2007).

Solar radiation is responsible for the accumulation of several secondary plant metabolites in broccoli. The concentration of flavonols in broccoli has been found to be dependent on the sum of solar radiation within the growing phase (Gliszczyńska-Świągło et al. 2007). Higher concentrations of chlorophyll and phenolic compounds, as well as a higher activity of peroxidase and catalase, were found in broccoli grown in spring compared to autumn in Krakow, Poland (Leja et al. 2002). The number of sunny days and the intensity of photosynthetic radiation were much higher in spring than in autumn and have therefore been identified as the main influencing factors (Leja et al. 2002). In contrast, no effect of radiation intensity during growth has been found on chlorophyll and carotenoids (Lut,  $\beta$ -Car) by Schonhof et al. (2007).

Similar to other crops, broccoli experiences a circadian rhythm during growth, leading to an accumulation of carbohydrate reserves during the day. After harvest, losses of sugars normally appear within the first 6 hours (King & Morris 1994a). In order to compensate for these losses, harvest at the end of the day has been suggested: It has been reported that harvest at 6 p.m. results in a better shelf life in terms of color, sugar and chlorophyll contents compared to morning and midday harvest (Hasperué et al. 2011; Hasperué et al. 2014). Even after harvest, cabbage crops are able to establish a circadian rhythm when irradiated with visible light at regular intervals. This diurnal training leads to enhanced plant defense mechanisms through a higher production of glucosinolates (Goodspeed et al. 2013). Broccoli curds from a local producer that were stored under continuous white light ( $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 22 °C showed a significantly better color retention and higher chlorophyll contents as well as higher contents of sugar and protein from day 3 onwards compared to curds stored in the dark (Büchert et al. 2011b; Büchert et al. 2011c).

Supplementary UV-radiation during vegetative growth led to an increase in ascorbic acid, phenolic and flavonoid contents according to Topcu et al. (2015). In addition, UV radiation during growth led to an increase in  $L^*$  and  $C^*$  values after 60 days of storage at 0 °C and modified atmosphere (Topcu et al. 2015). Treatments of broccoli with UV radiation during

postharvest result in a significantly enhanced shelf life (Costa et al. 2006a; Aiamla-or et al. 2010; Büchert et al. 2011b; Martinez-Hernandez et al. 2011). It is assumed that UV B radiation prevents chlorophyll degradation by suppressing the activities of chlorophyll degrading enzymes and possibly by increasing antioxidative enzyme activities (Aiamla-or et al. 2010). The findings of Büchert et al. (2011b) indicate that only the enzyme pheophytinase is affected by light treatments, while the degradation of chlorophyll a through chlorophyllase is not involved.

#### 3.1.4. Fertilization

*Brassica* crops are generally known to be high nutrient-demanding plants, so great amounts of nitrogen fertilizer are needed to produce high broccoli yields (Zebarth et al. 1995; Yoldas et al. 2008). Broccoli curds have been reported to increase in diameter and weight up to a certain level through increased applications of nitrogen fertilizer, depending on the harvest batch (Toivonen et al. 1994). Additionally, the number of leaves per plant increases and both higher fresh and dry weights are achieved through higher nitrogen fertilizer doses (Ouda & Mahadeen 2008). Li et al. (2011) report good correlations of higher nitrogen accumulation with both head yield (g/plant) and dry matter (g/plant). However, the diameter of broccoli curds was not affected by nitrogen accumulation (Li et al. 2011). Lisiewska and Kmiecik (1996) did not report higher dry weights of broccoli raised with 120 kg N ha<sup>-1</sup> when compared to 80 kg N ha<sup>-1</sup>. Intercepted radiation of broccoli, which was calculated considering the leaf area index, increased by increasing the nitrogen application rate (Vagen et al. 2004). The above-ground plant parts of broccoli accumulate great amounts of nitrogen during cultivation, so tissue nitrogen at harvest is high (Zebarth et al. 1995; Thompson et al. 2002; Bakker et al. 2009b).

High nitrogen rates have been associated with the occurrence of hollow stems, as well as with incidences of soft rot in broccoli (Hipp 1974; Fortier et al. 2010). Bakker et al. (2009a) report that increasing nitrogen rates improve floret color and decrease the number of misshaped heads on the one hand, but on the other hand support the incidence of hollow stem and head rot. Similar observations concerning color and hollow stems had already been made by Babik and Elkner (2000). There is, furthermore, strong evidence for boron deficiency playing an additional role in hollow stem disorder of broccoli (Shelp et al. 1992; Moniruzzaman et al. 2007).

Apart from an influence on the general quality of the harvested broccoli curd, reports about effects on storability at different nitrogen fertilizer levels are rare. In the investigations of Toivonen et al. (1994), no differences were found in broccoli which had received 5 different levels of nitrogen fertilizer on the field and was then stored at 1 °C and high RH (>95 %) for 20 days.

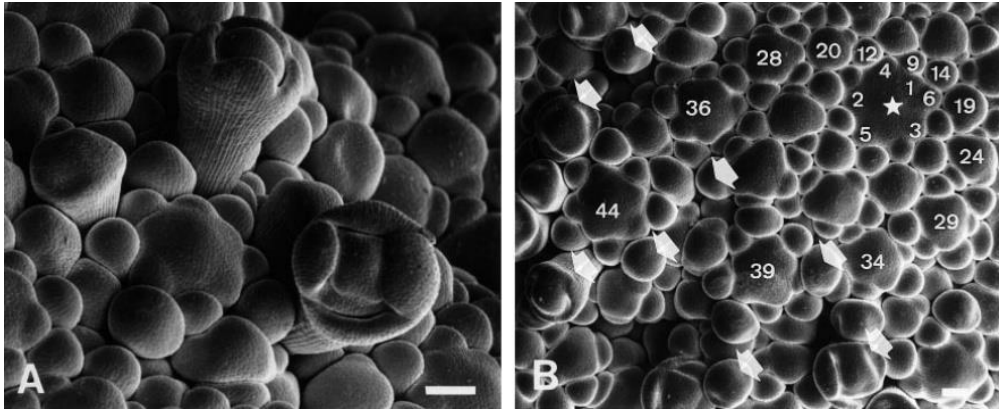
#### 3.1.5. Generative development

Broccoli represents one of the two subgroups (lat. *varietas*) of *Brassica oleracea* that form a large preinflorescence, which is used for consumption. The curd is regarded as “an

intermediate stage in the development of the flowers and seed” (Dark 1938). The development itself has been graded in different ranks, ranging from 1 (not visible) to 7 (flowering) with index 4 at optimum harvest maturity (Sørensen & Grevsen 1994). After the transition of the broccoli plant to the floral stage, the primordia produced by the apical meristem may form branches or flowers with a tendency towards more flower buds (Kieffer et al. 1998). Fujime and Okuda (1996) describe the phenomenon that during the late curd-forming stage, inflorescence primordia are newly initiated around the 1<sup>st</sup>-order inflorescence primordium. Branch primordia on broccoli are arranged in phyllotactic spirals following a (5, 8)-Fibonacci parastichy system (Fig. 4). At maturity, a single broccoli flower consists of 6 stamens, 4 sepals, 4 petals and one pistil (Carr & Irish 1997; Duclos & Björkman 2008). Finally, the surface of the marketable curd consists of a large number of flowers at bud stage with closed sepals. Fully closed buds are a required characteristic of broccoli sold as class I, while class II type of broccoli might have only “practically closed” floret buds (UNECE 2010).

It has been assumed that the maturity of broccoli is, among other factors, determined by the size of the individual florets (Marshall & Thompson 1987). Bud size and uniformity have been used in to rate the sensitivity of broccoli varieties to heat: Björkman and Pearson (1998) conducted ratings of bud injury from the “terminal whorl inwards, following the phyllotactic spiral”. In their examinations, bud length generally decreased towards the center of a whorl, with severely heat injured curds having shorter buds (down to about 0.8 mm) than uninjured curds (down to 2 mm). Farnham and Björkman (2011) measured broccoli buds with the help of a caliper on the intact curd. It is therefore to be assumed that they used bud diameter instead of bud length. The measured size was in the range of 1.34 to 1.98 mm, depending on the broccoli variety (Farnham & Björkman 2011). Buds of the broccoli cultivar ‘Monaco’ measured 1.72 mm in diameter on average (Kalisz et al. 2014). The chilling of juvenile broccoli plants led to curds with a significantly greater bud diameter of 1.78 to 2.45 mm, depending on chilling temperature and duration (Kalisz et al. 2014).

Tian et al. (1994) discovered a relationship between botanical maturity stages of broccoli flowers and their storability. When the stamens and pistil were removed from single flowers, the yellowing rate of sepals was reduced. Three days after harvest, the total chlorophyll content of sepals from intact flowers had decreased by 65 %, compared to a 50 % reduction in flowers where pistil and stamens were removed.



**Fig. 4: Transition of broccoli to the floral stage. Development of flower bud primordia (A + arrows in B) and arrangement of branch primordia following a phyllotactic spiral of 5 (left) and 8 (right) (B). Scanning electron micrographs, bar = 100  $\mu$ m (taken from Kieffer et al. 1998).**

Tian et al. (1995) determine the maturity stages of single broccoli flowers by grading pollen stages into three maturity groups. An accelerated rate of chlorophyll loss was noted in larger buds (separated from smaller ones with a sieve of 2 mm holes) when held in the air or when exposed to propylene. The larger buds most likely (63 %) contained mature pollen with pollen grains. From these findings it was concluded that yellowing of harvested broccoli curds is related to the development stage of the pollen and that the size of individual florets can be used as an index for floret maturity. Nevertheless, broccoli curds were covered with florets at various stages, 70 % of them being medium-sized florets which contained predominately immature pollen (Tian et al. 1995).

In other examinations of broccoli, e.g. growth models, the trait used as a maturity indicator most often is curd diameter (Wurr et al. 1995; Mourao & Brito 2000; Leja et al. 2002; Zutz et al. 2013). Leja et al. (2002) report the highest chlorophyll concentrations in broccoli curds with diameters of 10 to 12 cm and the highest amounts of ethylene produced in curds at the pre-florescence stage (34 to 42 cm). It therefore appears obvious that broccoli curds are increasing in size while developing towards botanical maturity. Nevertheless, curds might also flower before reaching their maximum size when exposed to unfavorable conditions (Gauss & Taylor 1969; Heather et al. 1992).

### 3.2. Determination and prediction of broccoli quality

In order to rate the quality of broccoli curds by visual appearance, subjective scores are frequently used. The scale from Wang (1979) comprises a huge number of quality attributes, considering the appearance of freshness, generative development and decay. Dufault (1996) uses a scale containing the density, color, shape and leafiness of the broccoli curd, as well as its bead size, following the standards of the USDA (1977). The scales invented by Shewfelt (1993) which range from 1 to 5, addressing color, texture and odor, have also been used in more recent examinations (Olarte et al. 2009; Zhan et al. 2012). The scale used in Wurr et al. (2002) ranges from 0 to 3 for the attributes of color, turgor and bud characteristics.

Apart from the lack of comparability of the scores given in the different examinations, rating scores in general have the disadvantage of not being suitable for many kinds of statistical analysis, as they represent ordered categorical instead of metric data (Piepho & Kalka 2003). In addition, slight variations are impossible to express with the help of such scales, so that the potential for objective decision making is extremely limited.

In the following subchapters, the determination of broccoli quality during storage is reviewed. The focus is laid on optical methods and on the retention of green color in general, including chlorophyll degradation processes.

### 3.2.1. Reflection measurements

Color measurements based on reflection have been applied in the rating of broccoli quality for more than three decades and are widely accepted in academic research nowadays. Shewfelt et al. (1983) were the first authors to use colorimetry for broccoli, but judged it to be less sensitive to color change than visual evaluation. By now, the fact that color measurements are fast and easy to conduct and have a high repetitious accuracy is agreed upon. They have been conducted in almost every publication dealing with broccoli in recent years to evaluate cultivation influences (Zaicovski et al. 2008; Cogo et al. 2011; Hasperué et al. 2011; Hasperué et al. 2014), suitability of varieties (Farnham & Björkman 2011; Fernandez-Leon et al. 2012) or effectiveness of postharvest treatments (Aiamla-or et al. 2010; Gómez-Lobato et al. 2012c; Fernández-León et al. 2013b; Jin et al. 2015).

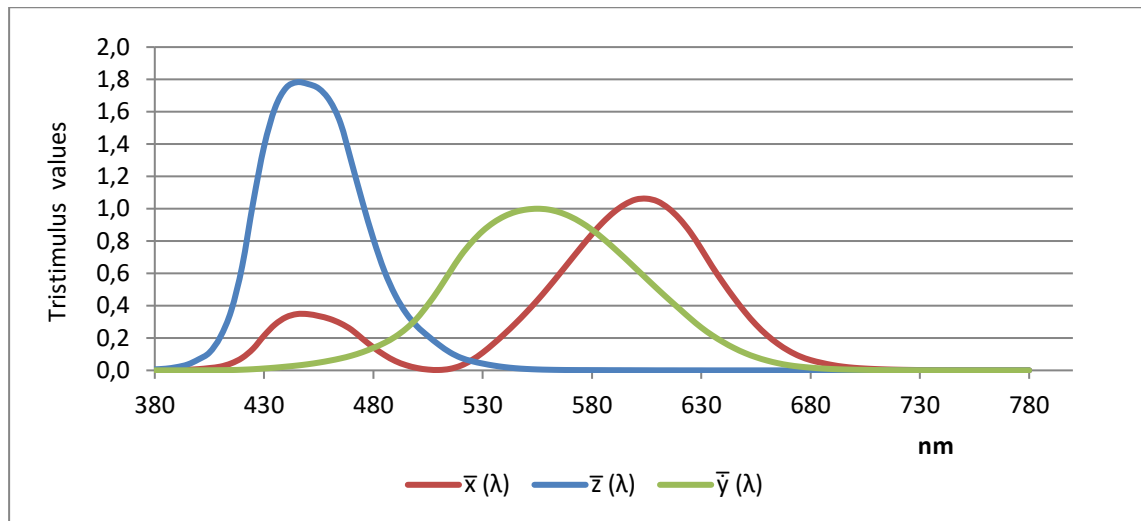
Color measurements are based on diffuse reflectance readings, either as spectral measurements when attained with a spectrophotometer, or as direct tristimulus values from simpler devices. For broccoli measurement, the color-spaces CIE 1976 ( $L^*$ ,  $a^*$ ,  $b^*$ ), abbreviated CIELAB, as well as the Hunter Lab are often applied. In order to demonstrate how reflection spectra are transformed to certain colors which are then assigned to objects, the basic calculation of the widely used CIELAB variables is briefly introduced here.

#### 3.2.1.1. Calculation of CIELAB values

The basis of CIELAB color definition dates back to 1931, when data from color matching experiments performed with test panels was adopted by the Commission Internationale de l'Eclairage (CIE) in order to establish color matching functions (Ohta & Robertson 2006a, p. 64). The results of the experiments were originally expressed as RGB color matching functions, later they were transformed in order to only result in positive values, as shown in Fig. 5. The original RGB color matching functions represent the amounts of the reference stimuli [R], [G] and [B], which are monochromatic lights of the wavelengths  $\lambda_R=700.0$  nm,  $\lambda_G=546.1$  nm, and  $\lambda_B=435.8$  nm, respectively, needed to match monochromatic stimuli of the white color stimulus (Ohta & Robertson 2006a, p. 64). Finally, the CIELAB was invented in 1976 in order to obtain a color-space with higher uniformity of perceived lightness on the one hand and a certain independence of the



spectral distribution of the illuminant on the other hand (Ohta & Robertson 2006c, p. 127 & 135).



**Fig. 5: Color matching functions of the CIE 1931 standard colorimetric observer (Minolta 1996).**

Accordingly, a range of calculations has to be executed for the conversion of the actual measured spectral reflection into CIELAB. First, values of the tristimulus values  $X$ ,  $Y$  and  $Z$  are calculated by the use of the above color matching functions as follows:

$$\begin{aligned} X &= K \int_{380}^{780} R(\lambda)P(\lambda)\bar{x}(\lambda)d(\lambda) \\ Y &= K \int_{380}^{780} R(\lambda)P(\lambda)\bar{y}(\lambda) d(\lambda) \\ Z &= K \int_{380}^{780} R(\lambda)P(\lambda)\bar{z}(\lambda) d(\lambda) \end{aligned} \quad (1)$$

where  $R(\lambda)$  is the spectral reflectance of the reflecting object,  $P(\lambda)$  is the spectral distribution of the illuminating light and  $d(\lambda)$  is the interval of wavelength. The value  $Y$  is normalized to a value of 100 for a perfect reflecting object with the help of constant  $K$ :

$$K = \frac{100}{\int_{380}^{780} P(\lambda)\bar{y}(\lambda)d(\lambda)} \quad (2)$$

Using those  $X$ ,  $Y$  and  $Z$  values, the parameters of CIELAB are ultimately calculated as

$$L^* = 116 \left( \frac{Y}{Y_n} \right) - 16 \quad a^* = 500 \left[ \left( \frac{X}{X_n} \right)^{\frac{1}{3}} - \left( \frac{Y}{Y_n} \right)^{\frac{1}{3}} \right] \quad b^* = 200 \left[ \left( \frac{Y}{Y_n} \right)^{\frac{1}{3}} - \left( \frac{Z}{Z_n} \right)^{\frac{1}{3}} \right] \quad (3)$$

with certain restrictions for small values (Ohta & Robertson 2006c, p. 130).  $X_n$ ,  $Y_n$ , and  $Z_n$  are the tristimulus values of the reference white point, representing a perfect reflecting diffuser. Out of the values  $a^*$  and  $b^*$ , the hue angle (4) and chroma  $C^*$  (5) of an object can be calculated (Minolta 1996). The hue angle is the parameter used most often in the ratings of broccoli color mentioned in this work.

$$h^\circ = \tan^{-1} \left( \frac{b^*}{a^*} \right) \quad (4)$$

$$C = \left[ a^{*2} + b^{*2} \right]^{\frac{1}{2}} \quad (5)$$

In conclusion, it should be noted that the origin of CIELAB lays in the color-receptive perception of human rods and cones, and that a series of calculations is needed to transform the original reflectance spectrum into CIELAB values.

### 3.2.1.2. Color modelling

In broccoli colorimetry has not only been used as a simple quality evaluation system, but is also part of a number of prediction models. In general, careful monitoring of a biological process might be able to predict its further development, when applied to independent data. Therefore, the occurring processes are estimated with the help of a mathematical function, which is the principal step of biological modeling (Lentz 1998). For broccoli it is often assumed that color changes depend on time and rate constants, principally following Arrhenius' law

$$k = e^{-E_a / (RT)} \quad (6)$$

where  $k$  is a reaction rate constant,  $E_a$  is the activation energy,  $R$  is the gas constant and  $T$  is (storage) time (Weemaes et al. 1999b; Tijssens et al. 2001; Ren et al. 2006; Schouten et al. 2009).

The reported models in the literature have been very useful with regard to the comparison and evaluation of storage impacts at different temperatures and gas surroundings (Ishikawa & Hirata 2001; Ren et al. 2006; Schouten et al. 2009). The model used in Schouten et al. (2002) and (2009) characterizes color change as the sum of broccoli color without green pigments, plus initial amounts of chlorophyll and protochlorophyllide, each of them multiplied by factors containing kinetic parameters meant to describe the pigment constitution as well as its degradation. The reaction rate constants of the color change were calculated with the help of a reference constant  $k_{ref}$ , which was the previously identified rate constant of broccoli color change at 18 °C. Ren et al. (2006) presume that the quality of an agricultural product is related to time and temperature during storage according to Arrhenius' law. Additionally, a pre-exponential equation constant was taken into account. Rate constants were then estimated, while the pre-factors as well as the activation energy were based on experimental data collected on purchased broccoli. The Arrhenius-function resulted in high adjustment degrees for the  $b^*$  value, while a polynomial model returned in a better a fit for hue values (Ren et al. 2006). Arrhenius' law was also used for color changes during the blanching of broccoli (Tijssens et al. 2001) and during the heating of broccoli juice (Weemaes et al. 1999b). In contrast, Ishikawa and Hirata (2001) applied multiple regression analysis instead of Arrhenius' law and propose a model to describe color development in broccoli as a function of in-package gas composition and integrated respiration rate.

Another approach described is the use of artificial neural networks for color prediction models. According to Hosaka et al. (2012) chlorophyll degradation of broccoli heads has been suitable for the prediction of color change at the start of storage when using a neural

network. The 2<sup>nd</sup> derivative of reflectance spectra from 380 to 1000 nm and the change in chlorophyll concentration were placed as input factors (Hosaka et al. 2012).

### 3.2.1.3. Image analysis

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

However, apart from colorimetry, the reflection spectra of broccoli have only recently been analyzed in more detail. Using a hyperspectral imaging system, Hosaka et al. (2012) selected 10 areas per broccoli curd for analysis. The 2<sup>nd</sup> derivative of reflectance spectra from 380 to 1000 nm and the change in chlorophyll concentration were the input factors in an artificial neuronal network by 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 through 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, with the use of NIR spectra, green asparagus (Sanchez et al. 2009). The NIR-spectrum has also been used to predict the water content of broccoli leaves (Thiel et al. 2011 + 2012). For the quality determination of broccoli postharvest, non-colorimetric spectroscopy has not been used thus far.

Concerning broccoli, there are approaches considering the reflection spectra of broccoli on a field scale in order to monitor water and nitrogen supply. Data are derived from a ground-based remote sensing system (El-Shikha et al. 2007) or from a satellite (Johnson & Trout 2012). In this context, the normalized difference vegetation index (NDVI) is the 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 the water and nutrient status of broccoli (El-Shikha et al. 2007; Johnson & Trout 2012).

The reported digital and hyperspectral image studies on broccoli, however, have rarely applied advanced image analysis that takes all of the information included in images into account. Whenever single parts of broccoli images have been investigated, it is supposed that mean values of spectral reflectance have been calculated, instead of using image data on single pixel basis. Thus, the values have been mainly treated similarly to readings from single spot devices, as being separated from spatial information. Concerning

broccoli, this may be a knowledge gap: Due to their complex morphology, senescence changes within individual broccoli curds can be expected to occur inhomogeneously on the curd surface related to single floret buds (see 3.1.5). Hernández-Hierro et al. (2014) applied image analysis for the determination of spatial glucosinolate distribution. However, they used a longitudinal slice of broccoli instead of the curd surface, where the senescence changes normally occur.

### 3.2.2. Chlorophyll fluorescence

The curd of broccoli is a green floral organ containing large amounts of chlorophyll. Consequently, it is capable of performing photosynthesis, and this characteristic is also maintained postharvest. Thus, chlorophyll fluorescence has been applied during storage and after postharvest treatments of broccoli in order to rate its quality. In this context, different fluorescence parameters have been used with varying results. Toivonen (1992) applied the variable fluorescence ( $F_v$ ), which represents the length of the slope from minimum ( $F_0$ ) to maximum fluorescence after dark adaptation ( $F_m$ ) (von Willert et al. 1995, p. 219). According to the study of Toivonen (1992),  $F_v$  proves to be a valuable score for the rating of broccoli during adequate storage (1 °C, 97 % RH), because a decline of freshness could be measured before visible yellowing occurred. Broccoli which was stored at 0-1 °C for 7 to 10 days and was then transferred to room temperature (18 °C) showed an immediate and continuous decline of parameters measured in the light-adapted state, whereas  $F_v/F_m$  did not show such a prompt reaction (DeEll & Toivonen 1999).  $F_v/F_m$  provides an estimate of the maximum of photosynthetic efficiency measured in the dark-adapted state. Healthy and highly active plants can exhibit a  $F_v/F_m$  ratio as high as 0.84 (Björkman & Demmig 1987).

When broccoli was subject to hot water treatments, treatment durations which reduced yellowing of the curds resulted in lower  $F_v/F_m$  ratios (Tian et al. 1996). Concerning the maturity of the curd, no differences of chlorophyll fluorescence parameters were observed between broccoli at optimum compared to overmature stage (Toivonen & DeEll 1998).

Differences between broccoli packed in two different plastic films were determined without opening the seals by the use of several fluorescence parameters both in the light- and dark-adapted state (DeEll & Toivonen 2000). It was assumed that the differences resulted from different respiration patterns, because one of the plastic films caused an accumulation of  $CO_2$  inside the package (DeEll & Toivonen 2000). Upon removal from the packages, values of fluorescence parameters might recover (Toivonen & DeEll 2001).

In leaves of broccoli, it is possible to assess the flavonol content through chlorophyll fluorescence measurements after excitation with two wavelengths (Bengtsson et al. 2006). Also polyphenolics in broccoli have been estimated by the use of fluorescence measurements (Fortier et al. 2010).

In conclusion, it can be assumed that chlorophyll fluorescence may be a valuable technique for quality determination of broccoli, although the application is not as common as color measurements. Based on the findings in the literature, it is uncertain if

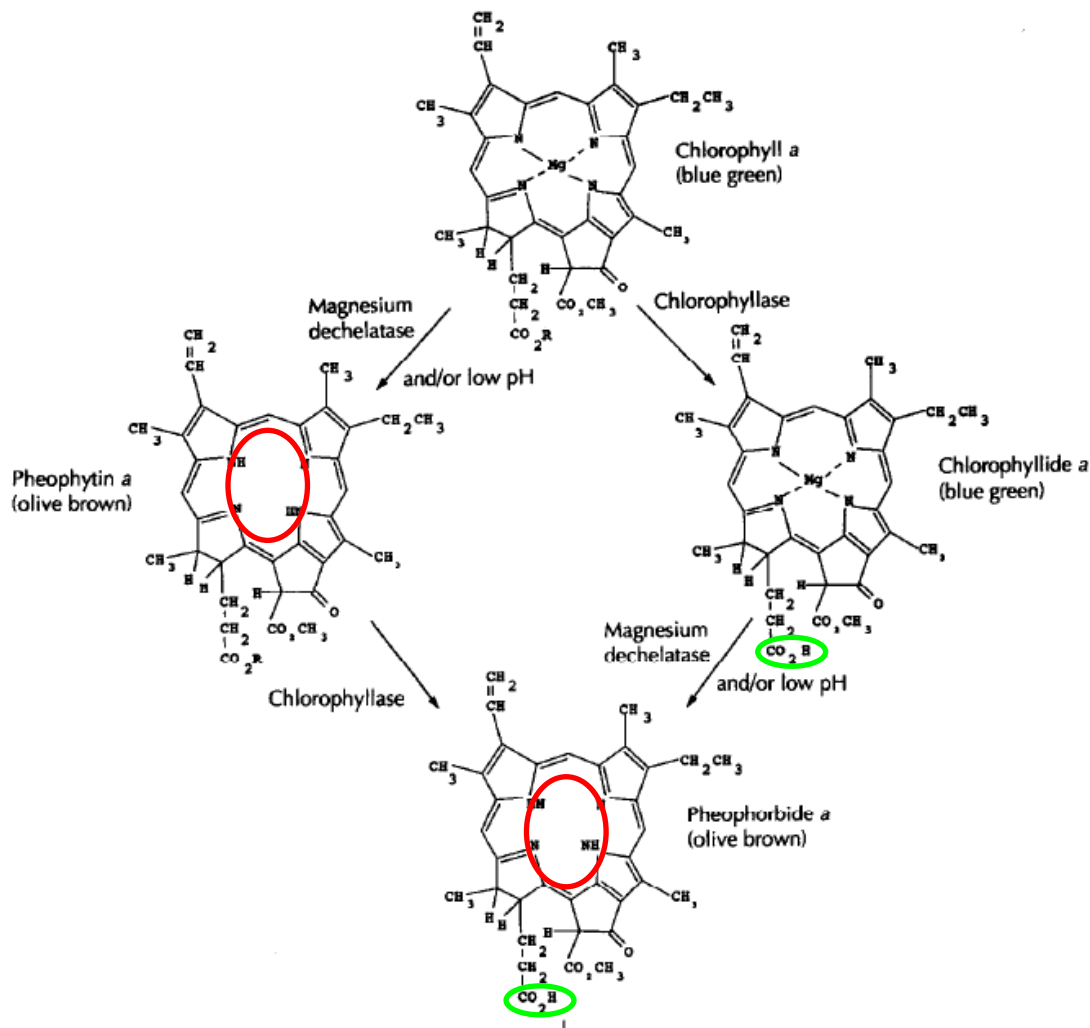
fluorescence analysis is generally more sensitive to changes during storage than reflection intensity and concomitant color determination. Although some authors report a higher sensitivity, it has also been stated that senescing tissue tends to maintain its photosynthetic capacity until near the end of its storage life (DeEll & Toivonen 1999).

### 3.2.3. Chlorophyll degradation

Regarding green vegetables, the maintenance of the photosynthetic apparatus serves as an indicator of freshness. Quality losses of green vegetables go hand in hand with the degradation of the photosynthetic system, which is probably activated in order to prevent cell death and to remobilize nitrogen from chlorophyll binding proteins (Hörtensteiner 2004, 2006). In the literature, chlorophyll catabolism has been in the focus of broccoli shelf life examinations. In this context, next to enzyme activities (Zhuang et al. 1994; Funamoto et al. 2002; Costa et al. 2005a; Büchert et al. 2011a; Aiamla-or et al. 2014) and corresponding gene expression patterns (Chen et al. 2008a; Gómez-Lobato et al. 2012b, a), also the actual presence of chlorophyll derivatives has been used to interpret the current senescence status of broccoli (Yamauchi et al. 1997; Costa et al. 2005a; Costa et al. 2006a; Aiamla-or et al. 2010; Kaosamphan et al. 2010; Aiamla-or et al. 2012).

Looking more closely at the early chlorophyll degradation pathway, it is known that the conversion from chlorophyll *b* into chlorophyll *a* is a general prerequisite (Scheumann et al. 1999; Tanaka & Tanaka 2006); Fig. 6. The light harvesting complex II (LHC II), which contains a relatively huge amount of chlorophyll *b*, is degraded. Therefore, the ratio of chlorophyll *a* to *b* may shift towards chlorophyll *a* during senescence (Schopfer & Brennicke 2006, p. 529). The last colored chlorophyll catabolite in the degradation pathway is Pheoide. In broccoli, Pheoide is later degraded into a red chlorophyll catabolite, followed by fluorescent (Mühlecker et al. 1997; Hörtensteiner & Kräutler 2011) and finally by three nonfluorescent catabolites (Roiser et al. 2015). The intermediate steps of early chlorophyll degradation have been the subject of much controversy: It used to be assumed that the initial step of chlorophyll catabolism was dephytylation, resulting in Chlide as the first product (Heaton & Marangoni 1996; Tanaka & Tanaka 2006; Kaewsuksaeng 2011). In broccoli, the higher expression of the enzyme pheophytinase compared to chlorophyllase was found by Büchert et al. (2011a). This indicates that the chlorophyll degradation follows the steps of the pathway presented on the left of Fig. 6 instead. A proof of this principle is achieved in *Arabidopsis*, where pheophytinase specifically needed Pheoin as a substrate *in-vitro* (Schelbert et al. 2009).

Other findings based on the respective enzyme activities in broccoli also indicate that the removal of the central Mg<sup>2+</sup> atom (pheophytinization) may take place prior to the dephytylation step (Costa et al. 2005a; Aiamla-or et al. 2012; Gómez-Lobato et al. 2012c). Nevertheless, the involvement of chlorophyllase resulting in Chlide formation is still regarded as an important intermediate step on the chlorophyll degradation pathway by other authors (Aiamla-or et al. 2010; Kaosamphan et al. 2010).



**Fig. 6: Schematic diagram of early chlorophyll degradation modified after Heaton and Marangoni (1996).**

From a practical point of view, pigments such as chlorophyll and carotenoids determine the color of plants and plant organs because of their light absorbing properties (Schoefs 2002). Next to chlorophyll, carotenoids and flavonoids are the dominant pigments in broccoli (Cogo et al. 2011; Fernandez-Leon et al. 2012). Chlorophyll a and Chlide are characterized as blue green, Pheoin and Pheoide as olive brown (Heaton & Marangoni 1996; Weemaes et al. 1999a) or even yellow-brown (Schouten et al. 2009). Carotenoids are of a yellow–orange color due to their intense absorption in the 400–550 nm range (Scheer 2013). The colors of carotenoids range from light yellow (xanthophylls) to dark orange (carotenes). During the storage of broccoli florets, carotenoid contents usually remain relatively stable compared to other compounds, but may also decrease at higher storage temperatures (Kidmose & Hansen 1999; Cogo et al. 2011; Nath et al. 2011). For unknown reasons, different packaging material caused great differences in the retention of total carotenes during six days of storage at 5 °C in one study (Barth & Zhuang 1996). While fruits are ripening, e.g. tomatoes or apples, carotenoid content is increasing simultaneously to chlorophyll degradation (Khudairi 1972). Although some believe that a conversion from chloroplast to chromoplast is also taking place in broccoli while yellowing

(Suzuki et al. 2005), an accumulation of carotenoids in broccoli during storage has not been observed so far and was therefore not expected in the present work. According to the assumptions of Matile et al. (1999) regarding senescent leaves, sepal discoloration and subsequent yellowing of broccoli is the result of chlorophyll degradation, which unmasks the remaining carotenoids rather than a synthesis of new yellow components.

In this work, the focus was put on early reactions of chlorophyll degradation in order to investigate a possible indication of early senescence in broccoli through the occurrence of green chlorophyll catabolites. Nevertheless, carotenoids were identified simultaneously in order to relate the yellowing of broccoli to specific compounds.

In addition to the analysis of these compounds, the storability of broccoli was examined with the help of different non-destructive quality rating methods, which were compared to each other regarding their sensitivity. Storage was conducted under controlled conditions of temperature and humidity in order to induce senescence. Plant material was harvested from different planting sites of one commercial grower and two scientific institutions. Information on climate, fertilization and irrigation was gathered and matched using a database in order to model significant impacts on subsequent storability. In the following chapter, the methods and material used in this work are described in more detail.

## 4. Methods and materials

The investigations in the presented work were conducted according to three major approaches: In the first approach, examinations were conducted to compare optical measuring methods. In the second, influencing factors on quality were rated using information related to the cultivation of the broccoli curds. Third, features of the curd physiology and pigment decomposition during storage were evaluated.

### 4.1. Plant material

All studies were performed with broccoli that was freshly harvested at different planting sites in northern Germany (4.1.2). Directly after harvest, broccoli curds 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, the broccoli stems were cut 5 cm below the first branch and all leaves were removed. Then, the broccoli were stored in controlled storage chambers at 16°C for 4 days (4.1.3). Non-destructive, optical measurements were taken on a minimum of three days during storage (4.4 - 4.5). For this purpose, broccoli were removed from the storage chambers but were kept in closed insulated boxes. For pigment analyses, samples of two to three broccoli were removed on the respective storage days (4.6). More detailed information on the methods applied and the materials used is provided in the following subchapters.

#### 4.1.1. Broccoli harvest batches

Broccoli used for investigations was cultivated by one commercial vegetable producer (Behr AG) and two research institutions: Leibniz Universität Hannover (LUH) and Leibniz Institute of Vegetable and Ornamental Crops (IGZ). The institutions were part of the previously stated research framework. Broccoli was harvested during the years 2011 to 2013 at different planting sites of the cultivating institutions (4.1.2). Harvested batches were then used for multiple investigation purposes as indicated in Table 1. For all investigations, the broccoli variety 'Ironman' was used.

Depending on the harvest batch, a varying number of broccoli was harvested according to different capacities of the institution. Sample sizes are designated by investigation.



**Table 1: Overview of broccoli harvest batches used for various investigation purposes.**

Planting date <sup>2</sup>	Harvest date	Institution	Field	Investigation purpose				
				Cultivation alterations	Curd development	Comparison of optical measurements	Climate influences	Pigment analysis
03Jun2011	04Aug2011	Behr AG	Scharbow	-	x (+ bud size)			
17Mar2012	08Jun2012	Behr AG	Schwanneheide	-	x	x	x	x
17Apr2012	25Jun2012	LUH	Herrenhausen	Net cover	x	x	x	
04May2012	09Jul2012	Behr AG	Tüschow	Fertilizer application	x	x	x	x
22May2012	18Jul2012	IGZ	Großbeeren	Plastic film cover	x	x	x	
06Jul2012	30Aug2012	Behr AG	Schwanneheide	-	x	x	x	x
11Jul2012	11Sep2012	IGZ	Großbeeren	Plastic film cover	x	x	x	x
23Jul2012	24Sep2012	Behr AG	Kogel	-	x	x	x	x
04Jul2013	09Sep2013	Behr AG	Backhaus Wald	-		x (hyperspectral images)	x	
11Jul2013	26Sep2013	Behr AG	Kogel	-			x	

Harvested broccoli, in general, perfectly met the quality standards concerning shape, firmness and bud appearance following trade regulations (UNECE 2010), with one exception: Broccoli in the batch harvested on July 18<sup>th</sup>, 2012 did not meet the requirements for shape and color as they had elongated floral stems (Fig. 7 B). Broccoli from this batch was, however, integrated in the investigations, as the predetermination of product quality was a considerable part of the experimental approach. In this sense, the physiological quality defects represented a valuable source of information. As regards the comparison of non-destructive quality determination, however, this harvest batch should not be considered as being of marketable quality.

<sup>2</sup> Planting dates of broccoli may be delayed by one or two days for the broccoli batches of the commercial grower (Behr AG). This may have occurred when entire field plantings were not completed on the same day.



**Fig. 7: Broccoli fully meeting marketing standards of UNECE (2010) harvested at September 26<sup>th</sup>, 2013 (A) and broccoli of non-marketable quality harvested on July 18<sup>th</sup>, 2012 (B).**

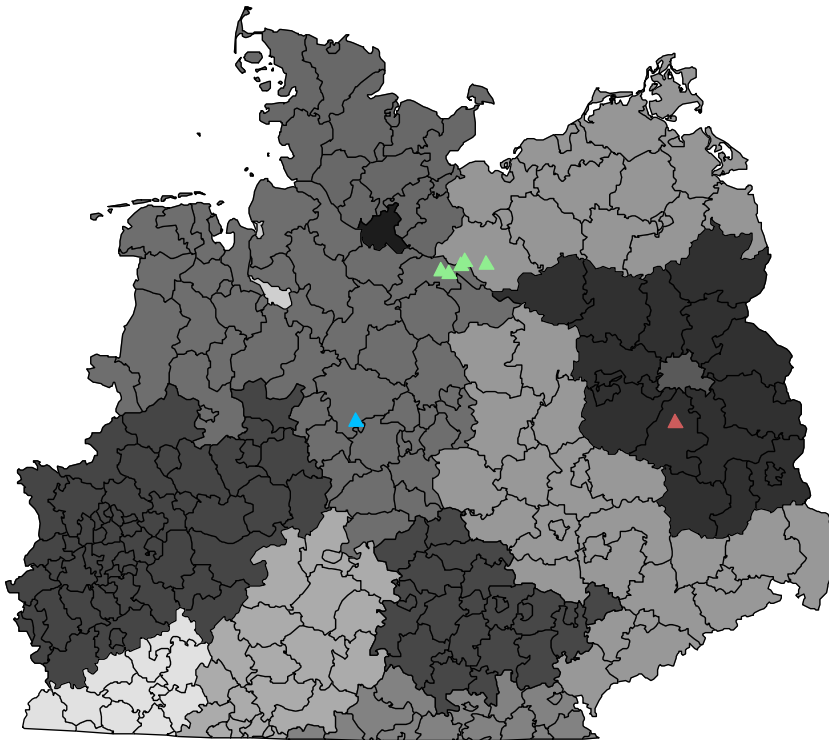
In some harvest batches, storage diseases occurred. When spots exceeded 1 cm<sup>2</sup>, the broccoli curd was discarded. In addition to the broccoli used in sampling for pigment analysis, this resulted in decreasing sample sizes during the course of storage.

#### 4.1.2. Experimental sites

Broccoli was grown on 7 experimental sites in northern Germany (Fig. 8). The commercial grower's fields were, in general, very large (27-37 ha), while research institutions provided small-scale trial plantations of 108 m<sup>2</sup> (LUH) and 240 m<sup>2</sup> (IGZ). Fertilization, irrigation and plant protection was applied by the growers according to common practice.

Climate data for all experimental sites during the growing seasons 2012 and 2013 were recorded from weather stations on an hourly basis at a height of 1.2 m. Weather stations from the two research institutes were located in the direct proximity of the fields. For the commercial grower, data from four weather stations located nearby was used. For the fields Schwanheide and Tüschow (Table 1), data from the two nearest weather stations was averaged.

Irrigation data was provided by IGZ and by Behr AG. Due to the significant size of the fields, irrigation data from Behr AG cover a time slot of 4 consecutive days, during which plants were irrigated with 18 mm. The data resolution for irrigation at IGZ is the respective irrigation day.

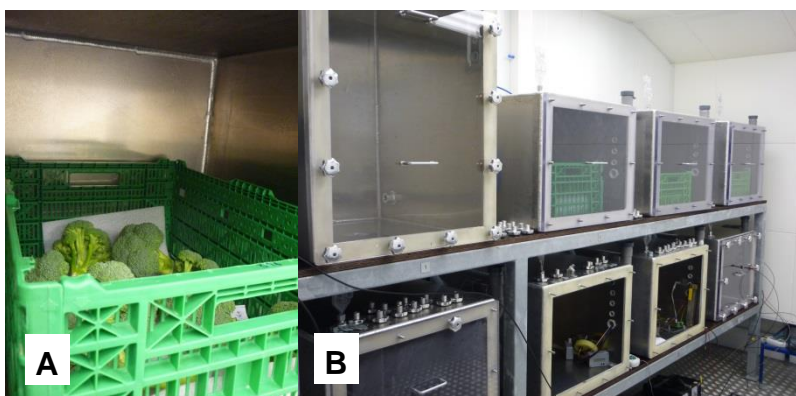


**Fig. 8: Location of trial fields in northern Germany. Colors of symbols indicate institution affiliation: green – Behr AG, red – Leibniz Institute of Vegetable and Ornamental Crops, blue – Leibniz Universität Hannover.**

#### 4.1.3. Storage conditions

To induce senescence, curds were stored in climate chambers at 16 °C and 95 % RH over 4 days. From 2012 on, storage was conducted in single chambers of storage rooms (Fig. 9, FRIGOTEC, Landsberg, Germany). Up to twelve broccoli were stored in a single chamber.

The storage temperature of 16°C was chosen based on the literature review and preliminary experiments. The aim was to choose a suitable storage temperature for differentiation of broccoli batches regarding their storability within a period of 4 days after harvest.



**Fig. 9: Storage of broccoli in a single chamber (A) of the storage room (B).**

Lids of single chambers were not fully closed, in order to allow air exchange between the chambers and storage rooms. Through this chamber storage, RH was kept high in order to prevent water loss in the broccoli. The selected storage intended to place the temperature as trigger for the induction of senescence. Based on daily weights starting from harvest day, broccoli lost 1.2 % of its weight per day on average.

Regarding the batch harvested in 2011, storage was done at 10 °C and high RH (> 95 %) for 8 days using a SB 222/300 climate chamber (Weiss Umwelttechnik GmbH, Reiskirchen-Lindenstruth, Germany) because the storage rooms (Fig. 9) were still under construction.

## 4.2. Cultivation factors

The influencing factors during the cultivation of broccoli on its subsequent quality and storability were investigated in cooperation with the cultivating institutions. The commercial grower had observed minor quality and storability changes when using high amounts of fertilizer. Therefore, fertilization was altered on one field following a practical approach (4.2.1). At IGZ, light qualities were varied during curd growth through the use of different plastic film covers (0). Investigations on cultivation protection nets against the pollen beetle *Meligethes aeneus* were conducted at LUH (4.2.3). In the present work, these variations in cultivation were investigated with regard to their impact on quality and storability. Details are explained in the following subchapters.

### 4.2.1. Fertilization

During cultivation, fertilizer was generally applied by the growers according to the nutrient demand of the broccoli, considering the respective soil properties. Amounts of fertilizer varied from 3.7 and 16.5 g nitrogen, 0 and 5.2 g phosphorus and 0 and 6.6 g potassium per plant. For one broccoli batch, which was cultivated from May 4<sup>th</sup> to July 9<sup>th</sup>, 2012, fertilizer application was varied by the commercial grower as indicated in Table 2. Accordingly, different quantities of nutrients were received by the single broccoli plant during cultivation. The level of nutrition elements was calculated based on information about fertilizer products applied by the grower.

**Table 2: Fertilizer application amounts on broccoli cultivated from May 4<sup>th</sup> to July 9<sup>th</sup>, 2012 (g/plant).**

	Nitrogen	Phosphorus (P <sub>2</sub> O <sub>5</sub> )	Potassium (K <sub>2</sub> O)
Best practice	9.4	2.8	4.8
Low fertilizer application	5.8	1.7	3.9
High fertilizer application	16.5	5.2	6.6

#### 4.2.2. Plastic film cover

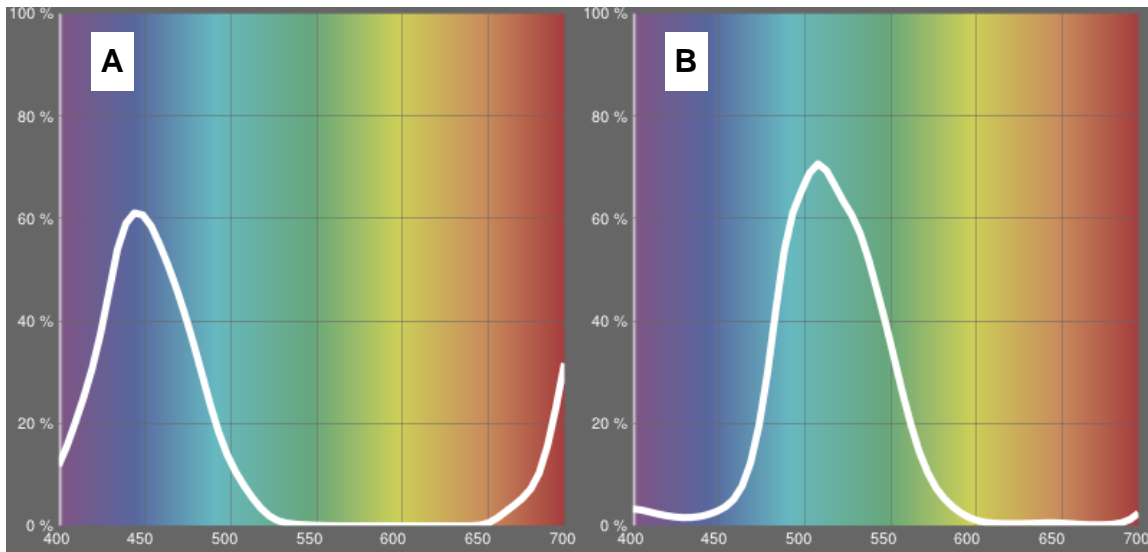
Broccoli curds cultivated by IGZ were covered with plastic film boxes (LEE filters, Andover, UK) in order to vary received light quality. Curds were grown under blue (Mikkel blue, No. 716) and green (Dark Green, No. 124) covers (Fig. 10, A). These films reduced irradiation and transmitted the visible electromagnetic spectrum selectively (Fig. 11).



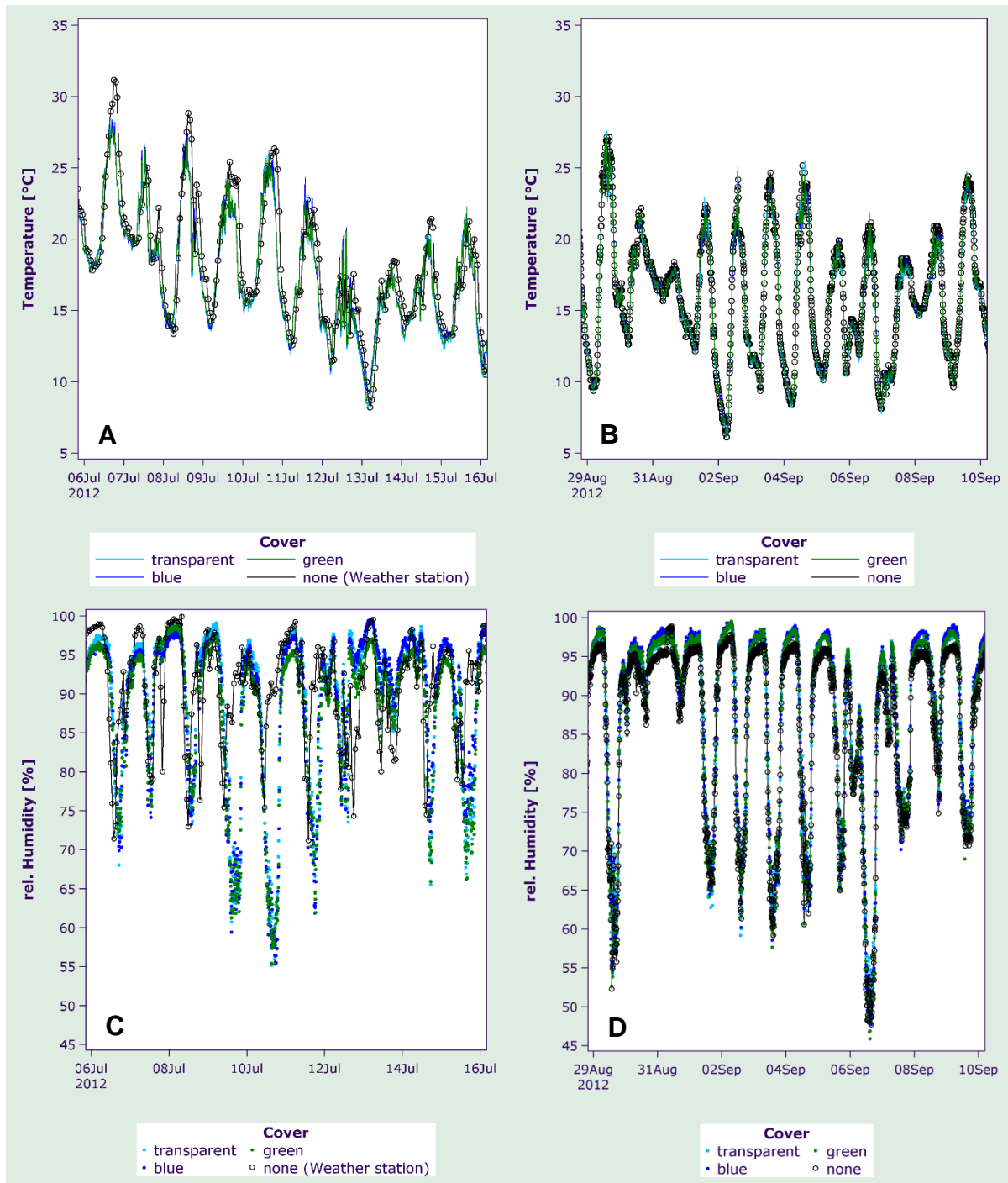
**Fig. 10: Plastic film covers placed on broccoli for variation of light quality (A) and fully transparent covers without light variation (B).**

Broccoli covered by boxes with fully transparent plastic film (Fig. 10, B) and uncovered broccoli were used as control in the investigations. Cover boxes were initially placed on broccoli plants when curds measured 5 cm in diameter and were used for 6 hours per day on sunny weekdays only. The trial followed a randomized block design with four repetitions per cover material.

Sensors (iButton, Maxim Integrated, San Jose, U.S.A) were used to monitor temperature and humidity under plastic film covers. In September, sensors were additionally placed in the uncovered crop stand. Readings from two sensors per cover type were averaged. Regarding RH, sensors occasionally recorded values higher than 100 %. Values were then corrected. In June, readings from sensors were compared to data of a weather station (Fig. 12 A+C). Temperature peaks recorded by the weather station were slightly higher than in the crop stand (Fig. 12 A). Temperature in the crop stand was identical under different cover types and without covering (Fig. 12 A+ B). The range of RH recorded by the weather station was narrower than that recorded by sensors in the crop stand (Fig. 12 C).



**Fig. 11: Light transmitted (%) by blue (A) and green (B) plastic film cover for each wavelength (LEE filters, Andover, UK).**



**Fig. 12: Climatic conditions under plastic film boxes placed on broccoli plants during curd growth. Temperature (A) and humidity (C) of a batch harvested on July 18<sup>th</sup> was compared to conditions recorded by a weather station. Temperature (B) and humidity (D) of a batch harvested on September 11<sup>th</sup> was compared to conditions recorded in the plant stand without plastic film cover.**

This was possibly due to a different principle of measurement, because the humidity in crop stands is generally expected to be higher than in the atmosphere. RH measured using iButton sensors in the uncovered crop stand peaked slightly lower than under plastic film covers (Fig. 12 D). Nevertheless, differences were found to be negligible and it was therefore assumed that temperature and humidity were not significantly altered by colored or transparent plastic film covers.

#### 4.2.3. Net cover

Transparent cultivation protection nets against *M.aeneus* made of polyethylene with 0.8 x 0.8 mesh size (HADI, Marschacht, Germany) were placed on the broccoli plants grown at LUH. They were covered for either the last two or three weeks before harvest. Additionally, all plants were covered by a net with wider margins to protect against bird and rabbit damage. Different coverage durations were carried out in a randomized block design. No differences were found in the photosynthetic active radiation (PAR) measured under different net covers. However, PAR under covers was significantly different from PAR without any cover (for more information, see Klinzmann & Voigt 2013).

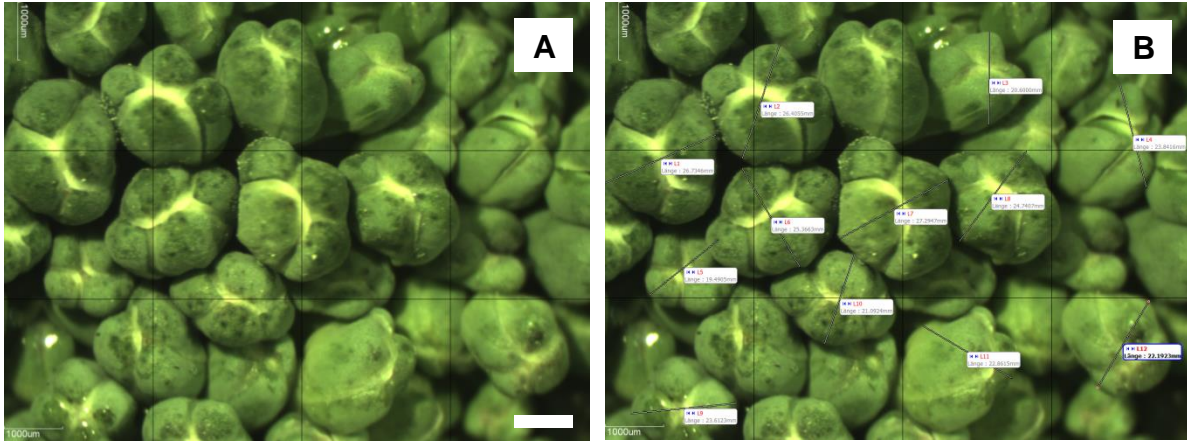
### 4.3. Maturity stages

As explained in chapter 3.1.5, broccoli curds are inflorescences at bud stage. The goal was to analyze their generative development and to relate it to subsequent storability. Two different methodological approaches were used. In 2011, microscopically recorded images of the curd were analyzed in one harvest batch (4.3.1). During the subsequent investigations in 2012, diameter and circumference of curds were measured after harvest (4.3.2).

#### 4.3.1. Bud size determination

One broccoli batch used for bud size determination was harvested on August 4<sup>th</sup>, 2011 on a field from the commercial grower (Table 1). The whole crop stand had been planted two months earlier, however, slight variations related to plant development and curd size were observed on different allotments of the field. Therefore, curds were labeled differently according to the field allotment from which they were harvested. In order to determine the individual bud diameter, photographs of broccoli florets were taken using a Moticam 2500 digital microscope camera (Motic, Hong Kong, China) in combination with a SM 33 microscope (Helmut Hund GmbH, Wetzlar, Germany). Bud diameter was measured using the software Motic images plus 2.0 as shown in Fig. 13 B. Twelve buds were measured in one microscopic image following the grid that divided it into twelve sectors (Fig. 13). Five images of each broccoli curd were recorded, two in the curd center and three at arbitrary chosen areas of the outer edge. In sum, 60 buds represented the developmental stage of a single broccoli curd.





**Fig. 13: Microscopic image of a broccoli curd harvested on August 4<sup>th</sup>, 2011 (A) and analysis of bud diameters (B). The white bar in (A) represents 1 mm length in the focal plane.**

#### 4.3.2. Curd size determination

The diameter and circumference of broccoli curds were recorded using a folding ruler and a tape measure, respectively. Measurements were done with an accuracy of 0.5 cm. Diameter was measured at the distance of highest expansion. Because the circumference was sufficiently correlated with diameter ( $r = 0.84$ ) and, furthermore, is used as an indicator of developmental stages in many existing works (3.1.5), only diameter was considered in further analysis of curd development.

### 4.4. Reflection measurements

Three different approaches based on reflection in the visible (VIS) and near infrared (NIR) electromagnetic wavelength range were analyzed regarding their suitability for the quality evaluation of broccoli. Color determination is widely applied in academia (see 3.2.1) and, therefore, is referred to as a reference measurement (4.4.1). Spot measurements of diffuse reflection were conducted using an OEM spectrometer (Fig. 14). Using hyperspectral imagery, reflection patterns within single broccoli curds were analyzed (4.4.3).

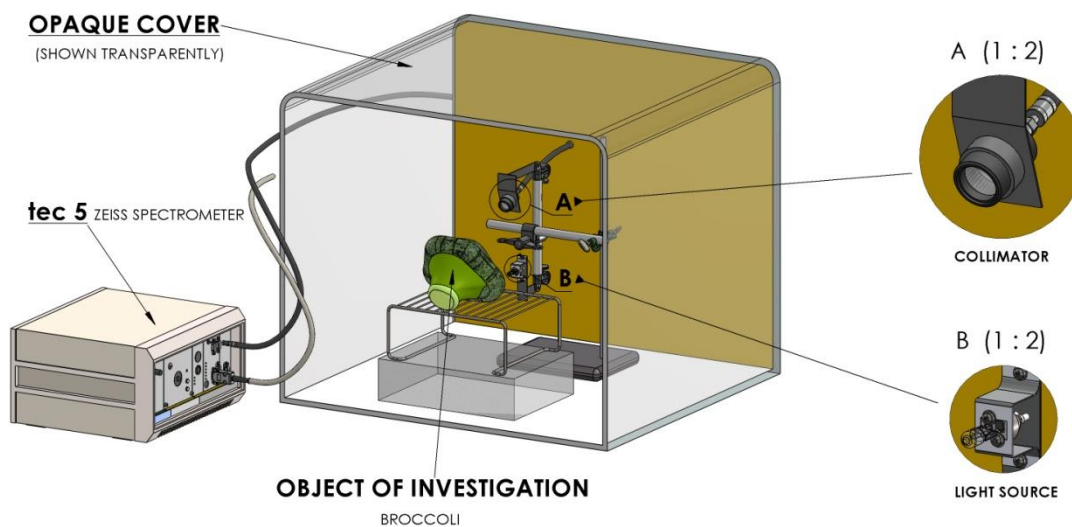
White adjustment of both the spectrometer and the hyperspectral camera system was done with the help of a spectralon standard (Zeutec, Rendsburg, Germany).

#### 4.4.1. Color determination

The surface color of broccoli curds was measured using a CM-2600d spectrophotometer (Konica Minolta Sensing Inc., Tokyo, Japan). Six superficial color measurements were conducted randomly on the broccoli curd and analyzed as parameters of the CIELAB ( $L^*$ ,  $a^*$ ,  $b^*$ , hue angle and chroma). For the calculation of color parameters (3.2.1.1), the spectrum of CIE Standard Illuminant D65 was used. Black/white calibration of the device was done using the supplementary device equipment.

#### 4.4.2. Spot measurements of reflection

Diffuse reflectance of broccoli was measured on individual spots of 2.5 cm<sup>2</sup> 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. 14). Broccoli curds were placed at a distance of 10 cm to a collimator, which narrowed the derived reflection signal from the measuring spot. For 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.



**Fig. 14: Schematic diagram of spot reflection measurements of broccoli curds.**

#### 4.4.3. Hyperspectral images

Hyperspectral images were recorded according to the methodology proposed by Bauriegel et al. (2011a) in the range of 400 to 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 (Pixelflyqe, PCO AG, Kelheim, Germany). Curds were illuminated with a stabilized halogen lamp (150 W) and were recorded from a 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 with hyperspectral images. The control of the camera system as well as black/white calibration was accomplished using LabView 8.2 (National Instruments Corporation, Austin, TX, USA). The spatial resolution of images was 696 x 512 pixels.

### 4.5. Chlorophyll fluorescence measurements

Measurements of chlorophyll fluorescence were conducted to assess maximum photochemical efficiency ( $F_v/F_m$ ) and the portion of it that is effective under illuminated

conditions, commonly referred to as photosynthetic yield ( $\Phi_{\text{PSII}}$ ,  $(F_m' - F_t)/F_m'$ ) (Nebdal et al. 2000). Chlorophyll fluorescence of two broccoli batches harvested in June 2012 was analyzed using a fluorescence camera imaging system (4.5.1). The remaining five batches in 2012 were assessed using spot measuring devices (4.5.2).

#### 4.5.1. Fluorescence imaging

Grayscale images of fluorescence emitted by broccoli curds were recorded using a modular FluorCAM 690MF camera system (PSI, Brno, Czech Republic) as described in (Bauriegel et al. 2011b) and Baier et al. (2013). Measurements were performed on dark adapted broccoli curds taken from storage rooms (4.1.3) following a template with user-defined timing of set points. A filter set enabled the detection of fluorescence in the range of 695 to 780 nm. A non-actinic measuring light from orange light emitting diodes (LED) ( $\lambda_{\text{max}} = 620 \text{ nm}$ ) induced basic fluorescence ( $F_0$ ). After a saturation pulse from a 250 W halogen lamp, maximum fluorescence ( $F_m$ ) was recorded. Actinic, non-saturating irradiation from LED-generated, steady-state photosynthetic conditions provided the parameter  $F_t$ . Single saturation pulses led to maximum fluorescence in light ( $F_m'$ ). Maximum photochemical efficiency  $F_v/F_m$  with  $F_v = F_m - F_0$  and effective quantum yield of photochemical energy conversion in PSII  $\Phi_{\text{PSII}} = (F_m' - F_t)/F_m'$  were calculated from the fluorescence signals.

#### 4.5.2. Fluorescence spot measurements

Fluorescence parameters of dark adapted broccoli curds ( $F_0$ ,  $F_m$ ) were measured using a Pam 2000 device (Heinz Walz GmbH, Effeltrich, Germany). For fluorescence parameters in simulated daylight conditions ( $F_m'$ ,  $F_t$ ), broccoli were illuminated for at least 4 min by LED-Panels (SL 3500-A, PSI, Brno, Czech Republic), which emitted a cool white color spectrum. A Mini-Pam device (Heinz Walz GmbH, Effeltrich, Germany) was used for the measurement of light-induced fluorescence signals. The PAR striking the broccoli surface during light-adaptation and subsequent measurement was 220 to 230  $\mu\text{mol m}^{-2} \text{s}^{-1}$  according to a Cosine Corrected Mini Quantum Sensor MQS-B (Heinz Walz GmbH, Effeltrich, Germany). A distance piece was used for the uniform performance of both measurements. Three measurements were conducted on each broccoli curd for fluorescence, both in dark and light conditions. In August and late September the Mini-Pam device was not available. Therefore, no light measurements were conducted on the broccoli batch harvested on August 30<sup>th</sup>, 2012. For the broccoli batch harvested on September 24<sup>th</sup>, the Pam 2000 device was used alternately for dark and light measurements of curds.

### 4.6. Pigment analysis

Broccoli curds from five harvest batches were used for pigment analysis (Table 1). In June and early September, two curds were sampled per storage day. Three curds per

storage day were sampled in the remaining harvest batches. Bud material was cut from the broccoli surface. Two samples of 1 g each per broccoli curd were used for repeat determination. Samples were then frozen in liquid nitrogen.

For extraction, frozen broccoli samples were ground in a buffered acetone solution following the method of Niinemets et al. (1998). Samples were then homogenized in 10 ml 70 % buffered acetone solution. Tris (30 mMol) was used as a buffer. Three further extraction steps were conducted, resulting in an 80 % acetone solution of chlorophyll and carotenoid pigments.

For the determination of pigments, a liquid chromatograph (Shimadzu, Kyoto, Japan) was used. Chlorophyll *b* and carotenoids were detected by a SPD-M20A UV/VIS photodiode array detector. For the detection of chlorophyll *a* and chlorophyll derivatives, a RF-20A xs fluorescence detector at excitation/emission wavelengths of 420/675 nm and 680/720 nm was used. The influence of harvest date and storage day on concentrations of chlorophylls and derivatives was calculated by means of analysis of variance (4.7.4).

Samples from the same broccoli curd were extracted and measured by HPLC separately on different days. The concentrations of pigments determined in the two samples of a broccoli curd were then averaged. A prerequisite for using the average was that deviation between the two samples of the same curd did not exceed 15 % of the minimum value of the respective pigment concentration. Through this double checking process, the accuracy of the results gained from the newly established HPLC method was ensured.

The deviation between samples of single broccoli curds harvested on June 8<sup>th</sup>, 2012, did exceed 15 %. Therefore, only one pigment concentration for each broccoli curd was included in further analysis. It was chosen for the samples that were only deviating by 5 % from the general value-range of the pigment concentrations in other batches.

#### **4.7. Data processing**

Methods of data analysis were chosen according to the respective investigation purpose (Table 1). Analysis of variance and multiple pair wise comparisons were used to evaluate the influence of both storage and cultivation alterations on optical measuring methods and pigment content (4.7.4). Pearson's correlation coefficient was used to determine the linear dependence between different optical measuring methods as well as between curd diameter and color of broccoli, measured as hue angle. To rate curd development, frequency distributions of bud size classes were displayed. For the analysis of spectral data, pre-treatment steps were conducted (4.7.2). Then, data were categorized by principal component analysis (PCA) (4.7.3).

##### **4.7.1. Rating of storability**

Analysis of variance (ANOVA) and multiple pair-wise comparisons were conducted using SAS 9.3 (SAS Institute Inc., Cary, USA). Influences of both cultivation factors (4.2) and

postharvest storage days at 16 °C and 95 % RH (4.1.3) were evaluated in one statistical model using the statistical procedure "PROC MIXED". This procedure was the most suitable for ANOVA because repeated measurements were taken on the same broccoli curds during storage.

Considering the repeated measurements on each broccoli curd, it was taken into account that measurements were correlated to

$$\sigma^2\rho t \quad (7)$$

where  $\sigma^2$  was the variance within the curd,  $\rho$  was the covariance parameter that should be estimated, and  $t$  was the absolute difference between the days of measurements.

For each quality trait that was examined (optical indexes, pigment concentrations) a statistical model was set up which assumed that the respective quality trait depended on both pre- and postharvest influences. The model was set up with the following dependencies:

$$q = \mu + h_{\text{batch}} + \text{store}_{\text{day}} + \text{fert} + \text{film}_{\text{cover}} + \text{net}_{\text{cover}} + h_{\text{batch}} \times \text{store}_{\text{day}} + \text{fert} \times \text{store}_{\text{day}} + \text{film}_{\text{cover}} \times \text{store}_{\text{day}} + \text{net}_{\text{cover}} \times \text{store}_{\text{day}} \quad (8)$$

where the dependent variable  $q$  was an examined quality trait and  $\mu$  was the estimated intercept.  $h_{\text{batch}}$  referred to the respective broccoli batch.

The variables  $\text{fert}$ ,  $\text{film}_{\text{cover}}$  and  $\text{net}_{\text{cover}}$  indicated the cultivation influences of fertilization (4.2.1), plastic film covers (4.2.2) and cultivation protection nets (4.2.3), respectively. The factor  $\text{store}_{\text{day}}$  denoted the respective day of storage (harvest day = day 0) and was additionally tested as interaction factor via multiplication with the other factors (8). Factors that were estimated to have a significant influence on the quality trait by hypothesis tests (F-tests, significance level  $\alpha = 0.05$ ) were further evaluated using multiple pairwise comparisons (permutative tests, significance level  $\alpha = 0.05$ ) conducted by the LSMestimate statement of SAS software. Least square means of quality traits were compared both with respect to a different amount of storage days in the same harvest batch and on the same amount of storage days in different harvest batches.

#### 4.7.2. Pre-treatment of reflection spectra

Spectra of spot measurements were normalized above 450 nm by min-max-normalization as follows:

$$R_{i\_norm} = \frac{(R_i - R_{MIN})}{(R_{MAX} - R_{MIN})} \quad (9)$$

where  $R_i$  is a measured value of reflection intensity,  $R_{i\_norm}$  is the normalized value, and  $R_{MIN}$  and  $R_{MAX}$  are the reflection intensities at the lowest and highest value on the spectrum, respectively.

For hyperspectral image analysis, masks were created to distinguish the curd from the background. All spectra of pixels within the masked area were normalized by equal area normalization as follows:

$$\sum_{i=R_1}^{R_{512}} i = 100\,000 \quad (10)$$

where  $R_1$  was the reflection intensity at camera band 1. For presentation purposes, image data were randomly reduced to 1 %.

#### 4.7.3. Analysis of reflection spectra

In order to identify changes in spectral reflectance related to quality changes in broccoli, PCA was conducted on the normalized reflection spectra of broccoli in the range of 450 to 1000 nm. All recorded spectra of broccoli harvested during the 2012 growing season and measured during 4 days of storage were used (N = 4305). First and second derivations of spectra from spot measurements were calculated by means of data base query on Microsoft SQL Server. Using Java programming language, normalized spectra and derivatives were employed as input variables of PCA, so that principal components (PCs) represented typical spectral patterns.

Concerning hyperspectral images, PCA was conducted in order to reduce redundant information. Here, normalized reflection intensities at camera bands 50-350 (wavelengths 441 to 806 nm) were employed as input variables of PCA. In order to grade differences in reflection within single broccoli curds during storage, classification procedures (Iso-Data, Fuzzy-c-means) were conducted on hyperspectral images, as well as on their first three PCs. Data processing of hyperspectral images including PCA, Euclidean distances and composite images was conducted using a software tool written in Java.

#### 4.7.4. Identification of external impacts on quality

Data collected from investigations including climate data, cultivation influences and quality parameters were used to establish a Microsoft SQL Server database. With the help of data base queries, influences on broccoli quality and storability were systematically examined. Selection for relevant quality influences was done based on literature review (3.1). Using SQL, individual events, e.g. specific climatic conditions, were counted (Fig. 15). Data base query was applied for cultivation periods that are known from the literature to be susceptible to the climate regarding their subsequent quality.

```
select
COUNT(DATEDIFF(hour, c.timestamp, u.harvest_date))
from unit u
WHERE
c.temperature > 25
AND DATEDIFF(day, c.timestamp, u.harvest_date) between 16 and 24
AND c.timestamp between u.planting_start AND u.harvest_date
GROUP BY u.id, ms.name
```

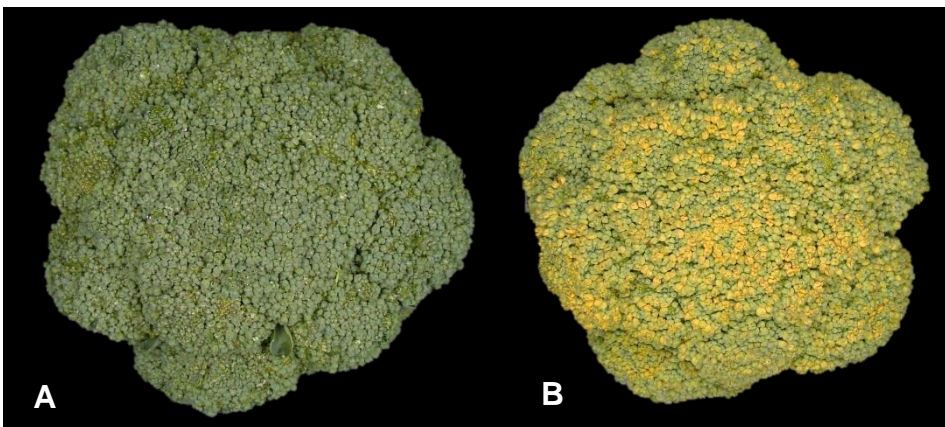
**Fig. 15: Example of SQL data base query: Hours with temperatures higher than 25 °C, occurring during a period of 16 to 24 days before harvest, were counted in the existing climate data (c.) for each harvest batch (u.).**

## 5. Results

In order to rate the postharvest quality of broccoli, different optical measuring systems were used during four days of storage at 16 °C and high RH (~95 %), as previously described (4.1.3). This storage method will be referred to as “defined storage” (DS) in the results chapter. Non-destructive optical methods are compared regarding both their sensitivity towards storage conditions and the reliability of their value levels.

### 5.1. Optical methods for quality rating

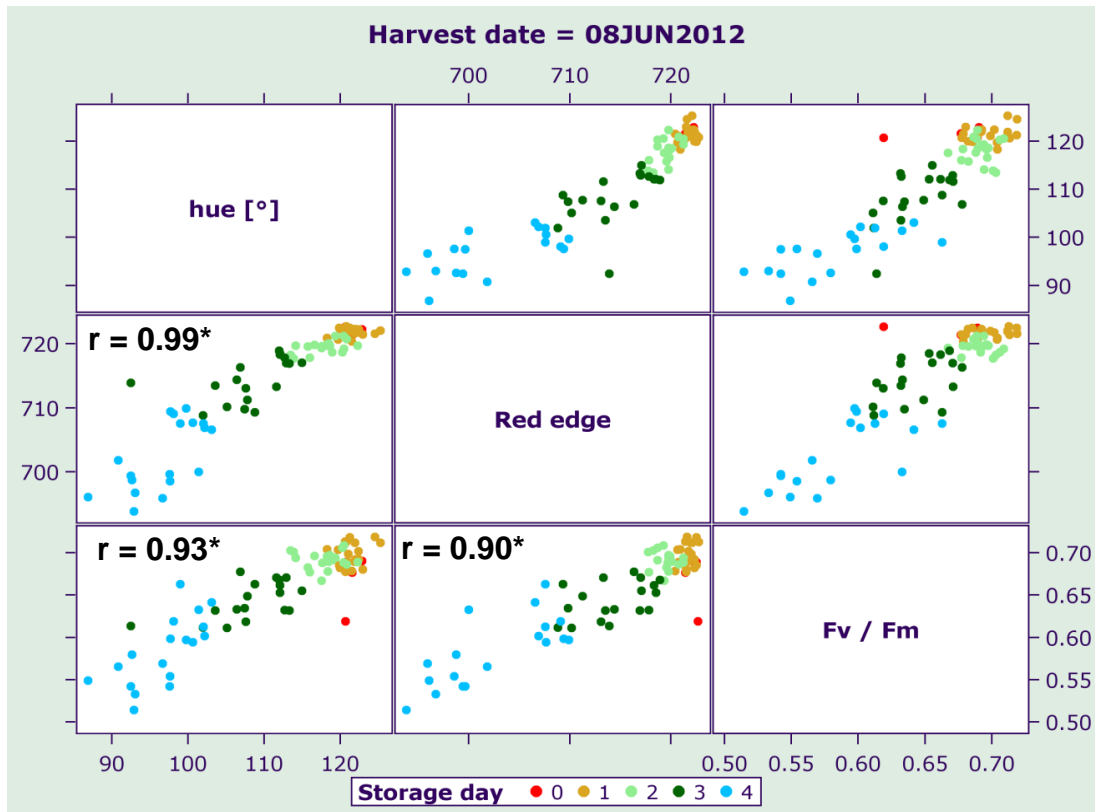
The outputs of different non-destructive optical measuring systems for two harvest batches were plotted against each other in a correlation matrix. These two batches represented broccoli with minor storability (June 8<sup>th</sup>, 2012; Fig. 16) and broccoli with only moderate changes during DS (June 25<sup>th</sup>, 2012). One parameter from each optical measuring system has been used: Hue, calculated from CIELAB readings, REIP of diffuse reflection spectra and Fv/Fm of chlorophyll fluorescence imaging.



**Fig. 16: Broccoli harvested on June 8<sup>th</sup>, 2012 at harvest day (A) and after 3 days of storage (B) at 16 °C and high RH (~95 %).**

The harvest batch from June 8<sup>th</sup>, which changed visual appearance during DS (Fig. 16) showed very high correlation coefficients of readings from the three measuring systems when all four storage days were considered together in one approach (Fig. 17). Pearson correlation coefficients calculated for each day individually increased during storage (Table 3). This indicated that progression in quality decline leads to a close relationship between non-destructive measuring methods.



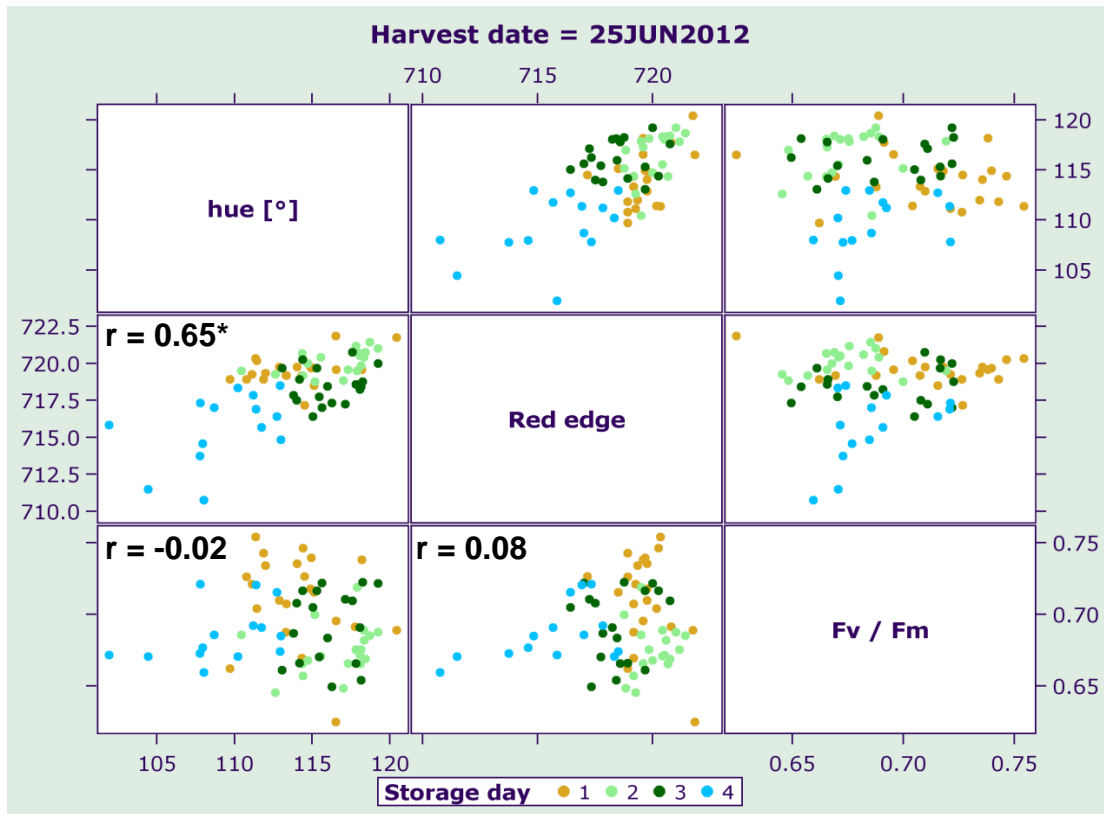


**Fig. 17:** Correlation matrix of non-destructive optical parameters representing the output of CIELAB (hue angle), of reflection spectra (Red edge inflection point) and fluorescence imaging (Fv/Fm). Broccoli was harvested on June 8<sup>th</sup>, 2012 and stored for 4 days at 16 °C and high RH (~95 %). Asterisks indicate significant Pearson correlations.

**Table 3: Pearson correlation coefficients of non-destructive optical parameters representing the output of the CIELAB (hue), reflection spectra (Red edge) and fluorescence imaging (Fv/Fm) measured over 4 days of storage at 16 °C and high RH (~95 %) on two broccoli harvest batches. Asterisks indicate significance.**

storage day	Harvest date = 08.06.2012			Harvest date = 25.06.2012			
	N	hue	Red edge	N	hue	Red edge	
1	19	hue	1	22	hue	1	
		Red edge	0.18		1	Red edge	0.47*
		Fv/Fm	0.23		0.22	Fv/Fm	-0.21
2	19	hue	1	19	hue	1	
		Red edge	0.53*		1	Red edge	0.53*
		Fv/Fm	0.00		-0.15	Fv/Fm	0.26
3	18	hue	1	18	hue	1	
		Red edge	0.52*		1	Red edge	0.14
		Fv/Fm	0.59*		0.46*	Fv/Fm	0.15
4	18	hue	1	14	hue	1	
		Red edge	0.66*		1	Red edge	0.11
		Fv/Fm	0.70*		0.69*	Fv/Fm	0.19

The harvest batch of June 25<sup>th</sup> showed only moderate visual changes during DS. In a correlation matrix, optical indexes showed weak correlations (Fig. 18). In this case, the range of readings was much smaller than in batches with obvious quality decrease, as can be seen in the axes of Fig. 18. Hue and REIP were correlated on the first two storage days, whereas maximum photochemical efficiency was not correlated to other optical parameters (Fig. 18; Table 3).



**Fig. 18: Correlation matrix of non-destructive optical parameters representing the output of CIELAB (hue angle), of reflection spectra (Red edge inflection point) and fluorescence imaging (Fv/Fm). Broccoli harvested on June 25<sup>th</sup>, 2012 and stored for 4 days at 16 °C and high RH (~95 %). Asterisk indicates significant Pearson correlations.**

In order to rate the sensitivity of particular measuring systems towards changes of broccoli curds caused by quality losses during DS, parameters were examined more in detail.

### 5.1.1. Color determination (CIELAB)

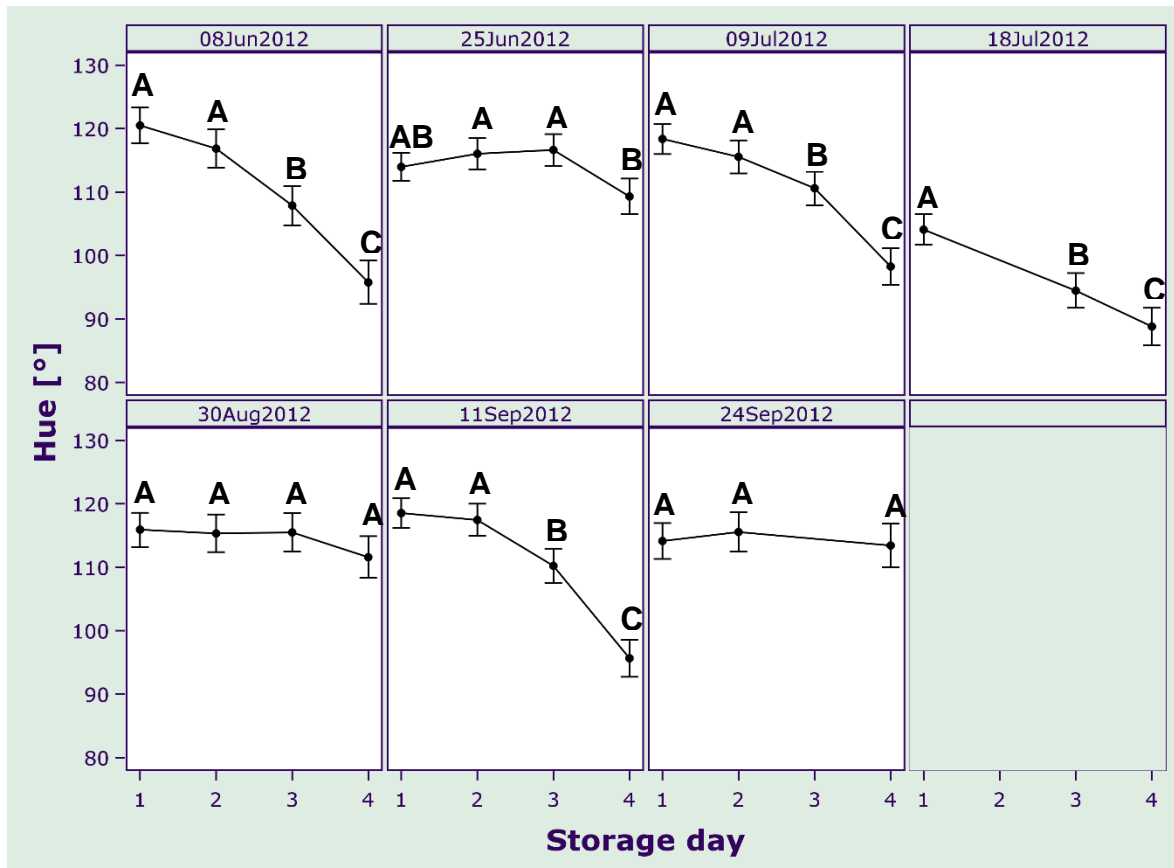
To facilitate a better understanding and comparison of CIELAB values, an example of a single broccoli curd during its color transition is given in Table 4. No subjective quality scores have been used in this work, therefore, color measurements function as a reference for quality condition. CIELAB has been established by CIE in order to simulate the perception of the human eye (see also 3.2.1.1).

**Table 4: CIELAB values of a single broccoli harvested on August 4<sup>th</sup>, 2011 and stored for 8 days at 10 °C and high RH (~95 %).**



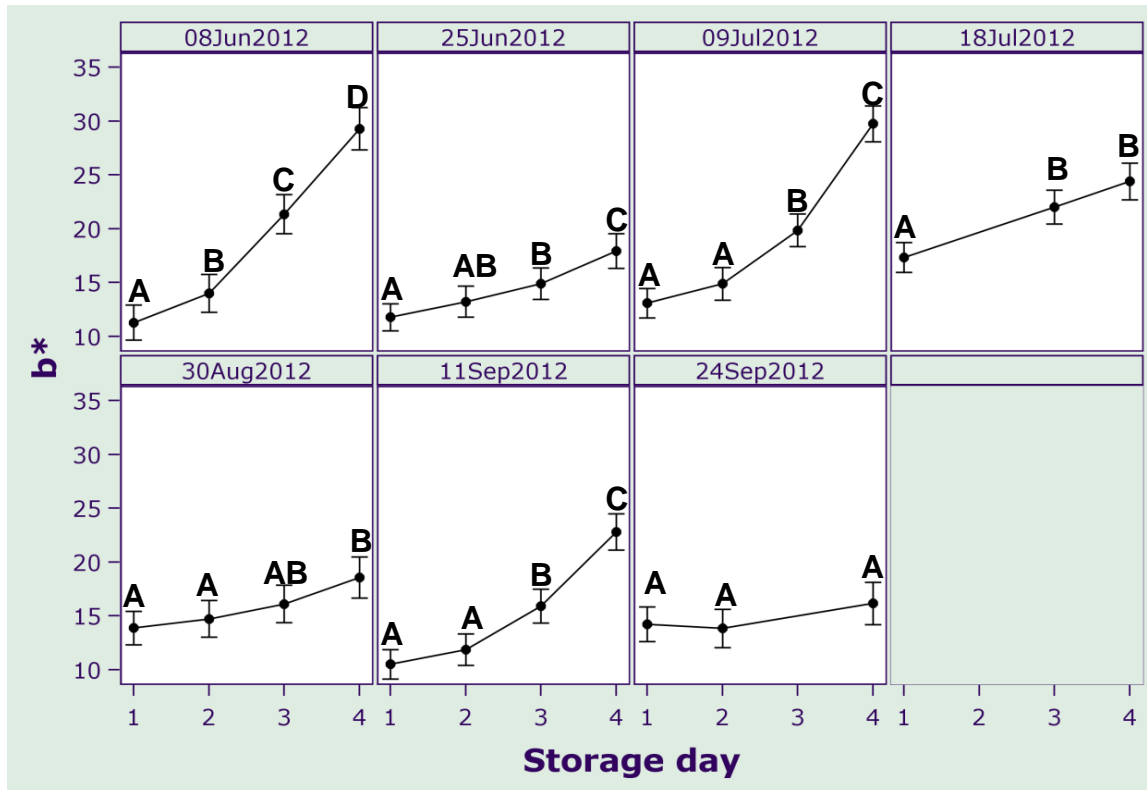
<b>L*</b>	38,85	44,82	49,22	51,49
<b>a*</b>	-7,37	-6,33	-5,51	-3,88
<b>b*</b>	15,07	12,93	17,84	22,84
<b>hue [°]</b>	116,14	116,25	107,72	99,39
<b>C*</b>	16,78	14,41	18,78	23,29

During DS, the mean hue angle values of five out of seven broccoli batches harvested throughout the year 2012 declined significantly (Fig. 19). Yet, in the batch from June 25<sup>th</sup>, the decline was not constant: The hue angle values of broccoli on day 1 did not differ from the other days, whereas values on storage day 2 and 3 were significantly different from storage day 4 (Fig. 19). Broccoli curds harvested on August 30<sup>th</sup> and September 24<sup>th</sup> showed no significant changes in hue angle during DS, therefore maintaining the overall broccoli color (Fig. 19). The first significant changes in hue angle were observed on day 3, although changes in the broccoli harvested on July 18<sup>th</sup> are likely to have already occurred earlier (Fig. 19).



**Fig. 19:** Hue angle (mean  $\pm$  confidence interval) of seven broccoli batches harvested throughout the 2012 growing season. Storage took place at 16 °C and high RH (~95%). Different letters indicate significant differences within one broccoli batch. Hue angle: 90° = yellow, 180° = bluish-green.

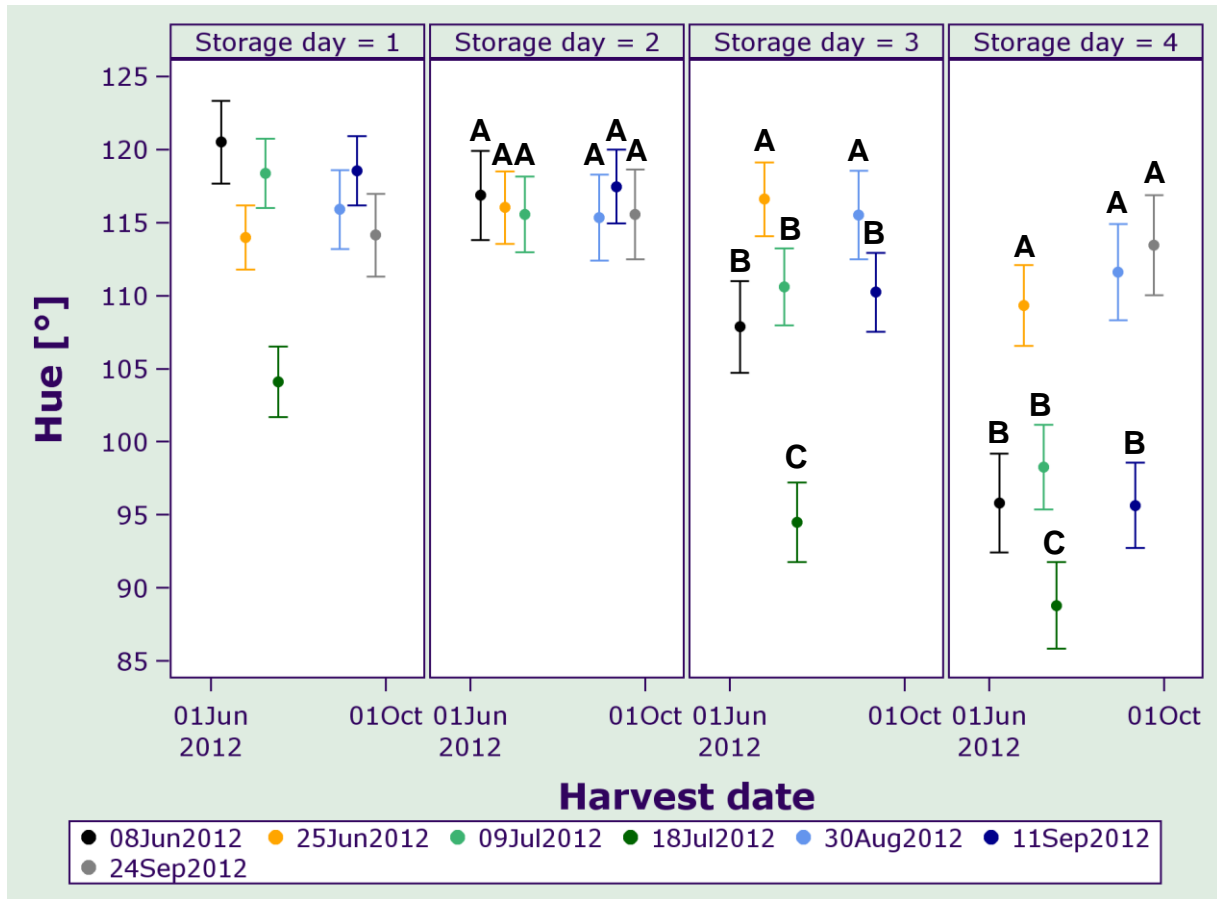
As explained in chapter 3.2.1.1, hue is a function of the color indexes  $a^*$  and  $b^*$ . In this work,  $b^*$  reacted faster to color changes during DS than hue (Fig. 20). The  $b^*$  value indicates color intensity on a blue to yellow scale. First changes in  $b^*$  were observed on day 2 in broccoli harvested on June 8<sup>th</sup> (Fig. 20). On day 3 of storage, 5 out of seven harvested batches indicated significant differences in  $b^*$ , whereas only 4 batches had changed regarding hue (Fig. 19 & Fig. 20). Additionally, in contrast to hue, the  $b^*$  values of broccoli harvested on August 30<sup>th</sup> changed significantly during DS, increasing at a constant rate (Fig. 20). On the other hand, broccoli harvested on July 18<sup>th</sup> did not show differences in  $b^*$  values from day 3 to 4, as was the case with hue angle values (Fig. 19 & Fig. 20).



**Fig. 20: Parameter  $b^*$  (mean  $\pm$  confidence interval) of seven broccoli batches harvested throughout the 2012 growing season. Storage took place at 16 °C and high RH (~95 %). Different letters indicate significant differences within one batch.**

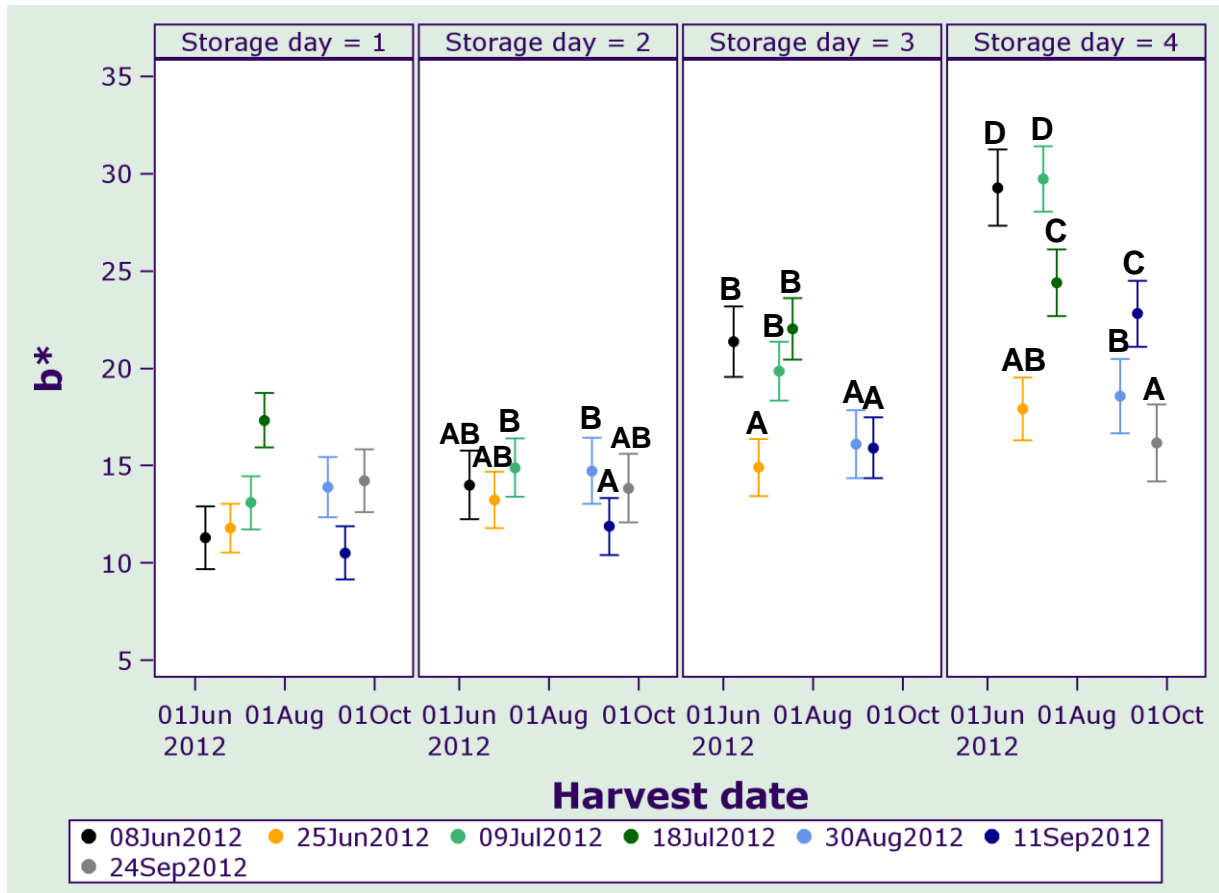
Both hue and  $b^*$  values of different harvest batches were compared among individual storage days with multiple pair-wise comparisons (Fig. 21 & Fig. 22). Values of hue were all within the same range in the first two days of storage, except for broccoli harvested on July 18<sup>th</sup>, which did not meet the high quality standards expected in the market (Fig. 21). The initial value range of the remaining batches over the first days was so narrow, that no consistent statistical subgroups of color could be formed through the statistics applied, even though some individual statistical differences were detected between single batches on day 1 (Fig. 22).

After 3 days, due to color changes, broccoli batches were divided into three homogeneous subgroups of hue values (Fig. 21). Those three groups were maintained on day 4 of storage (Fig. 21). Broccoli harvested on July 18<sup>th</sup> showed mean hue values of 104.1° on day 1 of DS and continued to display the lowest hue values of all broccoli batches (Fig. 21).



**Fig. 21: Hue angle (mean  $\pm$  confidence interval) of broccoli batches on 4 days of storage at 16 °C and high RH (~95 %). Batches were harvested throughout the 2012 growing season. Different letters indicate significant differences between broccoli batches on single storage days. On day 1, only the broccoli harvested on July 18<sup>th</sup> formed an independent statistical subgroup.**

The  $b^*$  values of six broccoli batches were in a similar range on the second day of DS, although the  $b^*$  values of harvest batch from September 11<sup>th</sup> were lower than those of the batches harvested on July 9<sup>th</sup> and August 30<sup>th</sup> (Fig. 22). On day 3, the values formed two statistical subgroups (Fig. 22). The  $b^*$  values of broccoli curds harvested on July 18<sup>th</sup> that were not of marketable quality did not differ from those harvested on June 8<sup>th</sup> or July 9<sup>th</sup> on day 3 (Fig. 22). After four days of storage, the  $b^*$  values of broccoli were divided into four subgroups (Fig. 22). The broccoli from July 18<sup>th</sup> was then in the middle of the range of  $b^*$  values (Fig. 22).



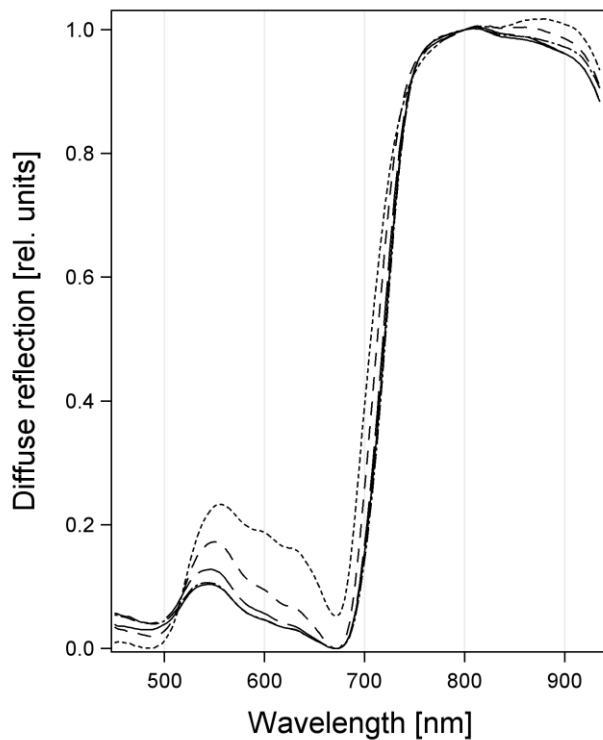
**Fig. 22:**  $b^*$  (mean  $\pm$  confidence interval) of broccoli batches on 4 days of storage at 16°C and high RH (~95%). Batches were harvested throughout the 2012 growing season. Different letters indicate significant differences between broccoli batches on single storage days. On day 1, only the broccoli harvested on July 18<sup>th</sup> formed an independent statistical subgroup.

### 5.1.2. Reflection spectra

Regarding the reflection spectra of broccoli curds in the range of 450 to 680 nm, an overall shift of spectra towards a higher reflection intensity was observed during storage (Fig. 29). All spectra showed a peak at 550 nm and a steep rise in reflectance between 680 and 750 nm (Fig. 23).

The PCA of all recorded reflection spectra in the 2012 growing season (N = 4305) indicated that 99.7 % of variance was explained by the first PC (Table 5). Using the original spectra for PCA, no change in the characteristics of spectral regions could be demonstrated. Accordingly, the first PC represented the typical shape of the original recorded spectra (Fig. 24).





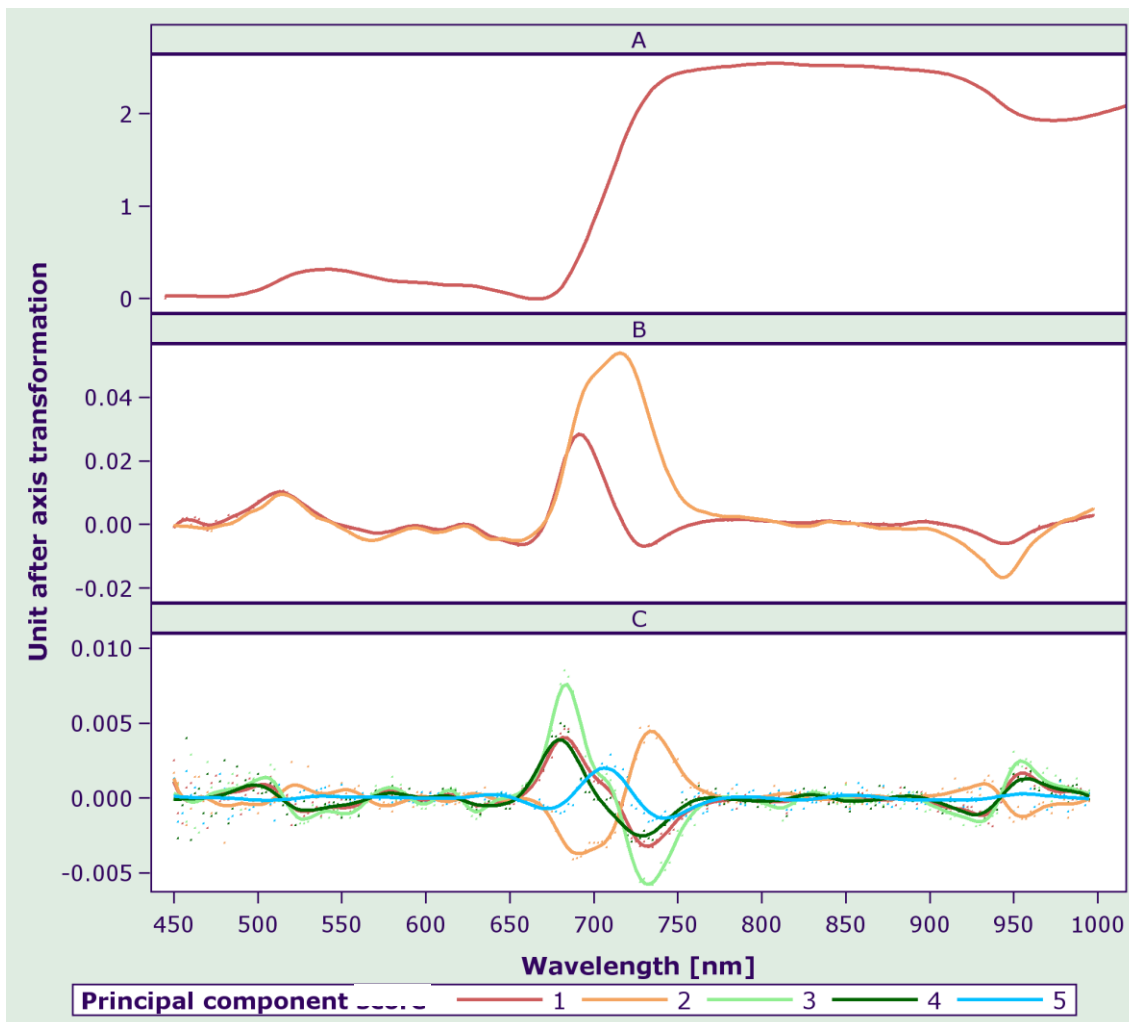
**Fig. 23: Normalized reflection spectra of one broccoli curd on harvest day (June 8th, 2012) to day 4 of storage at 16 °C and high RH (~95 %). Line patterns: harvest day – solid, storage day 1 – dash dot dash (hidden), day 2 – long dash, day 3 – medium dash, day 4 – short dash.**

PCA of the first and second derivative of spectra pointed to changes in the spectral signature. Proportions of explained variance were increasingly split into a higher number of PCs, when derivatives of spectra were used (Table 5). PCA of the first derivative of spectra resulted in 97.2 % of explained variance by the first and 2.5 % of explained variance by the second PC, respectively (Table 5). Regarding results of PCA on the first derivation of spectra, the first and second PC showed similarity throughout their overall spectrum. It was, however, observed that the maximum shifted in a range of 700-750 nm (Fig. 24).

When PCA was finally applied to the second derivative of recorded spectra, the explained variation was split into the first 5 PCs, the first PC accounting for only 80 % of variation (Table 5). PCs 3 to 5 of the second derivation of spectra still explained 1 to 1.5 % of variation (Table 5). The first five PCs had maxima and minima located at different positions in the range of 680 to 730 nm (Fig. 24).

**Table 5: Percentage variance of principle components of diffuse reflection spectra (and 1<sup>st</sup> and 2<sup>nd</sup> derivation of spectra) recorded on broccoli curds. Broccoli was harvested throughout the season 2012 and stored for 4 days at 16 °C and high RH (~95 %).**

Principal component	% variance		
	Reflection spectra	1 <sup>st</sup> derivation	2 <sup>nd</sup> derivation
1	99.7	96.8	80.0
2	0.2	2.8	7.0
3	0.0	0.1	1.5
4	0.0	0.1	1.1
5	0.0	0.0	1.0

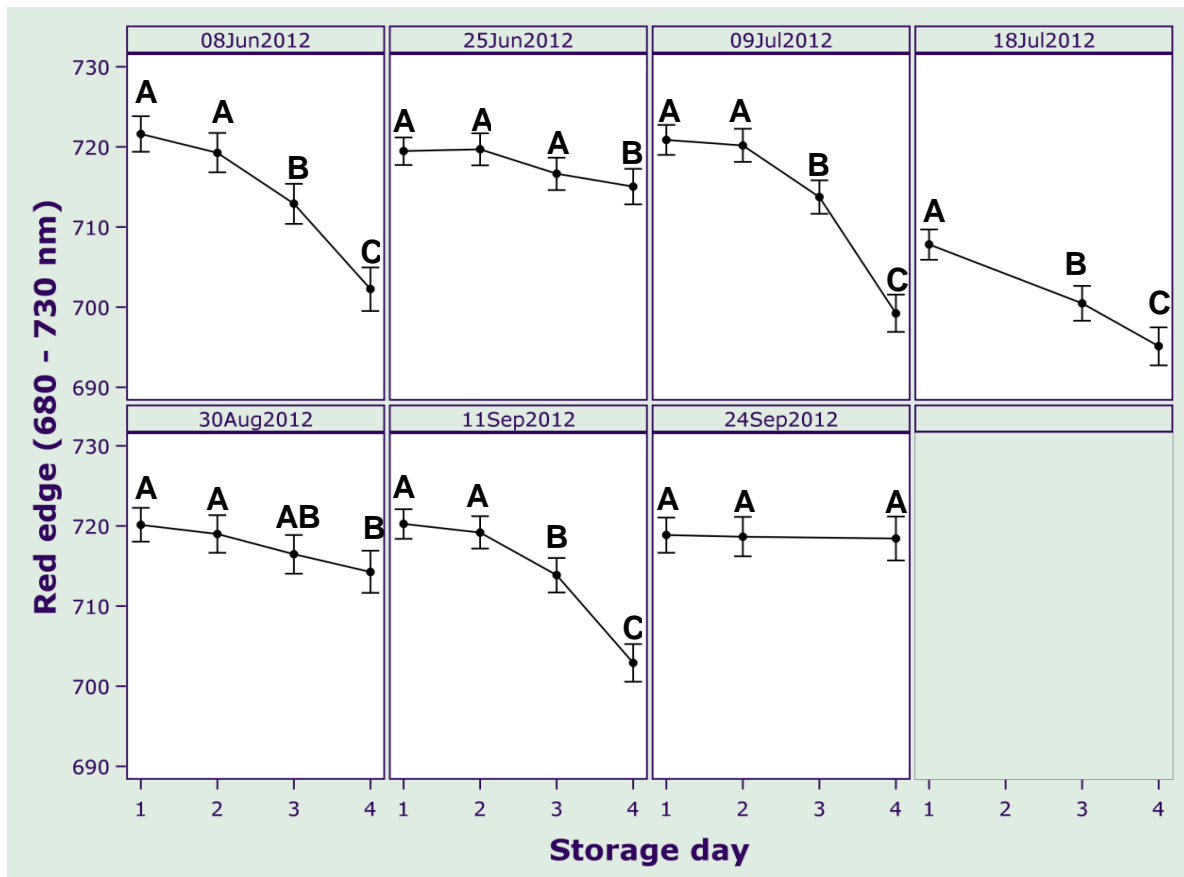


**Fig. 24: Principal components of the normalized reflection spectra of broccoli curds (A) and of their 1<sup>st</sup> (B) and 2<sup>nd</sup> (C) derivative (N=4305). Values of the 2<sup>nd</sup> derivative were spline-interpolated. Only PCs explaining  $\geq 1\%$  of variance were plotted. Broccoli was harvested throughout the season 2012 (June-September) and stored for 4 days at 16 °C and high RH (~95 %).**

These results indicated that the variation in the pattern of diffuse reflection spectra of broccoli in the range of 450 to 1000 nm was fully explained by the differences of inflection

point in the relatively narrow range of 680 to 730 nm. The diversity of the slope of spectra was particularly remarkable when PCs of the 2<sup>nd</sup> derivation of spectra were plotted (Fig. 24). The slope region is commonly referred to as the red edge and is also hosting the above mentioned vegetation index NDVI (3.2.1.3).

The REIP of the recorded spectra was located in the range of 692 to 723 nm. Further analysis of REIP of broccoli batches harvested in 2012 mostly showed a decline during DS (Fig. 25). Only REIP of broccoli harvested on September 24<sup>th</sup> stayed at the same level (Fig. 25). The first significant differences were observed on day 3 of DS (Fig. 25). REIP values at the beginning of storage were mainly on the same level. Only broccoli of unmarketable quality (harvest from July 18<sup>th</sup>) had much lower initial REIP values.



**Fig. 25:** Red edge inflection point of spectra in the range 680-730 nm (mean  $\pm$  confidence interval) of seven broccoli batches harvested throughout the 2012 growing season. Storage was done at 16 °C and high RH (~95 %). Different letters indicate significant differences within one batch.

The NDVI calculated on seven broccoli batches harvested in 2012 showed a significant decline during DS on four batches, whereas three batches remained on the same level (Fig. 26). Values of NDVI ranged from 0.59 to 0.87. The first significant changes were observed on day 3 (Fig. 26). In contrast to REIP, NDVI values varied greatly in different harvest batches measured at the beginning of DS. However, this did not influence further trends in the NDVI of the respective batches during storage (Fig. 26).

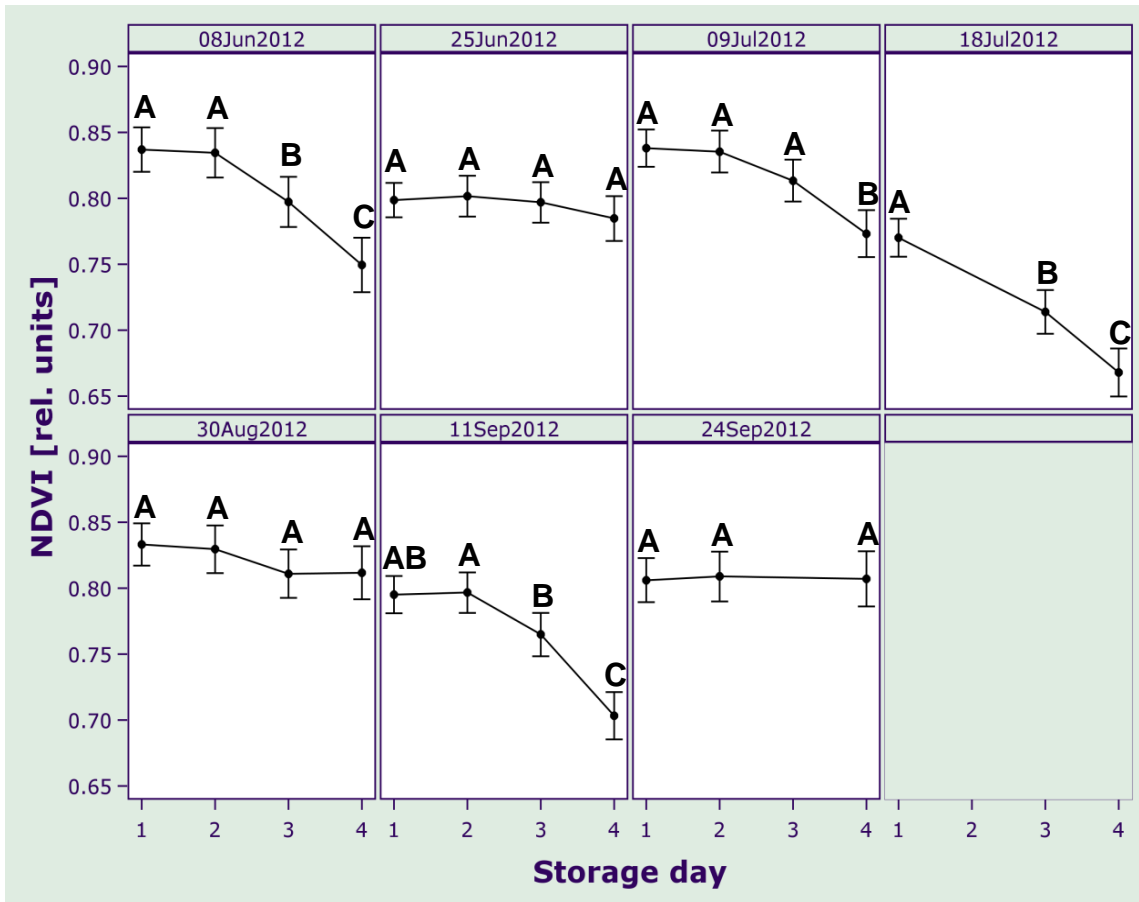
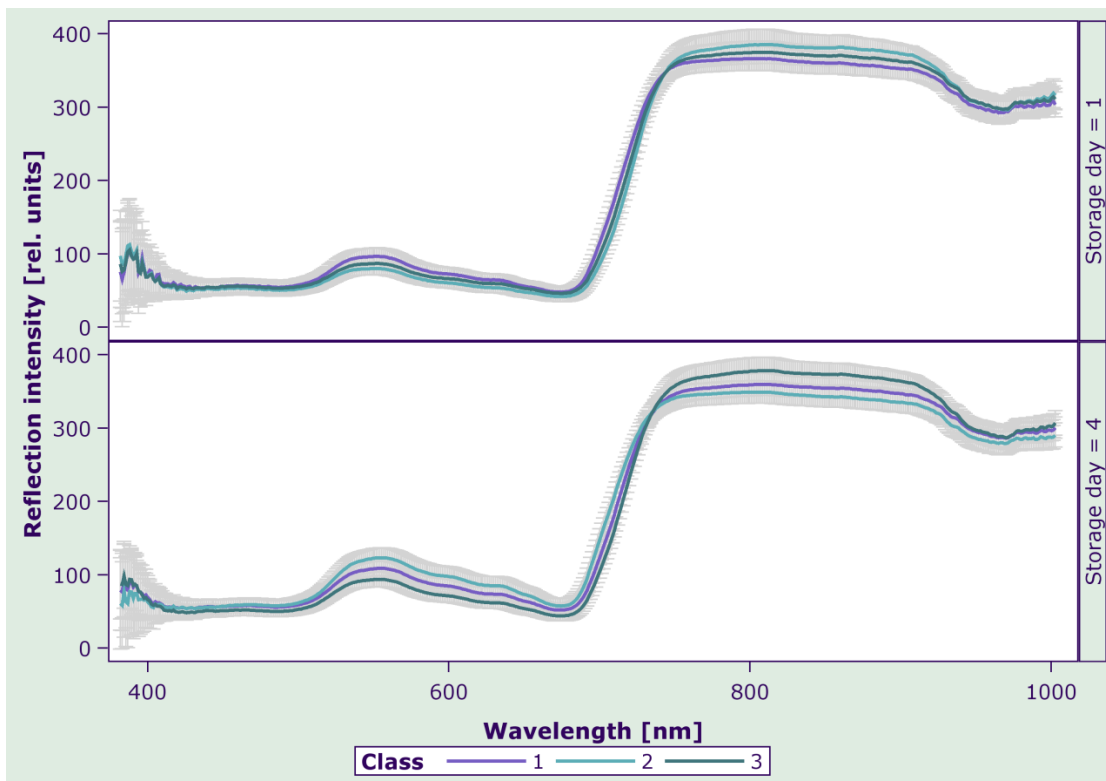


Fig. 26: NDVI (mean  $\pm$  confidence interval), calculated as  $[(R780 - R675) / (R780 + R675)]$ , where R675 is the reflection intensity at  $\lambda = 675$  nm. Seven broccoli batches were harvested throughout the 2012 growing season and stored at 16 °C and high RH (~95 %). Different letters indicate significant differences within one harvest batch.

### 5.1.3. Hyperspectral imaging

As shown by spot measurements, the analysis of the red edge region of broccoli spectra indicated changes within a reasonable period of time during DS. Due to their complex morphology, changes within individual broccoli curds can be expected to occur inhomogeneously, based on the senescence of single buds (3.1.5).

In order to grade differences in reflection intensity within single broccoli curds during storage, classification procedures (Iso-Data, Fuzzy-c-means) were conducted. For the classification of hyperspectral images, the first three PCs were used, which explained 93 and 94 % in total of the variance on day 1 and day 4 of storage, respectively. Different wavelength ranges including the red edge region were chosen for classification. However, the applied classification procedures did not lead to significant differences between classes, showing a high standard deviation within image classes (Fig. 27).

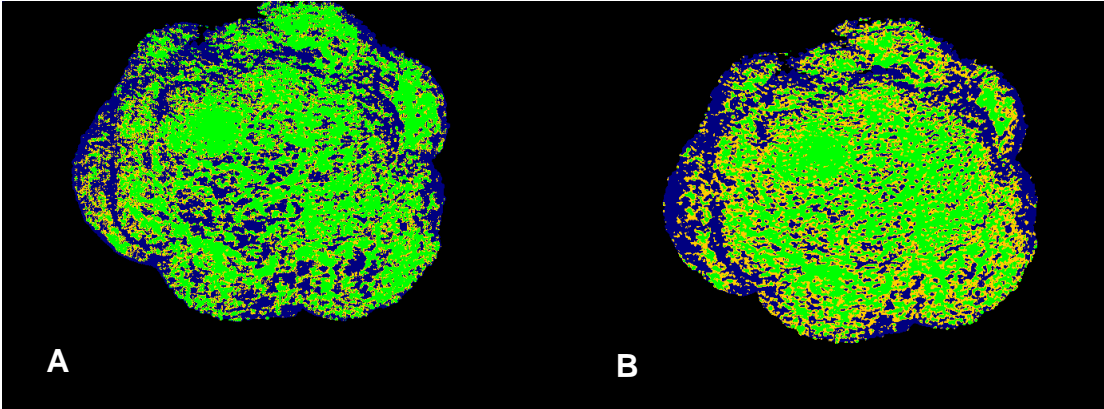


**Fig. 27: Reflection spectra of classes (mean  $\pm$  stdev) derived by the Fuzzy-c-means classification of bands 262-288 of hyperspectral images (~ wavelengths 700 - 730 nm) of one broccoli curd harvested on September 9<sup>th</sup>, 2013 after 1 and 4 days of storage at 16 °C and high RH (~95 %).**

The differences between reflection intensities were obviously not distinct enough to sort into classes. Instead, the classification procedures applied mainly distinguished reflection intensities from the outer and inner regions of the broccoli curd (Fig. 28). It can be assumed that the equal area normalization pre-treatment of the hyperspectral data mitigated the effect of the illumination to an extent, but did not eliminate it entirely. Due to the curved shape of the broccoli curd, equal area normalization might not be a sufficient pretreatment. Further data preprocessing techniques are applied in remote sensing

image analysis (Richards & Jia 2006, p. 39), but are considered too elaborate for application to broccoli.

When more than three classes were used as a classification target, the classified regions as shown in Fig. 28 were further subdivided, which likewise did not result in significant differences between classes.



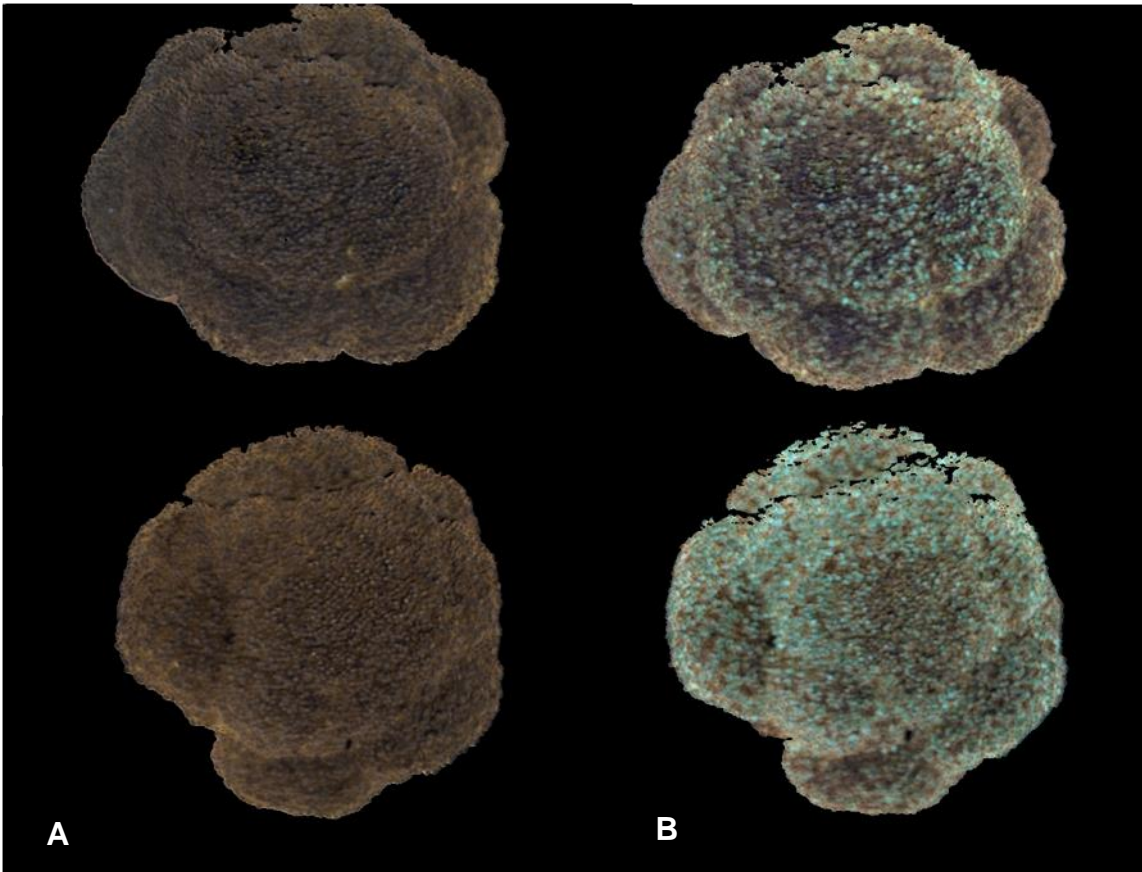
**Fig. 28: Result of Fuzzy-c-means classification (target: 3 classes) of principal components 1-3 after equal area normalization. Broccoli harvested on September 9<sup>th</sup>, 2013 after 1 (A) and 4 (B) days of storage at 16 °C and high RH (~95 %).**

These results demonstrated that the classification of hyperspectral images of broccoli curd surfaces was unsuccessful in rating quality differences within the curds. As an alternative, the Euclidean distances between reflection intensities of pixels within different wavelength ranges were measured. It was clearly observed that both Euclidean distances and variance of distances within broccoli curds increased during storage at 16 °C (Table 6). The mean and variance of Euclidean distances were much higher in hyperspectral images after four days, when compared to the beginning of DS (Table 6). This applied to the overall VIS spectrum and was most prominent in the range of 530 to 650 nm. In the range of the red edge, mean values and variance of Euclidean distances rose to a much lesser extent (Table 6).

**Table 6: Statistics regarding Euclidean distances within hyperspectral images of broccoli (n = 5) during storage at 16 °C and high RH 95 %.**

	Euclidean distance	Wavelength range		
		600-700 nm (2nd Absorption peak chlorophyll <i>a</i> + <i>b</i> )	530-650 nm	692-723 nm (Red Edge)
<b>day 1</b>	Mean	104.84	123.29	98.46
	Variance	4952.88	7696.01	4897.51
<b>day 4</b>	Mean	162.36	187.53	109.61
	Variance	13064.69	17248.84	5573.91

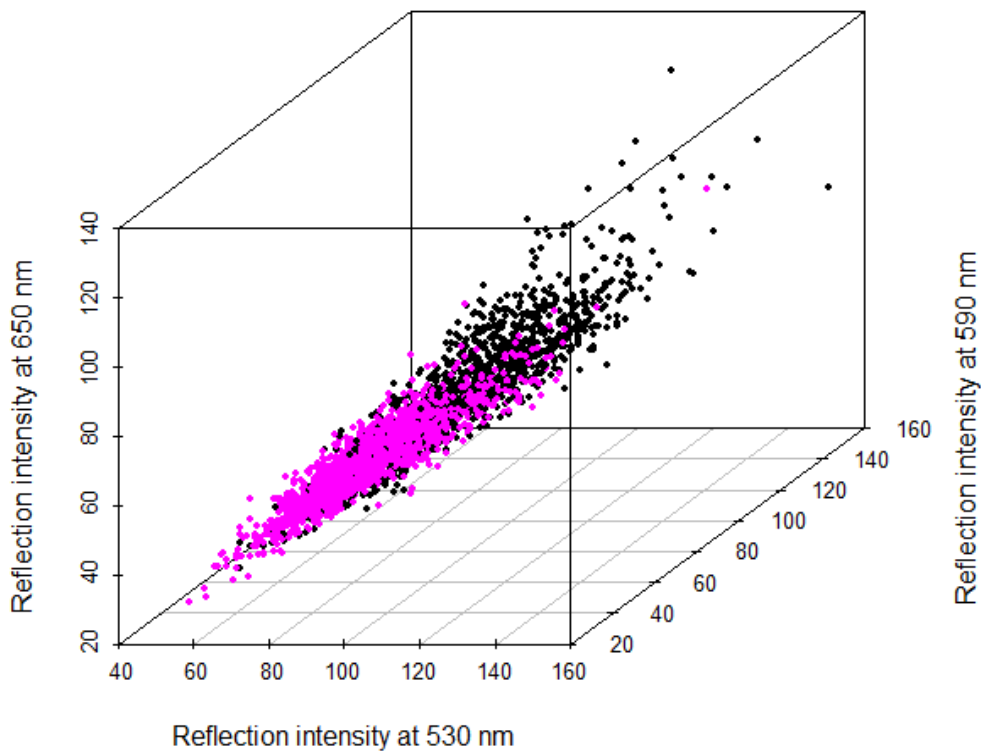
Indeed, the increase of Euclidean distances in the range of 530-650 nm might have resulted from an overall increase of reflection intensity in this region, as observed in the spot measurements of diffuse reflection (5.1.2). The increase, however, did not take place homogeneously, as the variance of Euclidean distances rose (Table 6). The variation among different reflection intensities of image pixels was therefore plotted as composite images, where reflection intensities at three different wavelengths (530, 590, and 650 nm) were placed as red, green and blue image channels, respectively (Fig. 29). The use of three wavelengths truly demonstrated the increase in the variation of reflection intensities in the images (Fig. 29). Within composites, brightness is shown in relation to the pixel with maximum recorded reflection intensity. Thus the variation in the images on day 4 of storage most likely resulted from higher reflection intensities, as more pixels showed brighter colors, since they were closer to the maximum recorded reflection intensity. The composite images obtained demonstrated that the overall increase in reflection intensities can be suitable for more elaborate quality ratings, although it was useful neither for the PCA of single spectra nor for the classification of images.



**Fig. 29: Reflection intensities of two broccoli curds after equal area normalization at wavelengths of 530, 590 and 650 nm on day 1 (A) and day 4 (B) of storage at 16 °C and high RH (~95 %).**

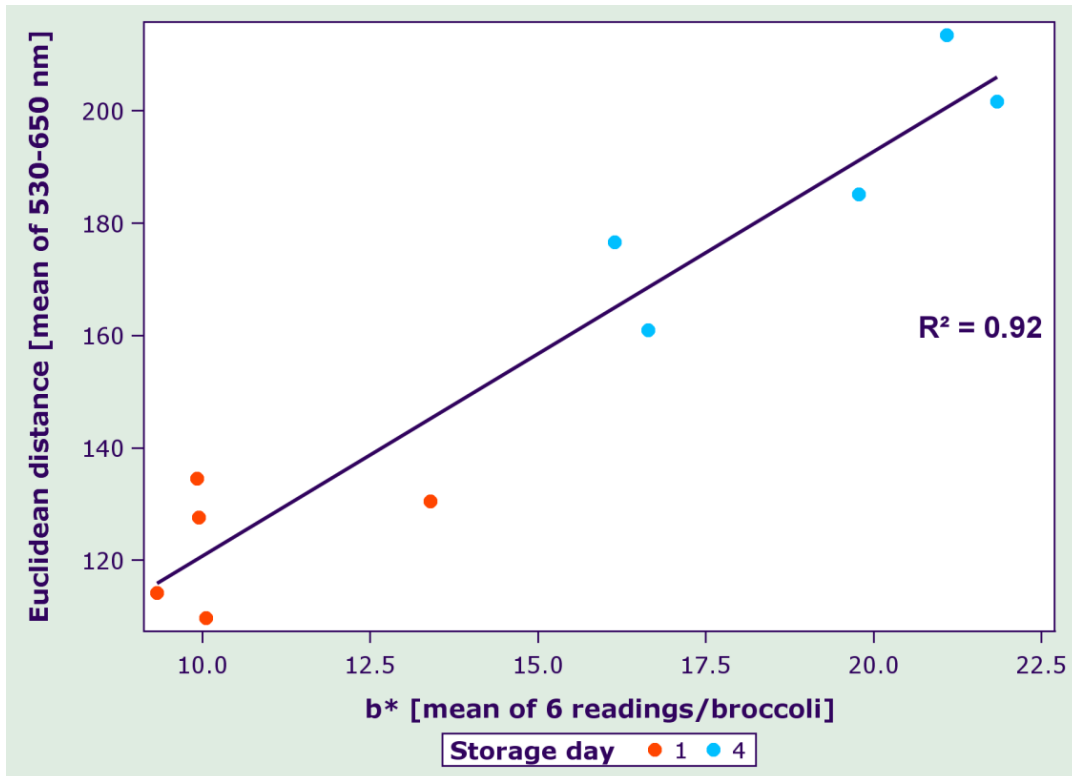
When the Euclidean distances were plotted on a three dimensional scatter plot, it was, however, observed that reflection intensities measured at initial and late storages overlapped in several regions (Fig. 30). The shift of reflection intensities to another sector of the three-dimensional space therefore occurred rather gradually. The scatter plot illustrates the fact that differences observed cannot be distinguished through classification procedures, as no distinguishable dot clusters were observed (Fig. 30).





**Fig. 30:** Array of reflection data of normalized hyperspectral images at wavelengths 530 nm, 650 nm and 590 nm. Pixel-wise reflection intensity of one broccoli curd after 1 (pink) and 4 (black) days of storage at 16 °C and high RH (~95 %). Image data were randomly reduced to 1 %.

The mean Euclidean distances of hyperspectral images were highly correlated with the mean  $b^*$  value of six arbitrary measurements per broccoli curd (Fig. 31). The information derived from the increasing variation within the broccoli curd was therefore similar to the information derived from spot measurements of  $b^*$  values.

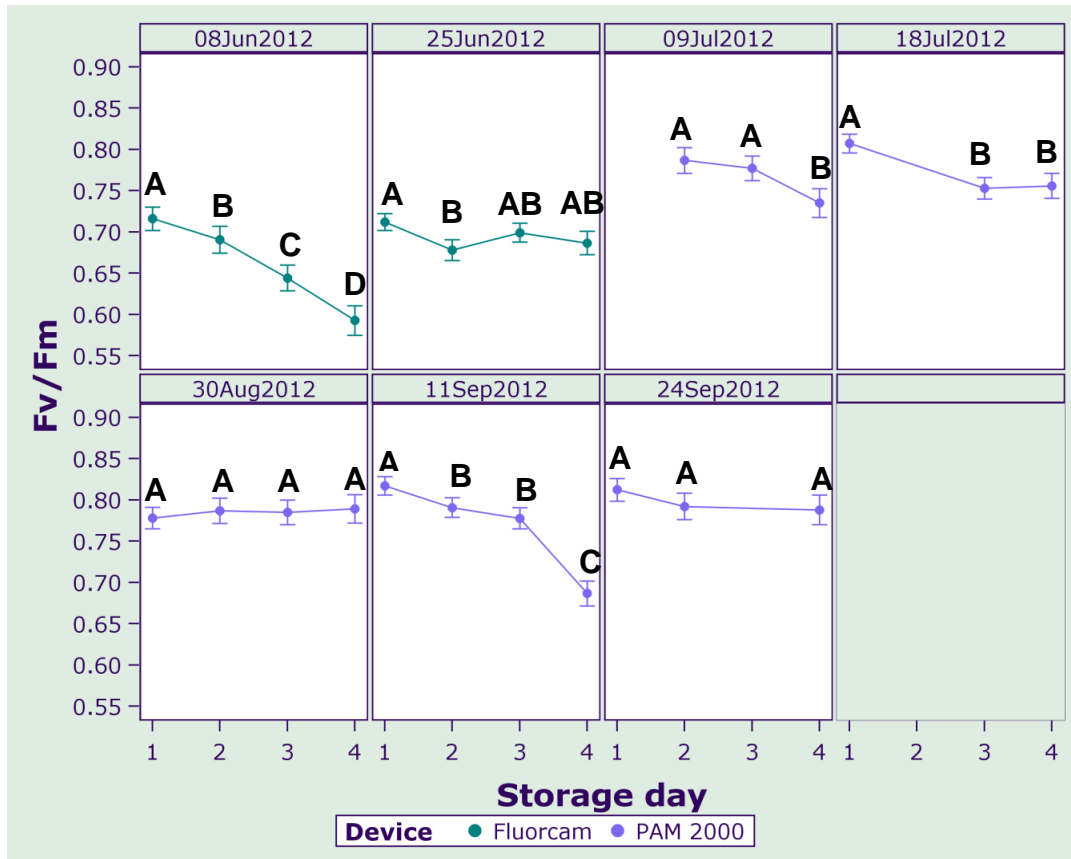


**Fig. 31: Relationship between means of the CIELAB parameter b\* (n = 6 measurements / broccoli) and mean Euclidean distances of hyperspectral images of broccoli on day 1 and 4 of storage at 16 °C and high RH (~95 %).**

#### 5.1.4. Chlorophyll fluorescence

Photosynthesis of broccoli curds harvested in 2012 was measured as maximum photochemical efficiency ( $F_v/F_m$ ) and as actual photon yield of photosynthetic electron transport ( $\Phi_{PSII}$ ) throughout DS. The parameter  $F_v/F_m$  provides information about the potential photosynthetic capacity, whereas  $\Phi_{PSII}$ , which is measured in light-adapted conditions, is a measure of the actual photosynthetic efficiency at that time. In June 2012, the chlorophyll fluorescence was measured by means of a fluorescence imaging system (4.5.1). From July onwards, spot measurements were conducted.

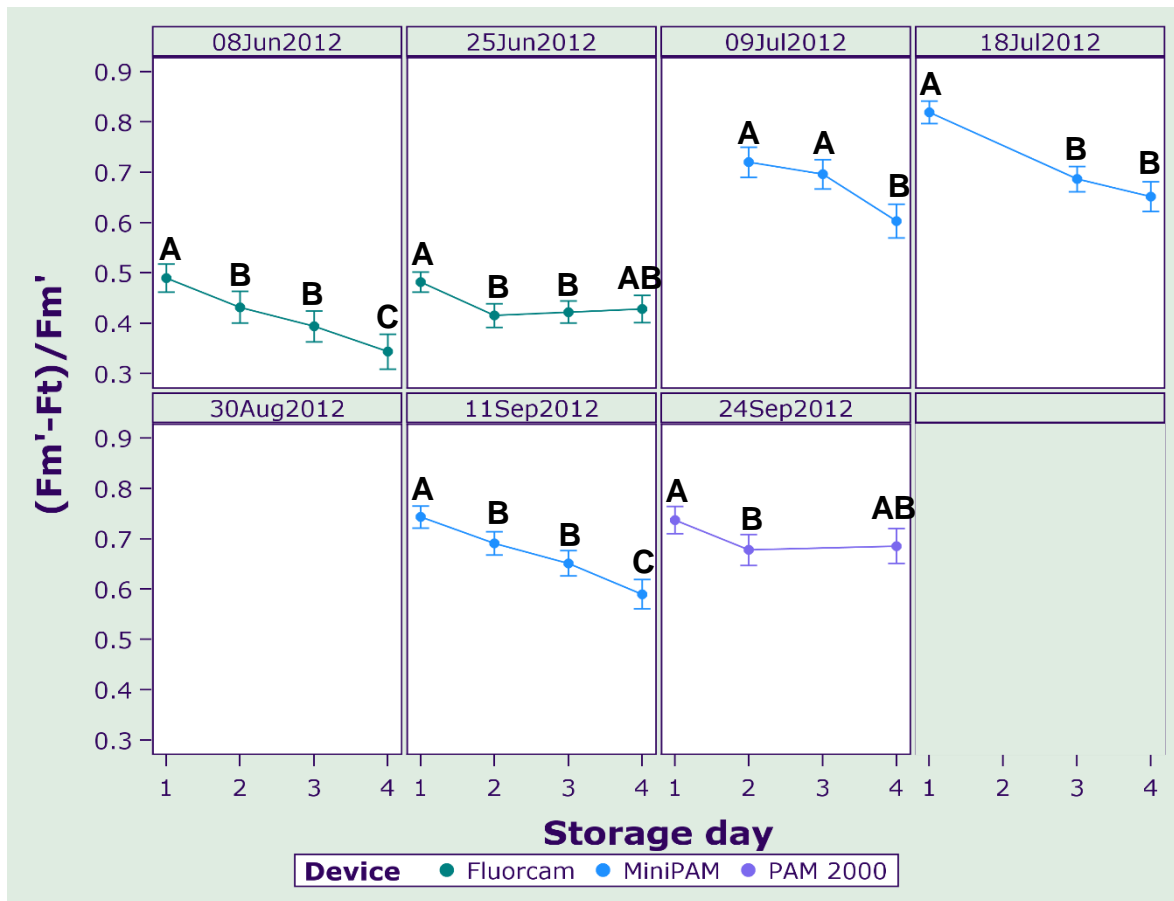
The values of  $F_v/F_m$  decreased significantly in five out of seven harvested broccoli batches during DS (Fig. 32). Two harvested batches (August 30<sup>th</sup>, September 24<sup>th</sup>) did not show any changes (Fig. 32). Significant changes in  $F_v/F_m$  were observed from day 2 onwards in three different harvest batches (Fig. 32).



**Fig. 32: Maximum photochemical efficiency (Fv/Fm) (mean  $\pm$  confidence interval) of seven broccoli batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). Different letters indicate significant differences within one batch between different storage days.**

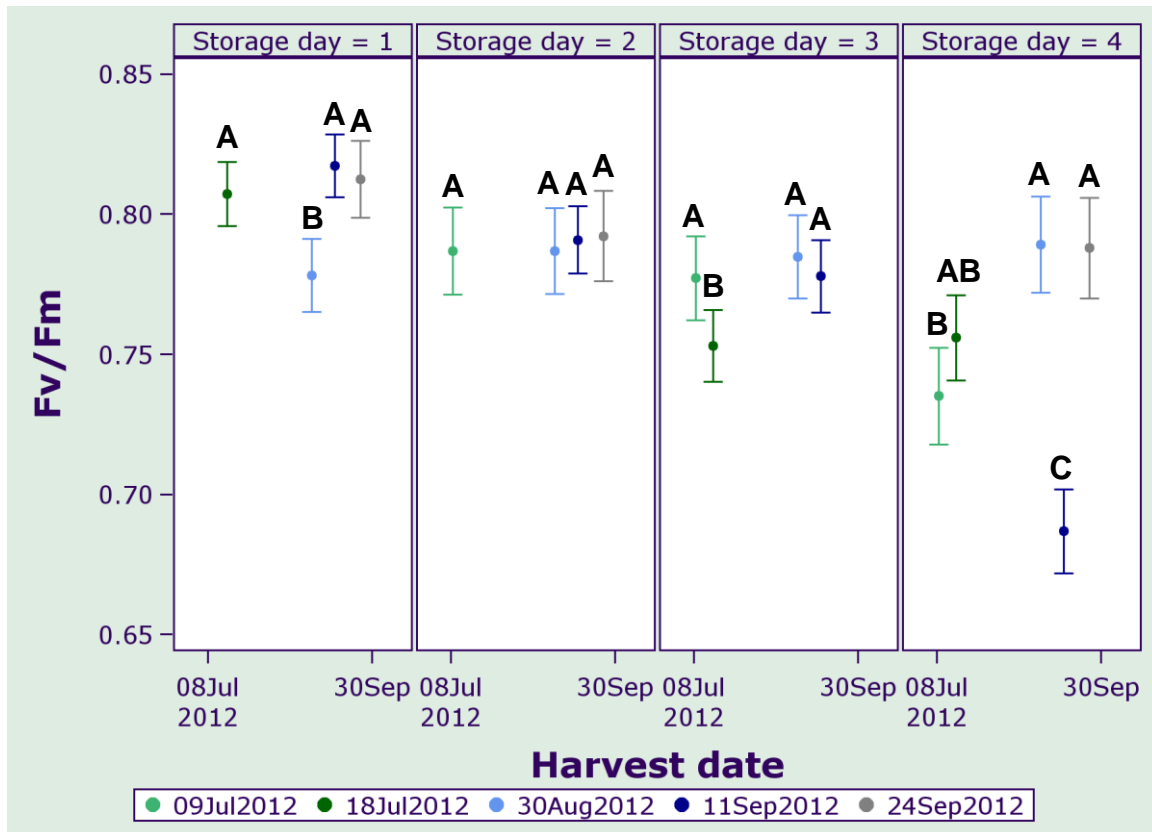
The values of Fv/Fm measured by means of fluorescence imaging were lower than values measured on single spots of the broccoli curd (Fig. 32). Broccoli harvested on July 18<sup>th</sup> that was not of marketable quality (4.1.1) exhibited high values of Fv/Fm (Fig. 32).

Values of  $\Phi_{PSII}$  declined in all investigated harvested batches, including that from September 24<sup>th</sup> (Fig. 33). However, in two batches (June 25<sup>th</sup>, September 24<sup>th</sup>) values on day 4 of DS had recovered and were then similar to initial values (Fig. 33).  $\Phi_{PSII}$  values of harvest batches measured by fluorescence imaging were much lower than in those measured by MiniPAM and PAM 2000 (Fig. 33). The non-marketable broccoli curds harvested on July 18<sup>th</sup> exhibited the highest initial values of photosynthetic yield  $\Phi_{PSII}$  (Fig. 33).



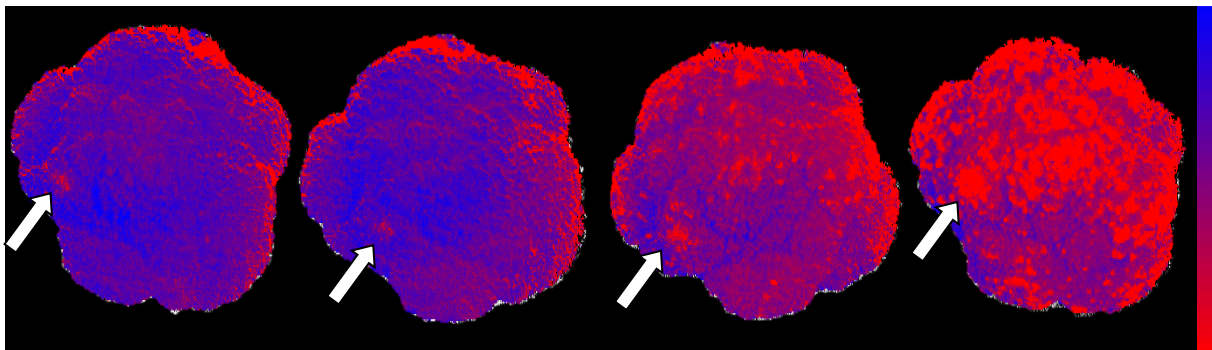
**Fig. 33: Actual photon yield of photosynthetic electron transport  $\Phi_{PSII}$  (mean  $\pm$  confidence interval) in six broccoli batches harvested throughout the 2012 growing season. The frame for August 30<sup>th</sup> was intentionally left blank (missing values). Storage was conducted at 16 °C and high RH (~95 %). Different letters indicate significant differences within one batch between different storage days.**

On the first day of DS, Fv/Fm values of broccoli harvested on August 30<sup>th</sup> were lower than values of three other batches (Fig. 34). On the next day, all broccoli batches were on the same level of maximum photochemical efficiency (Fig. 34). After 4 days, Fv/Fm values of broccoli harvested on July 9<sup>th</sup> and on September 11<sup>th</sup> had declined at different levels, whereas the broccoli batches from August 30<sup>th</sup> and September 24<sup>th</sup> remained on a high level (Fig. 34). Broccoli harvested on July 18<sup>th</sup> was at an intermediate level on day 4 of storage (Fig. 34).



**Fig. 34: Maximum photochemical efficiency (Fv/Fm) of five broccoli batches during 4 days of storage at 16 °C and high RH (~95 %). Batches were harvested throughout the 2012 growing season. Different letters indicate significant differences between broccoli batches on the respective storage days.**

Imaging of the chlorophyll fluorescence with the FluorCam 690 MF device resulted in lower values than devices which measured the fluorescence at single spots (Fig. 33 & Fig. 34). The consideration of spatial distribution of Fv/Fm in fluorescence images showed some variation within broccoli curds (Fig. 35). Apart from the possible illumination effects on the edge, single regions with lower Fv/Fm values as indicated by the white arrow in Fig. 35 may function as early indicators of further quality decline in this region of the curd.



**Fig. 35: Maximum photosynthetic capacity (Fv/Fm) of one broccoli curd harvested on June 8<sup>th</sup>, 2012 on days 1, 2, 3 and 4 of storage at 16 °C and high RH (~95 %). False color scale ranges from 0.55 (red) to 0.75 (blue). White arrow points at growing spot of lower Fv/Fm values.**

## 5.2. External influences

Except for one broccoli batch harvested on July 18<sup>th</sup> 2012, all broccoli perfectly met the quality standards following trade regulations (UNECE 2010) at the time of harvest. Nevertheless, there were great differences in the yellowing of broccoli among batches during DS, as demonstrated through the different optical measurements (5.1).

Assuming that conditions of harvest and transport were comparable for all batches, in this chapter the role that cultivation conditions play in postharvest processes is highlighted. Differences in cultivation, namely variations of covers and fertilizer as well as climate influences were considered regarding their effect on storability.

### 5.2.1. Cultivation factors

The broccoli used for examinations was field grown under best practice conditions, partly with a variation in cultivation methods (4.2). As demonstrated in chapter 5.1, all quality indexes measured by different non-destructive devices were significantly influenced by the interaction of harvest batch and storage day during DS. In the statistic model of ANOVA (4.7.1), the additional influence of other factors involved in the cultivation was rated simultaneously to both storage time and harvest batch. The model was applied to several optical quality indexes (Table 7). The impact of cultivation systems on broccoli varied, depending on the quality index used (Table 7).

**Table 7: Levels of significance (analysis of variance) for the main and interactive effects of storage and cultivation factors on non-destructive quality indexes.**

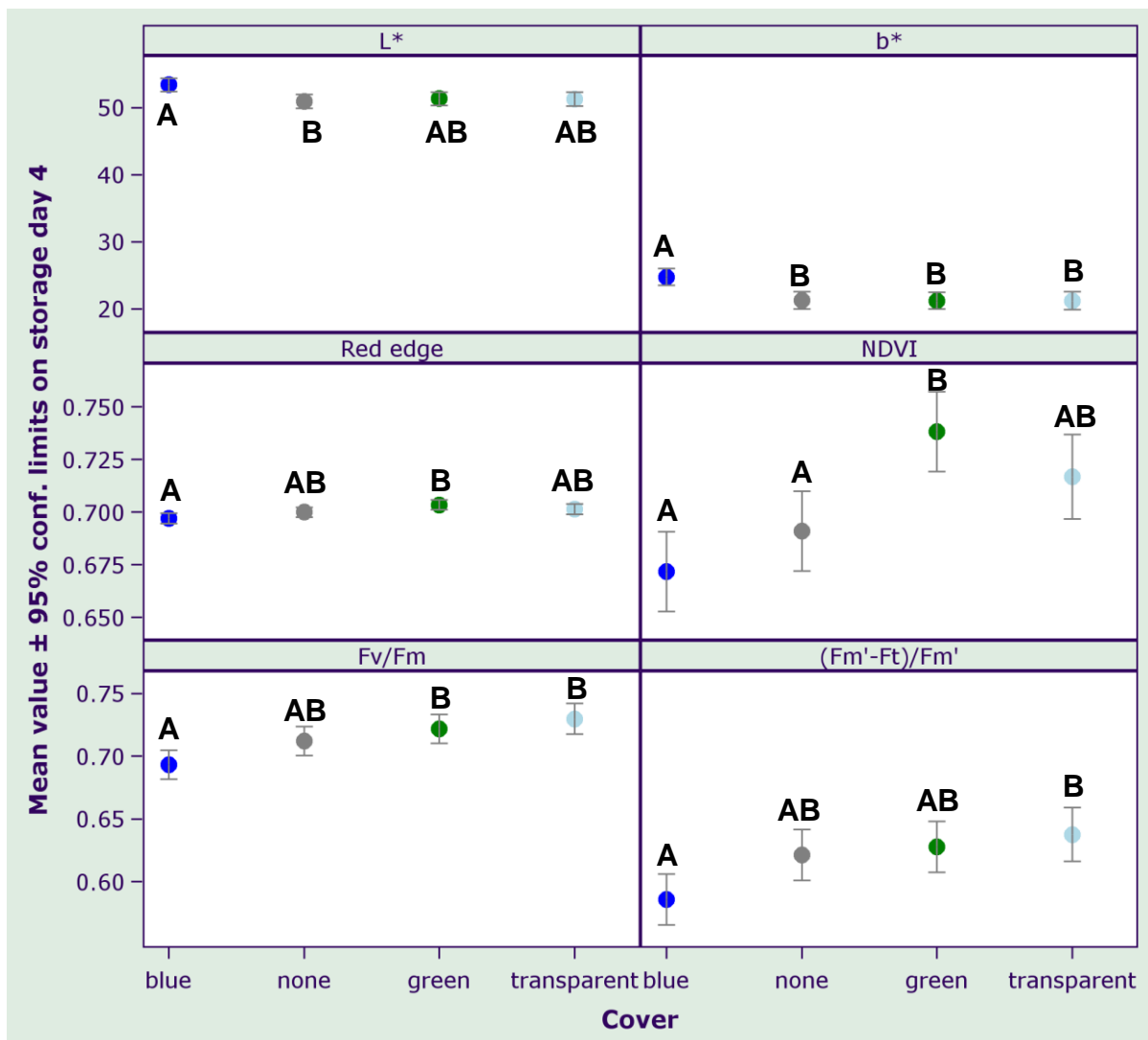
	Color values			Reflection indices	
	L*	b*	hue	NDVI	REIP
Harvest batch	*	*	*	*	*
Storage day	*	*	*	*	*
Net cover	n.s.	n.s.	n.s.	n.s.	n.s.
Plastic film cover	*	*	n.s.	*	*
Fertilizer	*	n.s.	n.s.	*	n.s.
Storage day x Harvest batch	*	*	*	*	*
Storage day x Net cover	n.s.	n.s.	n.s.	n.s.	n.s.
Storage day x Plastic film cover	*	*	*	*	*
Storage day x Fertilizer	n.s.	*	n.s.	n.s.	n.s.

n.s. – not significant; \*  $p \leq 0.05$

Covering the broccoli curds with protection nets during curd growth (4.2.3) did not have any effect on color or reflection indexes (Table 7). In contrast, the differences in light

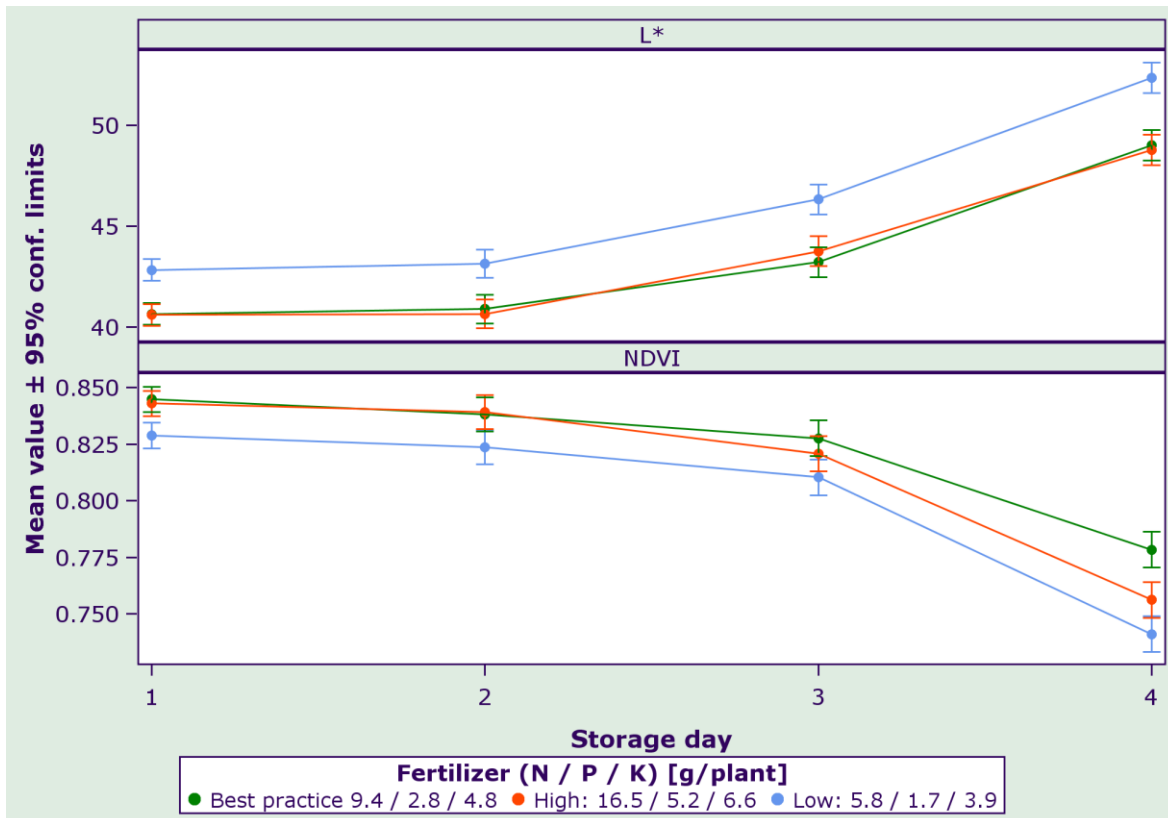
quality caused by different plastic film covers during the curd growth phase had a significant influence on all optical indexes in the course of storage (Table 7).

Different plastic film covers were applied from button stage onwards on two batches of broccoli during the 2012 growing season (4.2.2). Multiple pair-wise comparisons showed that broccoli covered with a blue film had significantly higher  $b^*$  values on day 4 of DS compared to broccoli grown under both green and transparent covers or left uncovered (Fig. 36). Regarding other quality indexes, broccoli grown under the blue cover showed differences to other variants: It was lighter in color compared to uncovered curds ( $L^*$ ) and exhibited both lower REIP and NDVI values compared to broccoli covered with green plastic films (Fig. 36). Furthermore, it had a lower maximum photochemical efficiency ( $F_v/F_m$ ) than broccoli grown under other cover films and lower  $\Phi_{PSII}$  values than broccoli grown under transparent covers (Fig. 36). A temporary reduction of the visible range of the electromagnetic spectrum to wavelengths of ~400 to 500 nm therefore induced an increase in senescence.



**Fig. 36: Non-destructive quality indexes of two broccoli batches grown under different covers from button stage onwards or uncovered ( $\triangleq$ “none”) after 4 days of storage at 16 °C and high RH (~95 %). Different letters indicate significant differences between cover types on storage day 4. Some error bars are hidden by symbols.**

Reduced fertilization had a significant effect both on the lightness parameter  $L^*$  and the NDVI of broccoli (Table 7). Other non-destructive indexes were not affected (Table 7). Analysis of NDVI-levels within the single broccoli batch which was cultivated under varying fertilization (July 9<sup>th</sup> 2012) indicated a relationship between NDVI and nutrient supply. The NDVI values of broccoli grown at low fertilization were significantly lower than those of broccoli with an optimum or increased fertilizer supply (Fig. 37). Additionally, on day 4 of storage, NDVI values of over-fertilized broccoli were significantly lower than those of curds with an optimum supply (Fig. 37).



**Fig. 37: Lightness  $L^*$  and NDVI  $(R780 - R675) / (R780 + R675)$  of broccoli harvested on July 9<sup>th</sup> 2012, during 4 days of storage at 16 °C and high RH (~95 %). Non-overlapping confidence limits are significantly different ( $\alpha = 0.05$ ).**

Regarding CIELAB readings, a similar relationship was observed concerning  $L^*$  values of broccoli with a different fertilizer supply (Fig. 37). Significantly lower values of NDVI in the low-level variant were only observed on day 1 and day 4 of storage, whereas  $L^*$  values were significantly higher throughout the whole storage period (Fig. 37).  $L^*$  values did not show differences between broccoli that was over-fertilized compared to optimum supply (Fig. 37). The reported differences in NDVI and  $L^*$  readings for different fertilizer levels were absolutely indistinguishable by visual rating.

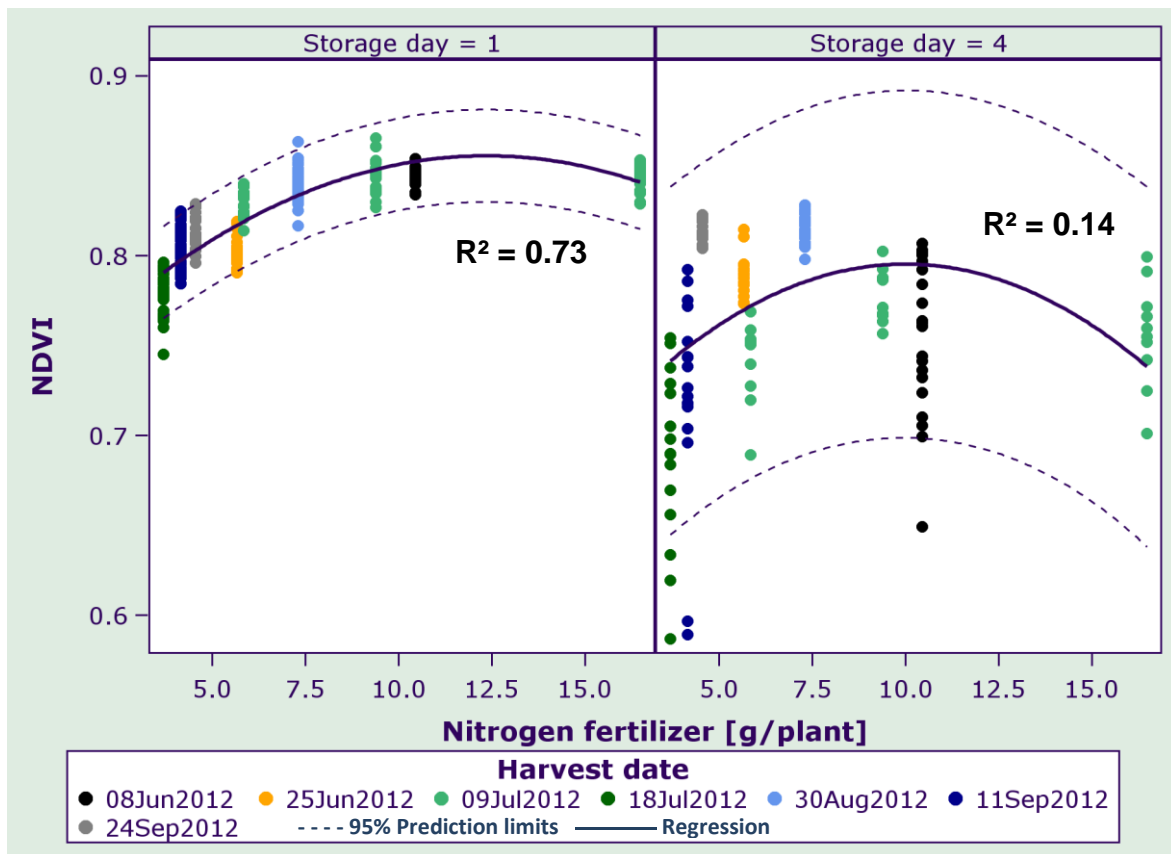
It should, however, be noted that different fertilizer doses in this batch did not show any effect on the storability of broccoli, because the interaction of the factors (storage day x fertilizer) did not influence NDVI or  $L^*$  values (Table 7).



The yellowing parameter  $b^*$  was influenced by different fertilizer levels of broccoli during DS (Table 7). However, multiple pair-wise comparisons did not indicate differences between differently treated broccoli curds on single storage days.

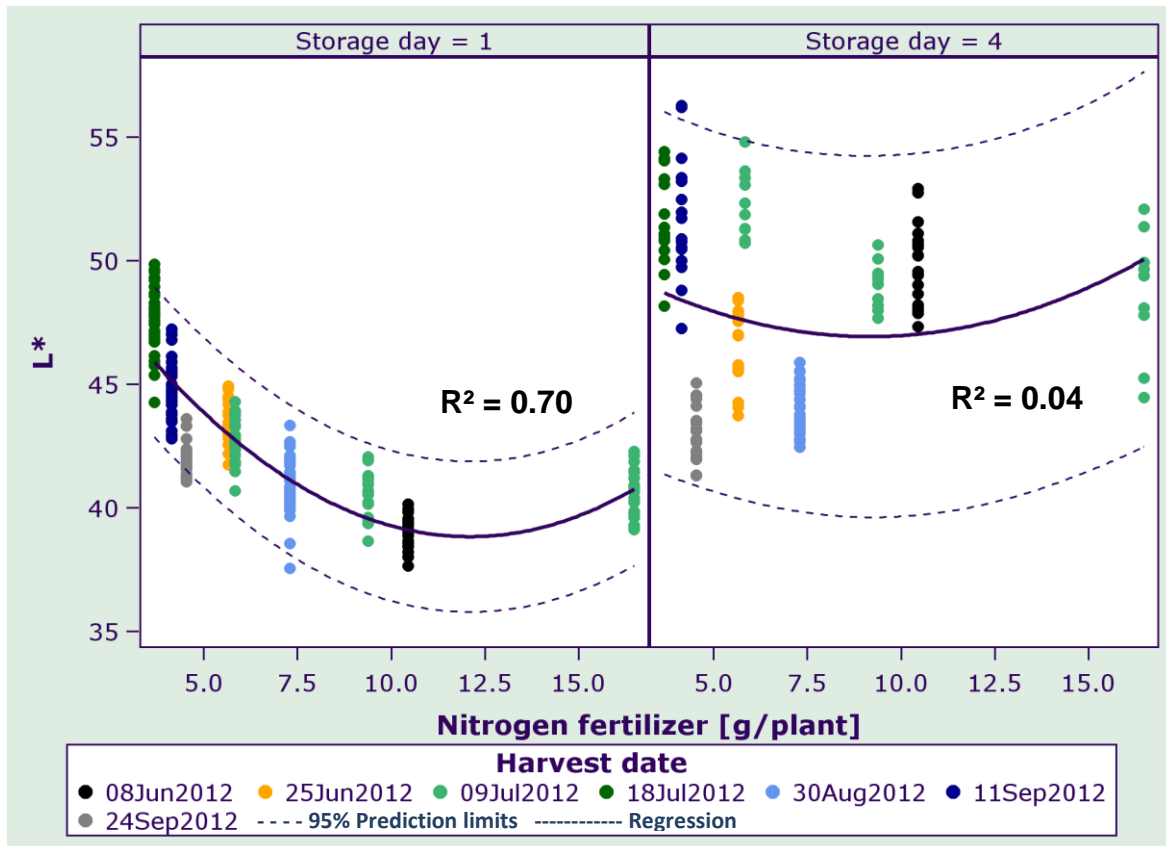
Levels of fertilization were also available for the other broccoli batches cultivated during the 2012 growing season. Although the conditions of application and soil characteristics varied between the planting sites, fertilization had a strong influence on both NDVI and  $L^*$  values (Fig. 38).

As presented in chapter 5.1.2, the initial NDVI values of broccoli batches harvested in 2012 varied greatly. Considering the amount of nitrogen fertilizer applied (Fig. 38), it was found that NDVI values on storage day 1 were highly dependent on fertilization, following a quadratic relationship ( $R^2 = 0.73$ ). The doses of nitrogen fertilizer applied throughout cultivation ranged from 3.7 to 16.5 g per plant (Table 2). Consecutive fertilizer doses had been summed up during the cultivation period of the respective harvest batch. On storage day 4 of DS, no further relation was found between fertilization and NDVI ( $R^2 = 0.14$ ; Fig. 38).



**Fig. 38:** NDVI ( $R780 - R675$ ) / ( $R780 + R675$ ) of seven broccoli batches on day 1 and 4 of storage corresponding to amounts of nitrogen fertilizer (g/plant) applied during cultivation. Batches were harvested throughout the 2012 growing season and stored at 16 °C and high RH (~95 %) for 4 days. Fertilization in batch harvested on July 9<sup>th</sup> was varied.

The relationship between nitrogen fertilization and  $L^*$  values was very similar to that of NDVI (Fig. 39). On storage day 1,  $L^*$  values followed a quadratic relationship as well, depending on the amount of nitrogen applied during cultivation ( $R^2 = 0.70$ ). In the progress of DS, the relationship was again completely dissolved (Fig. 39).



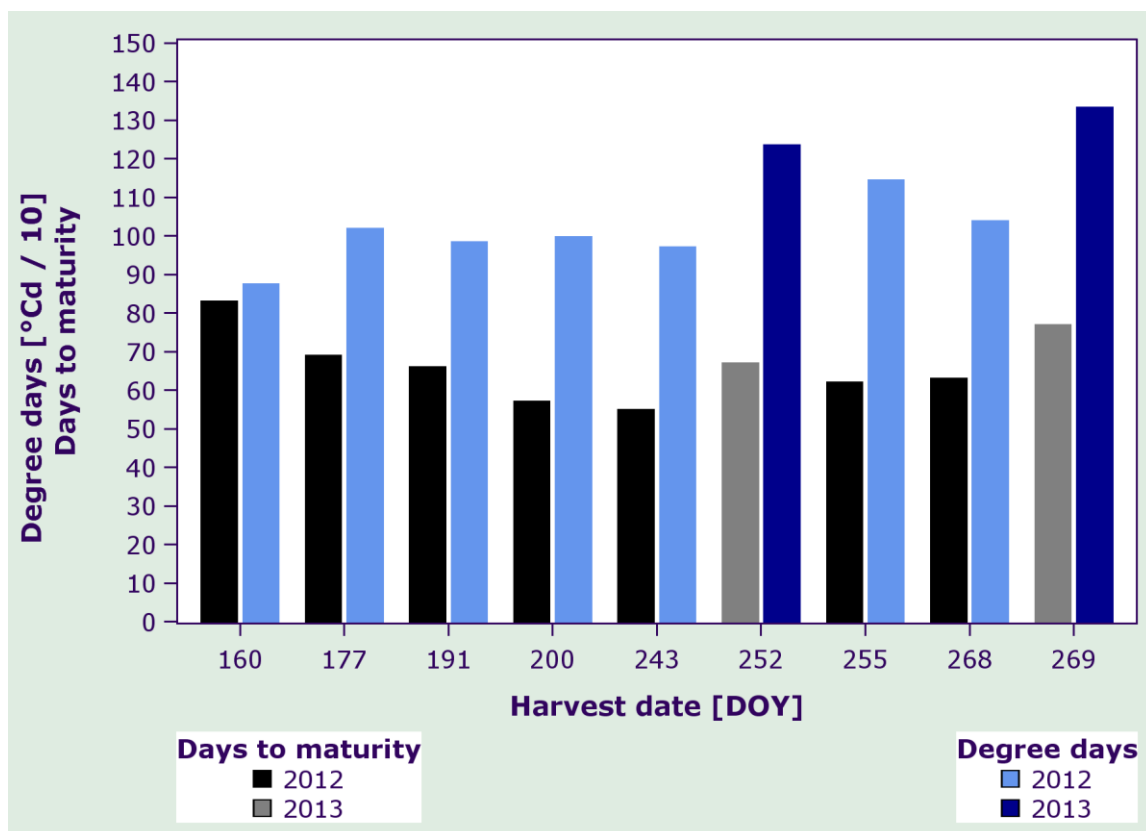
**Fig. 39: Lightness  $L^*$  of seven broccoli batches on day 1 and 4 of storage, corresponding to amounts of nitrogen fertilizer (g/plant) applied during cultivation. Batches were harvested throughout the 2012 growing season and stored at 16 °C and high RH (~95 %) for 4 days. Fertilization in batch harvested on July 9<sup>th</sup> was varied.**

It is clear that although the amount of applied nitrogen fertilizer highly determined both the lightness and NDVI of broccoli curds, it had no effect on the further development of these indexes during storage.

### 5.2.2. Climatic factors

Throughout the cultivation periods of broccoli curds in 2012 and 2013, data from electronic weather stations was recorded (4.1.2). In 2012, the cultivation period of individual broccoli batches decreased towards the peak growing season (Fig. 40). Thus, the number of days to maturity was lowest in the batch cultivated from July 6<sup>th</sup> to August 30<sup>th</sup>, 2012 (Fig. 40). Regarding comparable harvest dates in both years, the cumulative mean daily temperatures (degree days) were higher in 2013 than in 2012 (Fig. 40). Corresponding maturation periods were longer in 2013 than in 2012 (Fig. 40). The degree days during cultivation intervals ranged from 875.4 °Cd to 1332.3 °Cd (Fig. 40). The degree days of batches harvested in September were higher than in earlier batches (Fig. 40; DOY 243).

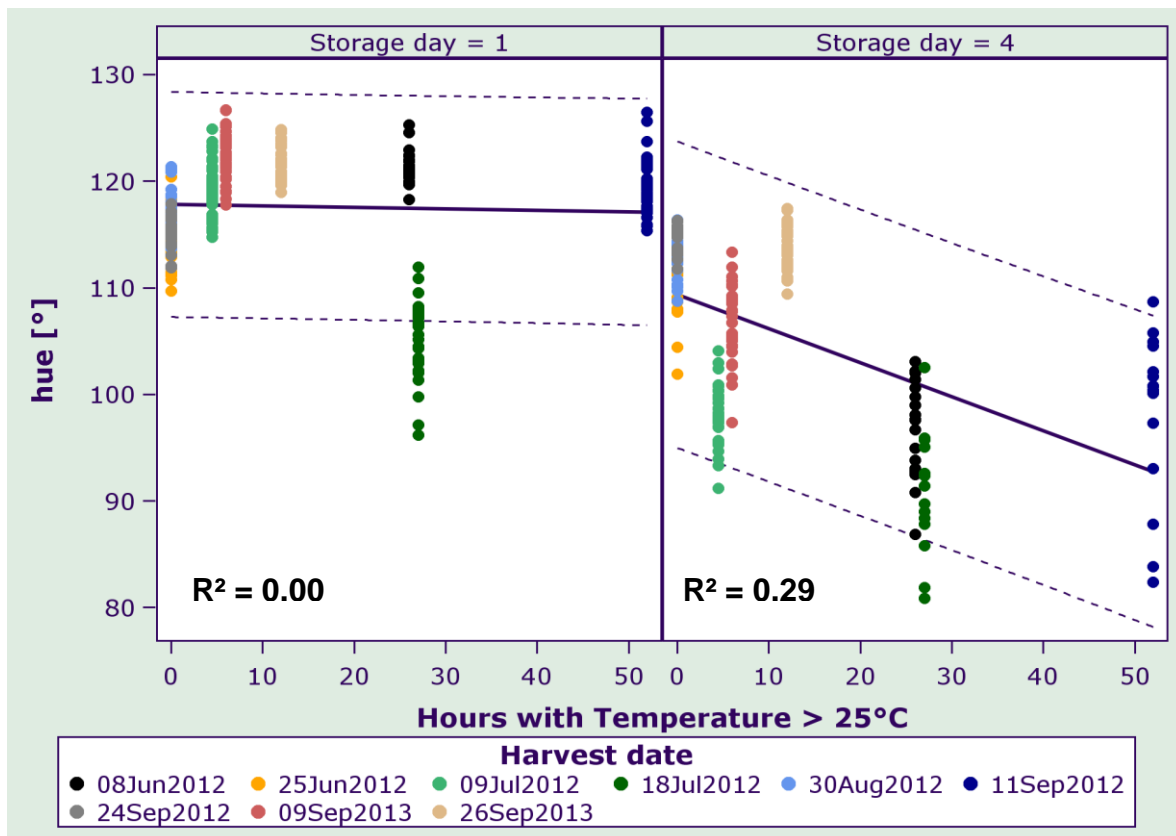
However, these seasonal trends in temperature sums did not correspond to the variation of broccoli quality indexes presented in chapter 5.1. Temperature sums as well as cultivation periods were not directly related to quality attributes.



**Fig. 40:** Time required for curd development (planting to harvest) and corresponding sum of temperatures during the growing season, lasting from day of year (DOY) 160 = June 8<sup>th</sup> to DOY 269 = September 26<sup>th</sup>.

Based on a literature review, the climatic characteristics that were identified as affecting the quality of broccoli were examined (3.1). The analysis was conducted by means of database query, enabling investigations of climatic conditions during particular phases of individual plantations through a combined approach (4.7.4).

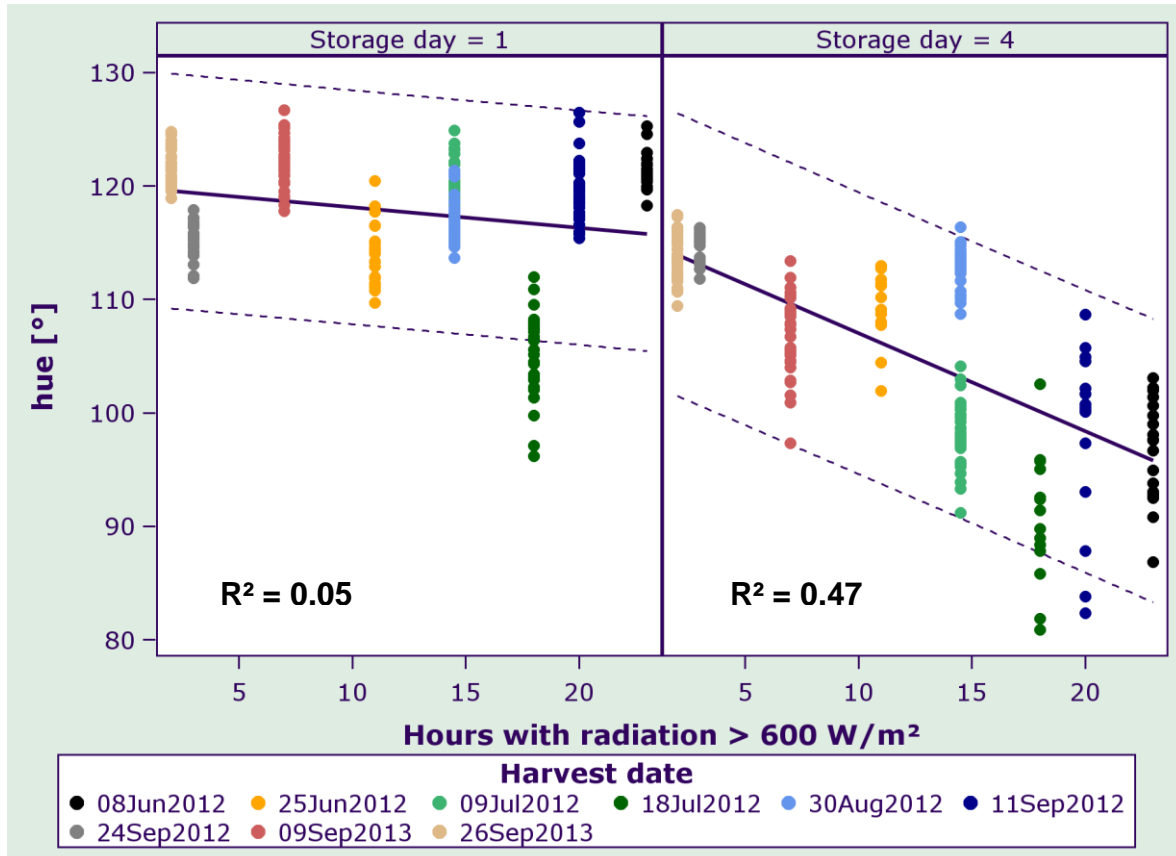
As the seasonal pattern of temperatures alone did not result in any information regarding subsequent broccoli yellowing, the weather conditions which were likely to influence postharvest quality were regarded in more detail. Hours with air temperatures higher than 25 °C, as provided by the attendant weather stations, were counted by data base query (4.7.4). During a growth phase of 16 to 24 days before harvest, temperatures higher than 25 °C were recorded up to 52 times (Fig. 41). In three batches from the year 2012 (June 25<sup>th</sup>, August 30<sup>th</sup>, September 24<sup>th</sup>) no temperatures above 25 °C were recorded during the respective phase (Fig. 41). On the first day of DS, different quantities of hours above 25 °C were of no consequence for the color of broccoli curds (Fig. 41). Instead, batches were within the same value range of hue angle, except broccoli harvested on July 18<sup>th</sup>, 2012 (Fig. 41). On day 4 of DS, there was a trend of decreasing hue values depending on the amount of hours with temperatures above 25 °C (Fig. 41;  $R^2 = 0.29$ ). Thus, when broccoli batches were cultivated in a hot period totaling more than 25 hours of temperatures above 25 °C during curd formation, yellowing during DS was more likely.



**Fig. 41:** The hue angle of broccoli curds related to the number of hours exceeding 25 °C during the cultivation period of 16 to 24 days before harvest. Broccoli batches were harvested in 2012 and 2013 and stored for four days at 16 °C and high RH (~95 %). Hue angle: 90° = yellow, 180° = bluish-green. Lines: solid = regression; dashed = 95 % prediction limits.

Regarding solar radiation during the same growth interval of 16 to 24 days before harvest, hourly values over 600 W/m<sup>2</sup> were recorded 2 to 23 times, depending on the harvest batch (Fig. 42). Similar to heat hours, on day 1 of DS, hue angle was not influenced by hours with high radiation (Fig. 42;  $R^2 = 0.05$ ). In contrast, after 4 days of DS, there was a linear dependency of hue angle values on radiation intensity (Fig. 42;  $R^2 = 0.47$ ). Thus,

the influence of high radiation on subsequent broccoli yellowing was more pronounced than that of heat. Harvest batches from June 08<sup>th</sup>, July 18<sup>th</sup> and September 11<sup>th</sup> of the year 2012 were grown under more than 17 hours of high solar radiation exceeding 600 W/m<sup>2</sup> in the respective period of time (Fig. 42). This resulted in lower hue angle values after DS (Fig. 42). It is also certainly the case that the two climatic influences of solar radiation and high temperatures are strongly interrelated.



**Fig. 42:** The hue angle of broccoli curds related to the number of hours with radiation exceeding 600 W/m<sup>2</sup> during the cultivation period of 16 to 24 days before harvest. Broccoli batches were harvested in 2012 and 2013 and stored for four days at 16 °C and high RH (~95 %). Hue angle: 90° = yellow, 180° = bluish-green. Lines: solid = regression; dashed = 95 % prediction limits.

Additionally, the influence of water provided during cultivation on broccoli yellowing was investigated, taking both precipitation and irrigation into account. The sum of rainfall and irrigation in the last 14 days of cultivation accounted for a range of 20.1 to 97.3 L m<sup>-2</sup>, depending on the harvest batch (Fig. 43). On the first day of DS, b\* values were not affected by differences in water availability before harvest (Fig. 43). After four days of DS, there was a strong linear dependency of the parameter b\* on the amount of irrigation and rainfall (Fig. 43; R<sup>2</sup> = 0.71). Water received by broccoli curds in the last two weeks before harvest therefore had a strong determination effect on further storability.

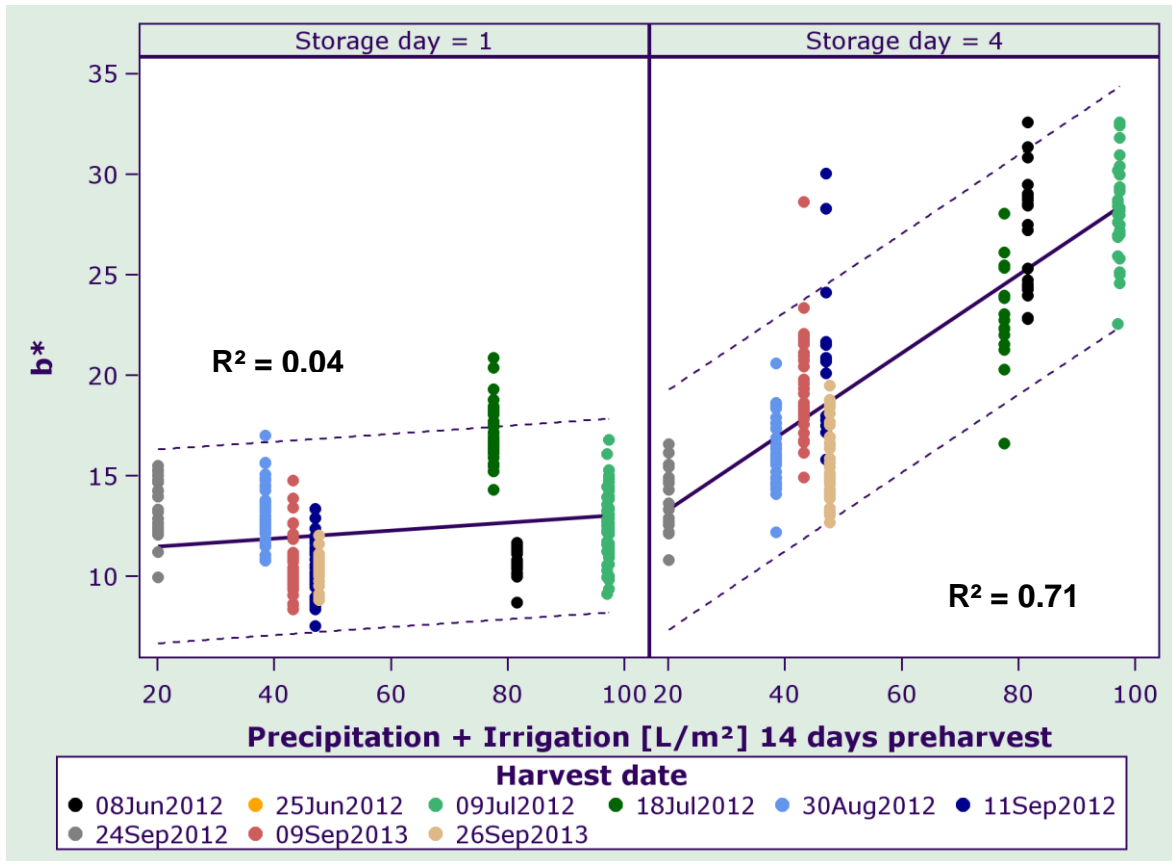


Fig. 43:  $b^*$  values of broccoli curds related to the sum of water (precipitation + irrigation) received during the fortnight preceding harvest. Broccoli batches were harvested in 2012 and 2013 and stored for four days at 16 °C and high RH (~95 %). No irrigation data was available for broccoli harvested on June 25<sup>th</sup>, 2012. Lines: solid = regression; dashed = 95 % prediction limits.

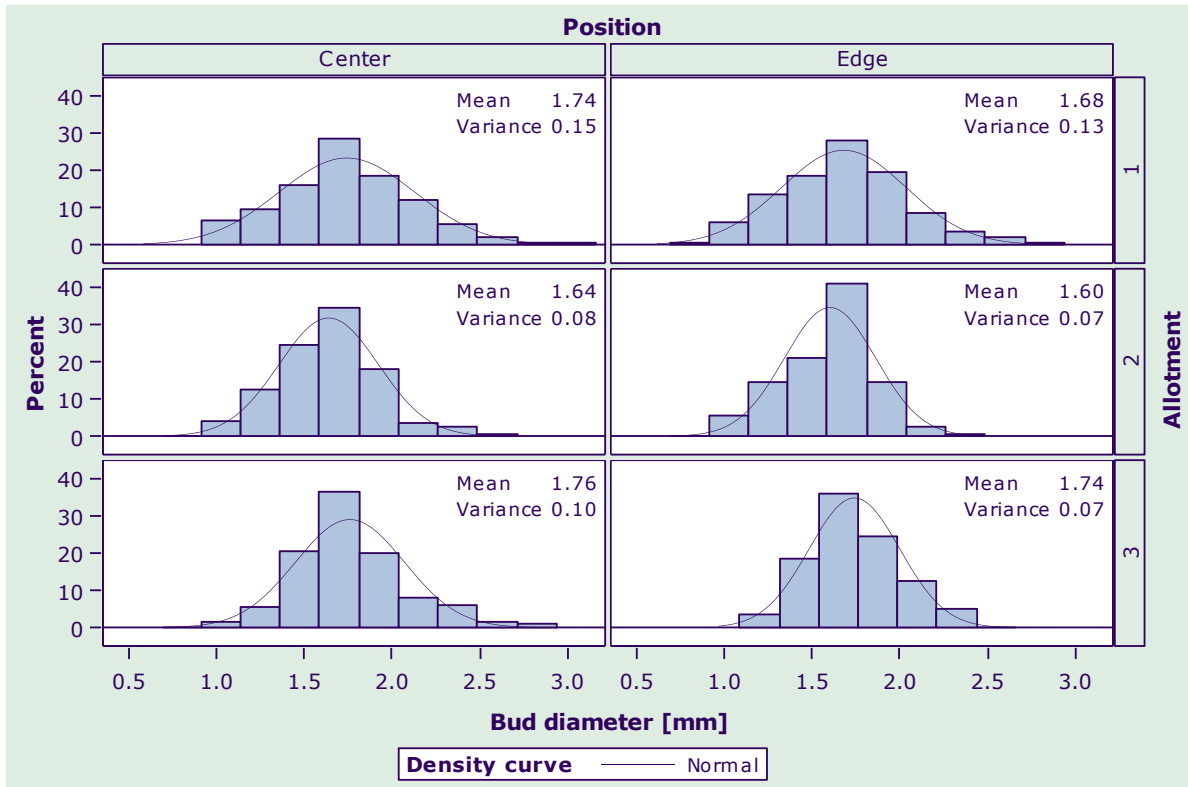
### 5.3. Physiology of broccoli curds

Yellowing not only varied between different broccoli batches, but also between individual curds in the same batch. The edible broccoli curd is a generative plant organ. In order to investigate if those differences among curds of the same batch were related to varying generative development, the diameters of both bud and curd sizes were measured. Results are presented in the following subchapter.

#### 5.3.1. Curd development

To determine the influence of the physiological curd stage on subsequent yellowing, broccoli with various curd sizes were harvested on three allotments of a commercial field in August 2011. All broccoli were planted at the same time, yet curds had clearly proceeded differently in growth and presumably in generative development (4.3.1).

The diameters of buds on the broccoli curds harvested in August 2011 ranged from 0.89 to 3.05 mm, following a normal distribution (Fig. 44). Regarding the three neighboring allotments of the field, the buds of broccoli curds harvested on the second and third allotment were more uniform compared to the buds of broccoli from the first allotment (Fig. 44). This was indicated by a greater variance of buds on broccoli harvested on allotment 1, as well as a flatter distribution of bud diameter classes (Fig. 44). The edge and center of broccoli curds showed a similar distribution of classes in accordance with the allotment where curds had been harvested (Fig. 44). Therefore, the position of the buds on the curd did not influence uniformity characteristics.

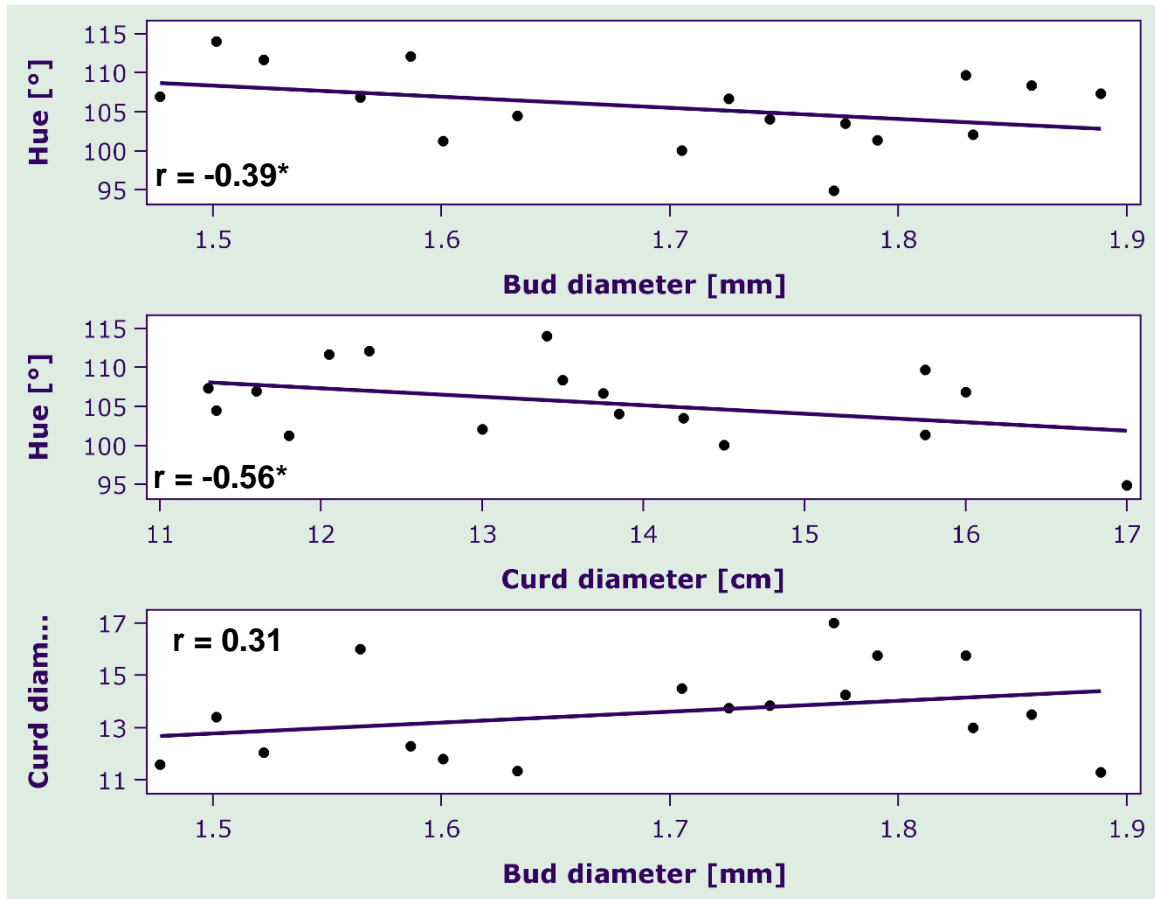


**Fig. 44:** The diameters of single buds of broccoli on different regions of the curd. Broccoli was harvested from different field allotments on August 4<sup>th</sup>, 2011.

The broccoli harvested from allotments 2 and 3 were further examined regarding the size of both curds and buds in relation to hue angle (Fig. 45). Curd diameters were between 11.3 and 17 cm. Bud diameters showed only a weak relationship to hue angle after 8 days of storage at 10 °C (Fig. 45). The relationship between curd diameters and hue angle was stronger, although still not very pronounced (Fig. 45). Curd and bud diameters did not correspond (Fig. 45).

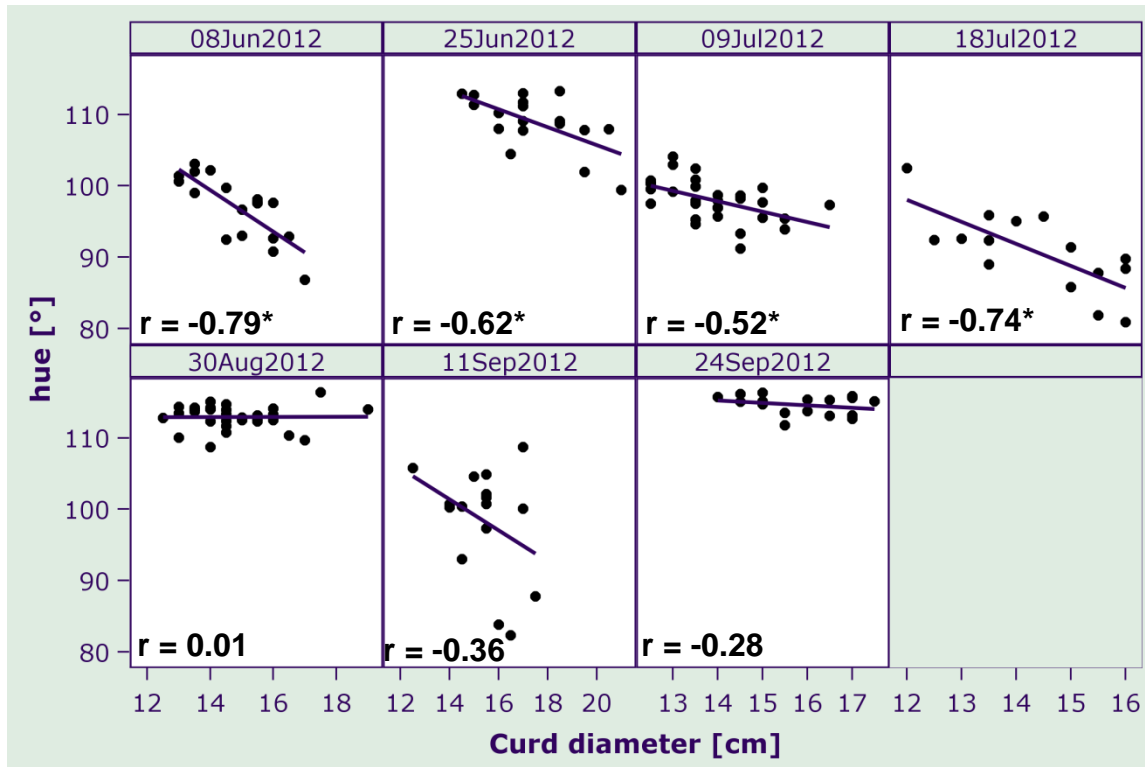
In investigations from the following growing season 2012, the relationship between curd diameter and hue angle was further evaluated. After harvest, the curd diameters of seven batches of broccoli harvested throughout the season 2012 (N = 264) were measured before they were transferred into storage chambers. The curd diameters were plotted against hue angle values after four days of DS (Fig. 45).





**Fig. 45:** The curd and bud diameters of broccoli harvested on August 4<sup>th</sup>, 2011, plotted against hue angle after 8 days of storage at 10 °C and high RH (~95 %). Asterisks indicate significant Pearson correlations.

There was a trend of larger curds resulting in lower hue angle values after four days of DS (Fig. 46). This was observed in four broccoli batches harvested during June and July (Fig. 46). The harvest batches of August 30<sup>th</sup> and September 24<sup>th</sup> retained their color throughout DS independent of curd size (Fig. 46). The hue values of broccoli harvested on September 11<sup>th</sup> showed a strong variation which was not related to curd size (Fig. 46).



**Fig. 46:** Curd diameter of seven broccoli batches harvested throughout the 2012 growing season and plotted against hue angle after 4 days of storage at 16 °C and high RH (~95 %). Asterisks indicate significant Pearson correlations.

Curd size played a clear role in subsequent yellowing during storage, although the effect was limited to broccoli grown in the earlier season (Fig. 46). The size of single buds, on the other hand, was only slightly correlated to yellowing during storage and had no relation to curd size.

### 5.3.2. Pigment composition

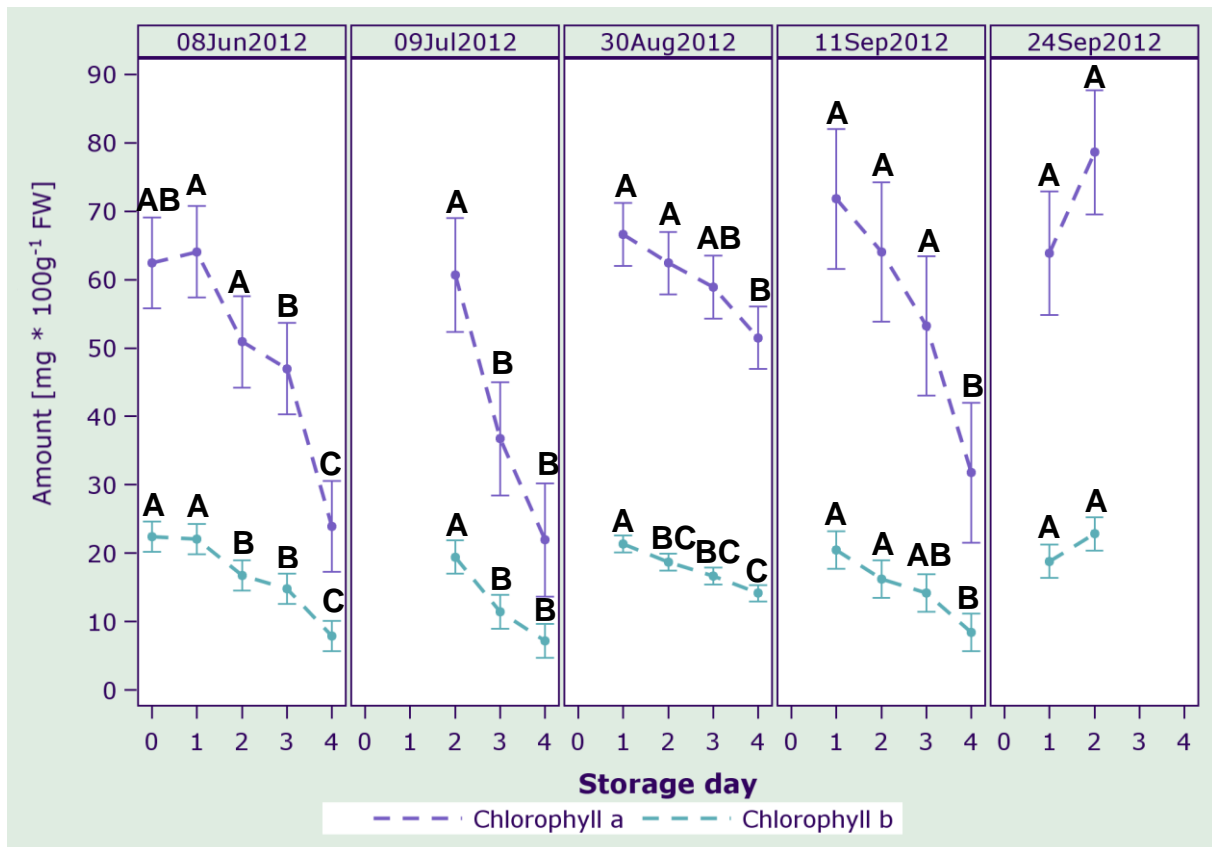
In harvested broccoli, photosynthetic pigments were analyzed by means of high pressure liquid chromatography (HPLC). Bud material of two to three broccoli curds from five harvest batches was used in repeat determination. The concentrations of green chlorophyll degradation products were determined by the use of a fluorescence detector next in line to the HPLC.

#### 5.3.2.1. Chlorophyll degradation

During DS, the chlorophyll concentrations of broccoli harvested throughout the 2012 growing season and studied for more than two storage days declined (Fig. 47). Chlorophyll concentrations of broccoli harvested in late September showed a tendency to increase from day 1 to day 2, which turned out to be insignificant (Fig. 47). On the first day of DS, chlorophyll concentrations in broccoli maintained their high level.

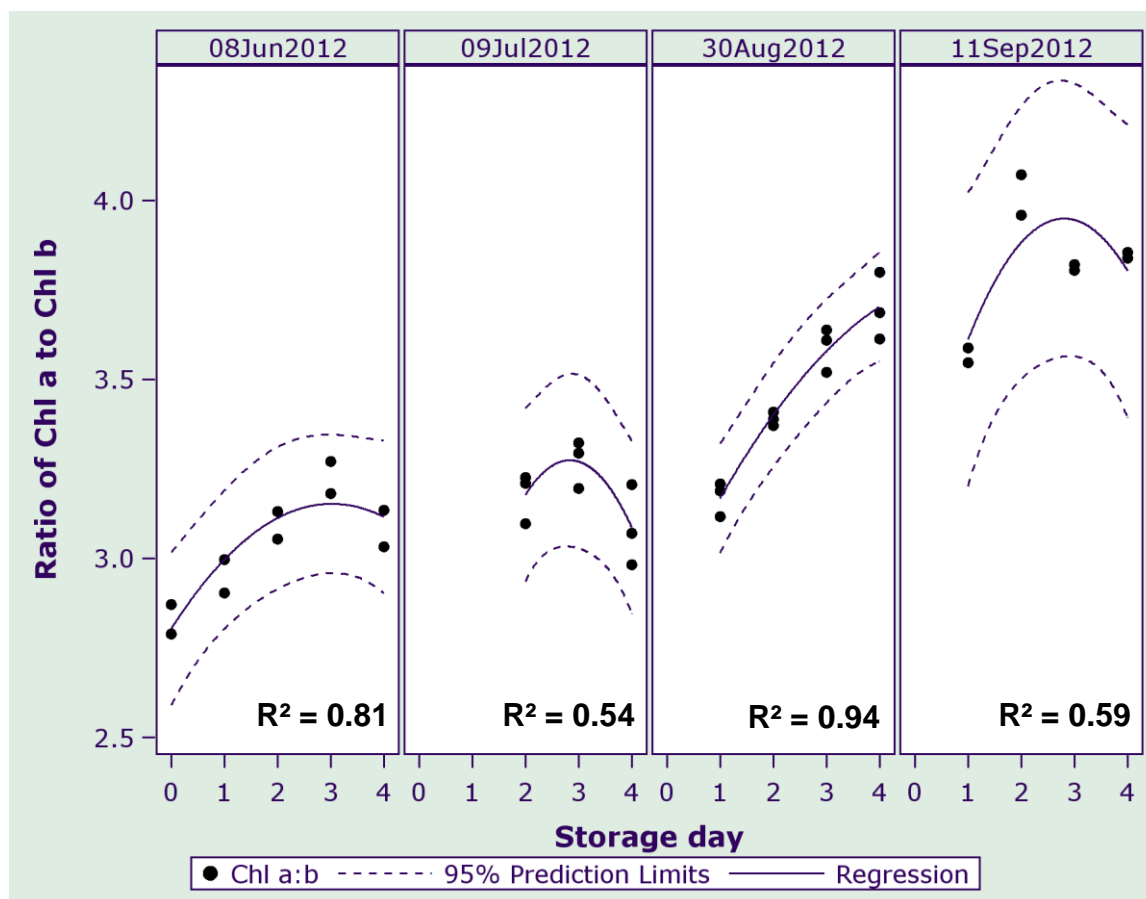
Concentrations ranged from 64 to 72 mg 100 g<sup>-1</sup> FW and from 19 to 22 mg 100 g<sup>-1</sup> FW for chlorophyll *a* and *b*, respectively (Fig. 47).

The first significant changes in chlorophyll *a* were observed on day 3, whereas significant changes in chlorophyll *b* already appeared on day 2 (Fig. 47). Broccoli florets from curds harvested in August showed a high level of chlorophyll *a* (52 mg 100 g<sup>-1</sup> FW) and *b* (14 mg 100 g<sup>-1</sup> FW) on day 4 of DS compared to other batches (Fig. 47).



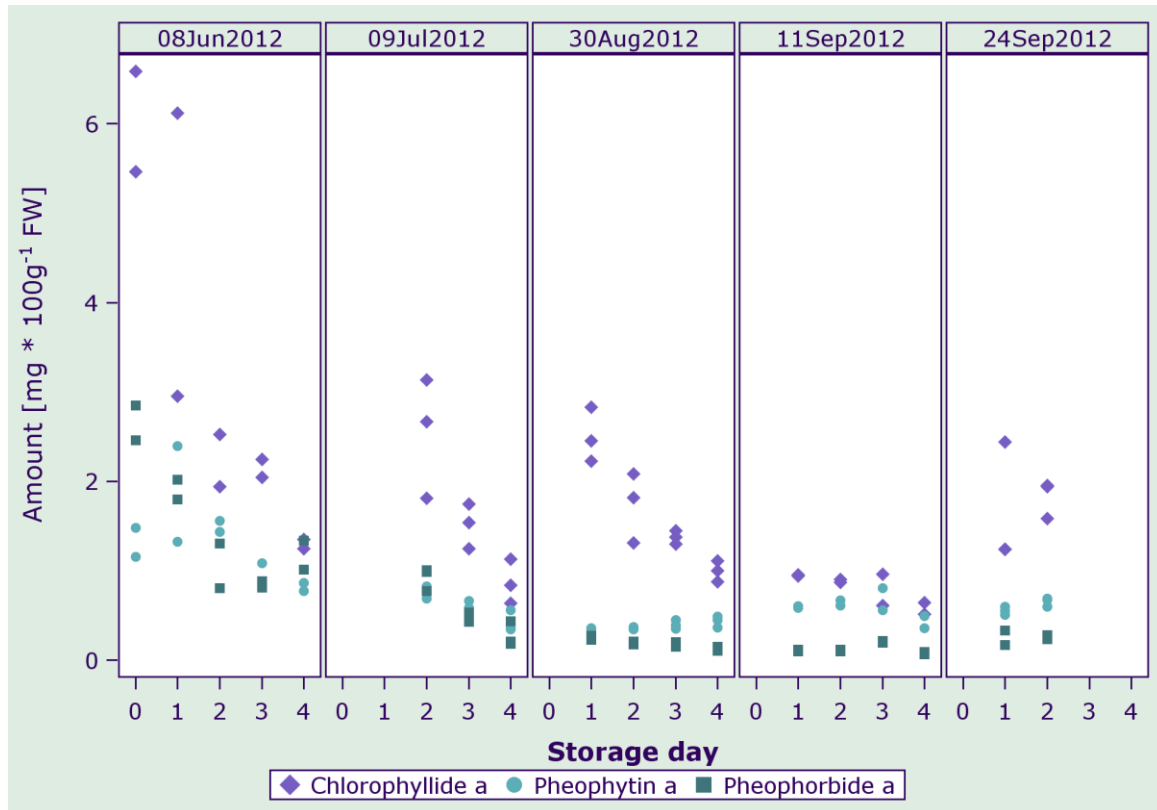
**Fig. 47: Concentrations of chlorophyll *a* and *b* in five broccoli batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). Mean ± Confidence interval (n = 2-3, repeat determination). Different letters indicate significant differences within one harvest batch.**

In order to highlight the specific relationship between chlorophyll *a* and *b* during DS, the ratio of detected amounts in single broccoli were calculated on the basis of molar terms (Fig. 48). By fitting a quadratic regression to the calculated ratios, it can be assumed that there was an initial phase where chlorophyll *a* rose in relation to chlorophyll *b* (Fig. 48). This phase of relative chlorophyll *a* enrichment was still present in the August harvest batch until the end of DS (Fig. 48). In broccoli harvested in June, July and September, the relative chlorophyll *a* enrichment appeared to have already passed its peak (Fig. 48).



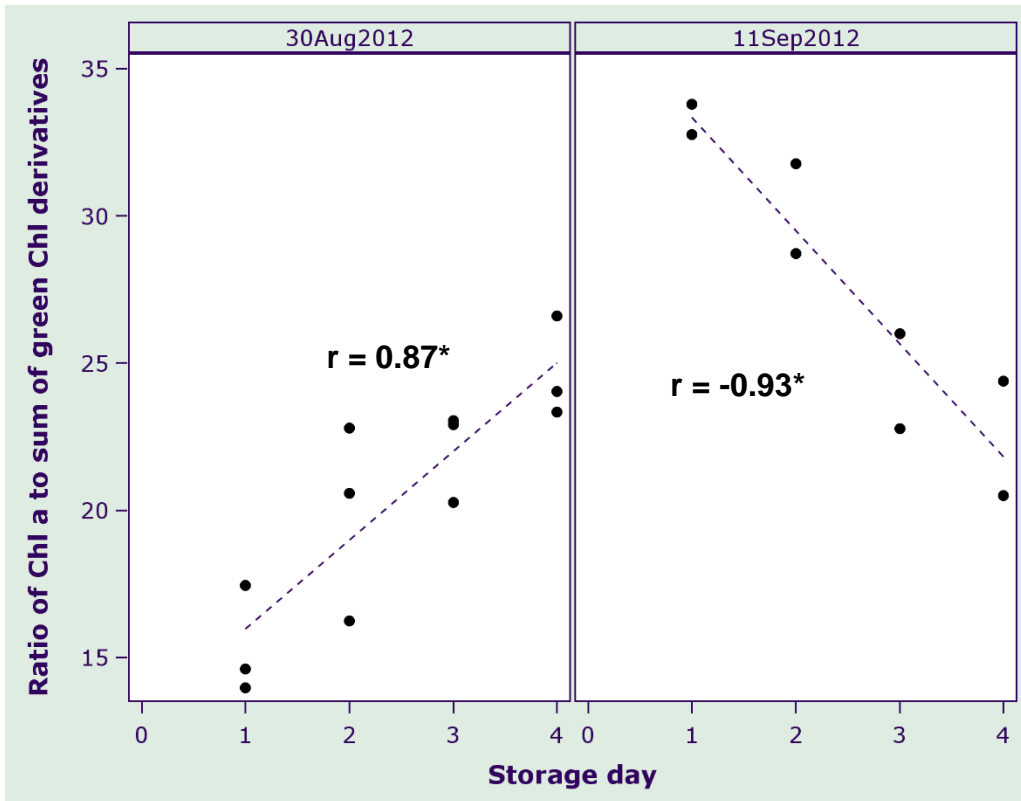
**Fig. 48: Chlorophyll *a/b* ratio based on molar terms. Concentrations determined in broccoli curds from four batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %).**

In the further degradation pathway, chlorophyll *a* may be degraded rather to Chlide or to Pheoin. The last green chlorophyll derivative is Pheoide, further catabolites are red or colorless instead (3.2.3). Detected concentrations of chlorophyll derivatives in the broccoli were in general very low compared to concentrations of chlorophylls *a* and *b* (Fig. 49). However, by using a fluorescence detector in combination with liquid chromatography, it was possible to study their amounts on ppm-scale (< 1 mg 100 g<sup>-1</sup> FW). Amounts of Chlide in broccoli harvested in June, July and August showed a declining trend during DS (Fig. 49). Chlide concentrations detected on the day of harvest, June 08<sup>th</sup>, were more than 4 times higher than on day 4 of DS. The next day one broccoli curd still contained a high amount of Chlide (6.12 mg 100 g<sup>-1</sup> FW), whereas the Chlide concentration of another broccoli was similar to that of other harvest batches on the first two days of storage (2-3 mg 100 g<sup>-1</sup> FW). These results suggest that Chlide might decline very quickly after harvest, compared to chlorophyll *a* and *b*. Absolute amounts of the chlorophyll catabolites Pheoin and Pheoid did not show much variation throughout DS (Fig. 49).



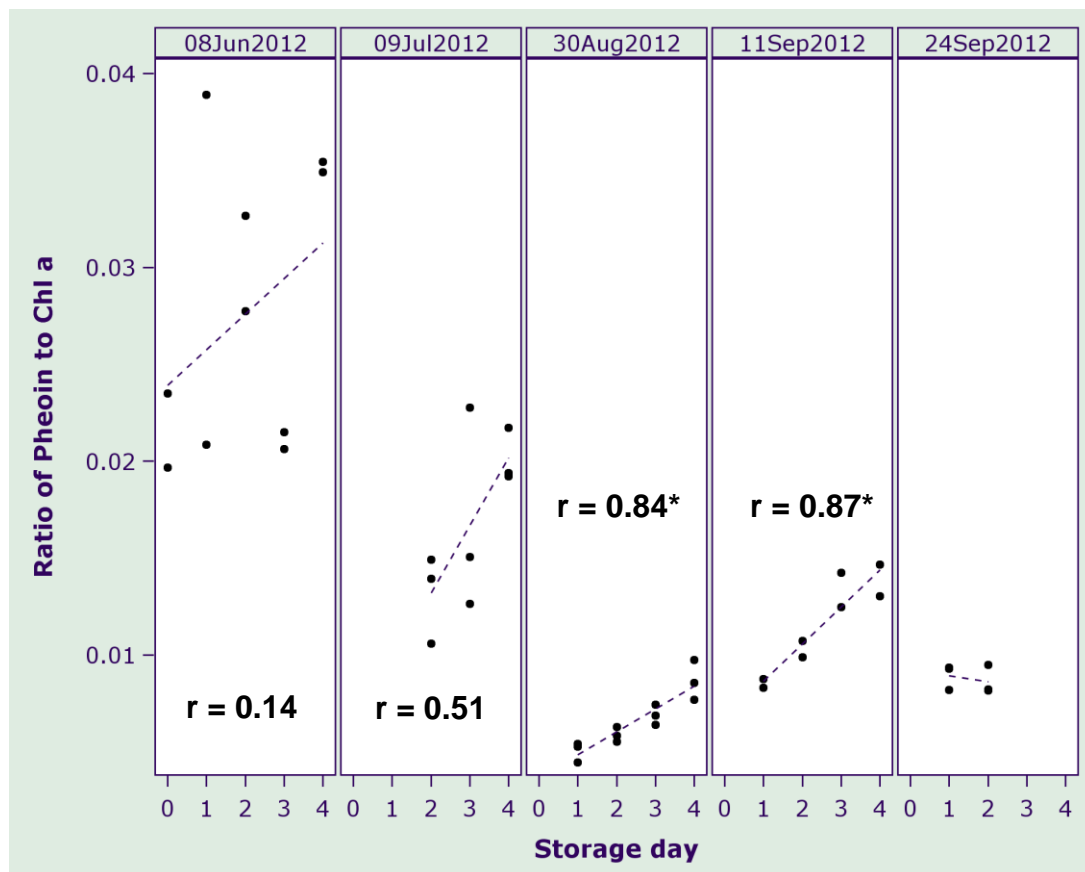
**Fig. 49: Chlorophyll derivatives detected in broccoli from five batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). Each dot represents the concentration in florets of a single broccoli.**

In addition to absolute amounts, the ratios of chlorophyll to its derivatives have also been considered in order to reflect possible decomposition patterns during storage (Fig. 50). Broccoli harvested in August showed an increase in the ratio of chlorophyll *a* to the sum of green chlorophyll derivatives during DS ( $r = 0.87$ ), whereas the ratio was declining in broccoli harvested in September ( $r = -0.93$ ; Fig. 50). As chlorophyll *a* is the precursor of green chlorophyll catabolites (3.2.3), a relative enrichment of chlorophyll *a* might indicate slow and moderate chlorophyll degradation. A quick trickle down along the chlorophyll degradation pathway would, on the contrary, result in a relatively higher proportion of chlorophyll derivatives over time and therefore a decline in the ratio. The gradients of chlorophyll *a* in relation to green chlorophyll derivatives appeared to accurately reflect both the green color retention (August) and color change (September) taking place in these two harvest batches (Fig. 19). In other harvest batches, no clear trend in the relation of chlorophyll *a* and its catabolites could be discerned.



**Fig. 50: Chlorophyll a divided by the sum of green chlorophyll derivatives in molar terms. Pigments were detected in broccoli curds harvested on August 30<sup>th</sup> and September 11<sup>th</sup>, 2012. Storage was conducted at 16 °C and high RH (~95 %).**

In figuring the ratio of Pheoin to chlorophyll a, a trend towards increasing proportions of Pheoin was detected (Fig. 51). However, correlations of storage day and ratio of Pheoin to chlorophyll a were only significant in the August and September harvest batches, but not in those of June and July (Fig. 51). The highest ratios of Pheoin to chlorophyll a were found in broccoli harvested in June (Fig. 51).



**Fig. 51:** Quotient of Pheoin divided by chlorophyll *a* in molar terms. Concentrations determined in broccoli curds of five batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %).

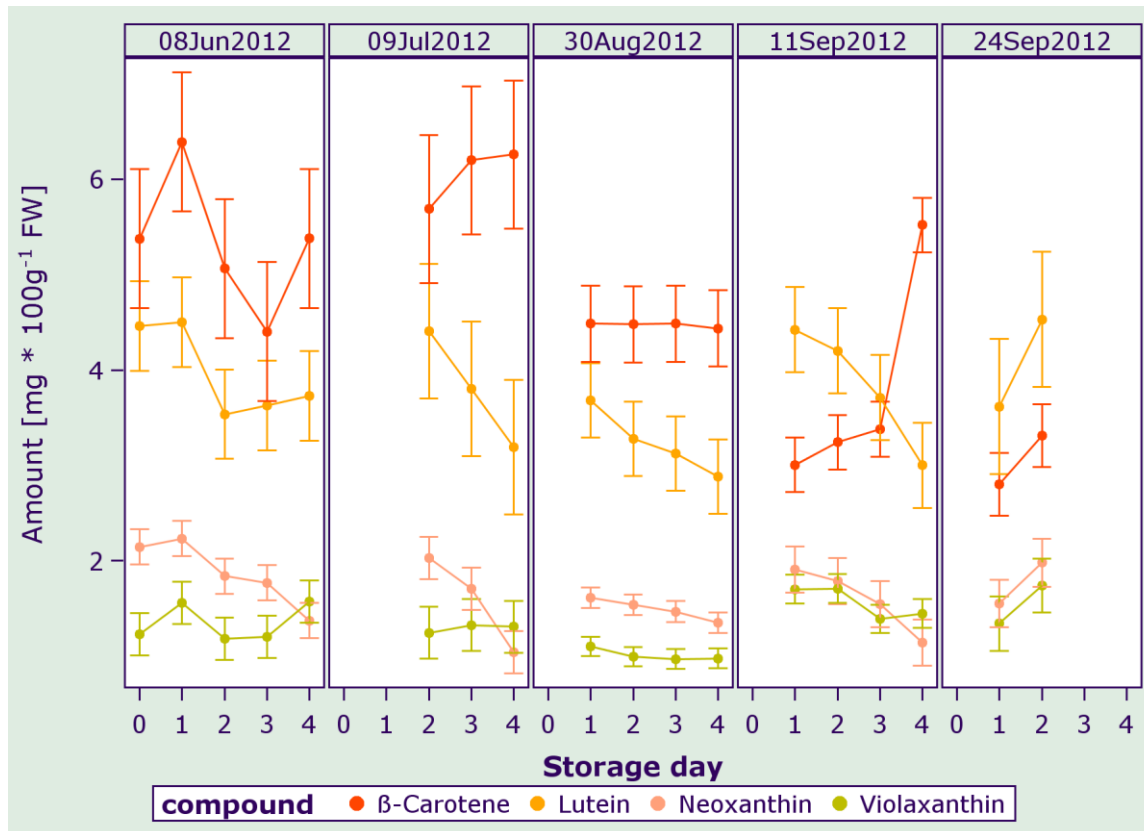
### 5.3.2.2. Carotenoids during storage

Carotenoids were analyzed in broccoli during DS in order to clarify which molecules were responsible for yellowing and if there was a rearrangement among them.  $\beta$ -Car, Lut, Nx and Vx were quantified in broccoli by means of HPLC in line with a diode array detector (Fig. 52). In June, July and August, the concentrations of  $\beta$ -Car were higher than concentrations of other carotenoids (Fig. 52). In broccoli harvested in September, Lut was initially found in higher amounts than  $\beta$ -Car (Fig. 52).

In addition to the carotenoids mentioned, the HPLC-chromatogram also indicated the presence of a carotenoid identified by its spectrum as auroxanthin (Aux). Aux was initially found in yellow varieties of *Viola tricolor* (Karrer & Rutschmann 1942) and may therefore also be located in the broccoli petals. Concentrations of Aux could not be quantified in the present work because standard solutions of Aux were not commercially available.

Regarding the progression of carotenoid concentrations in the harvested broccoli batches during DS, there was a trend of Lut decreasing during DS, which was significant in the early September harvest batch (Fig. 52). Additionally, Nx concentrations showed a decrease during DS, with significant changes on day 3 (June harvest) and day 4 (July and early September harvest) (Fig. 52). Vx concentrations remained at the same low level throughout DS (Fig. 52). Concentrations of Lut and  $\beta$ -Car in single broccoli varied a lot

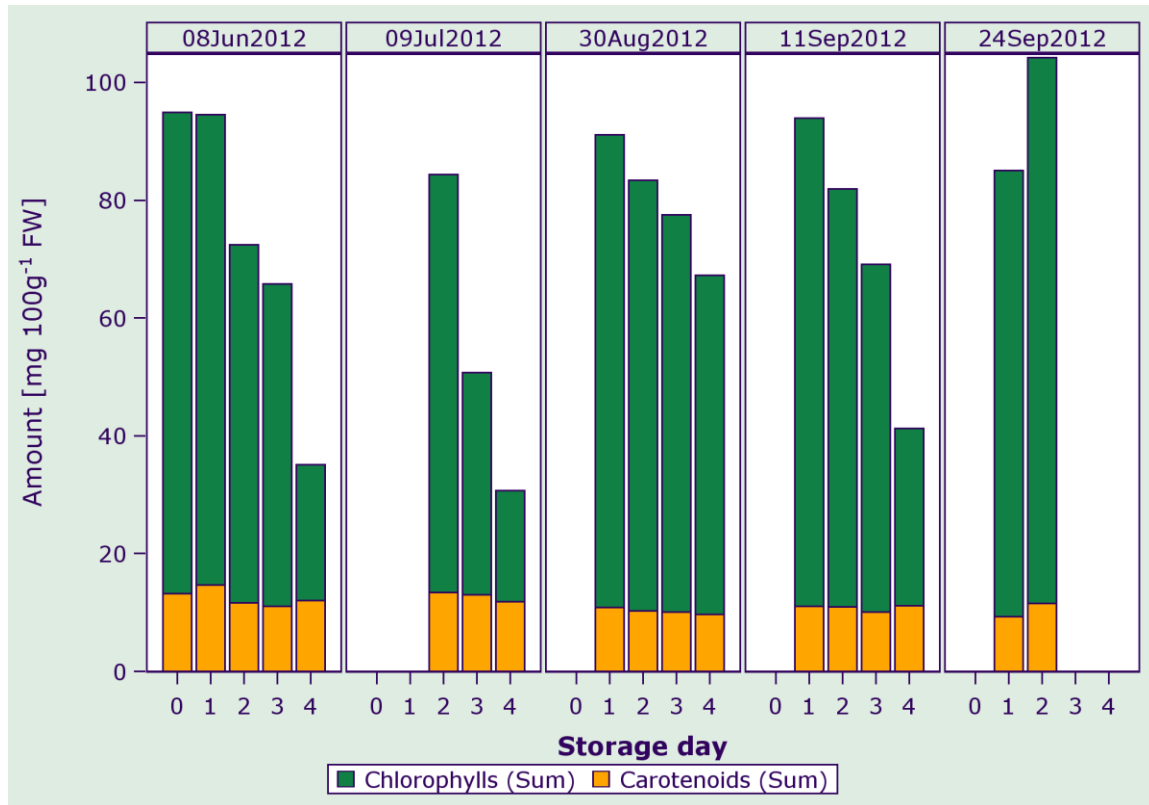
regarding harvest batches of June and July: In June,  $\beta$ -Car concentration was significantly higher on day 1 compared to day 3, but concentrations did not follow a reasonable pattern. In the early September harvest batch, concentrations of  $\beta$ -Car on storage day 4 rose significantly above the level of the other storage days (Fig. 52).



**Fig. 52: Levels of carotenoids in five broccoli batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %). Mean  $\pm$  95 % confidence interval (n = 2-3, repeat determination). Overlapping confidence intervals are not significantly different ( $\alpha = 0.05$ ).**

In summary, the changes in carotenoid concentrations were very small compared to changes in chlorophyll concentrations. When the two pigment groups were compared, the carotenoid changes were of no consequence, because chlorophyll was present in much higher concentrations and was, most notably, degraded to a much higher extent (Fig. 53). Chlorophyll is degraded down to colorless molecules which unmasked the carotenoids present in the plant tissue (3.2.3). It is therefore assumed, that the intensity of chlorophyll degradation was responsible for the level of yellowing in the broccoli curds analyzed.





**Fig. 53:** Sum of chlorophylls (mean chlorophyll *a* + *b*, Chlide, Pheoin, Pheoide) and carotenoids (mean  $\beta$ -Car, Nx, Vx, Lut) detected in broccoli from five batches harvested throughout the 2012 growing season. Storage was conducted at 16 °C and high RH (~95 %).

This relation can also be demonstrated by the chlorophyll and carotenoid profiles of single broccoli curds in relation to their optical appearance. On the first day of DS, chlorophyll *a* accounted for more than one half of total photosynthetic pigments, calculated in molar terms (Fig. 54). Chlorophyll and its derivatives together accounted for more than 80 % of photosynthetic pigments (Fig. 54). When the proportion of chlorophyll molecules decreased, carotenoids increasingly determined broccoli color (Fig. 54). It was therefore shown that the yellowing of broccoli was the result of the loss of chlorophyllous pigments and the concomitant increase in the proportion of carotenoids (Fig. 54).

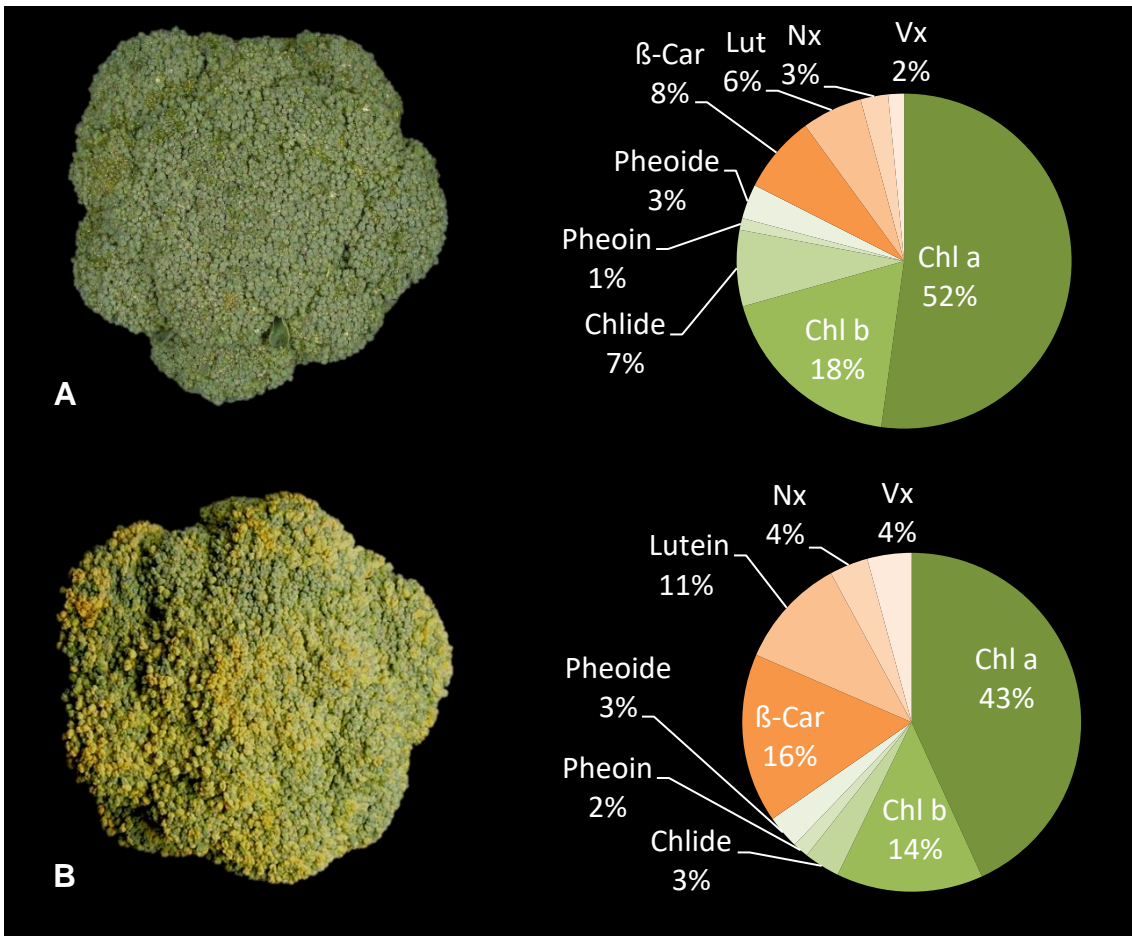


Fig. 54: The proportion of pigments (molar terms) in one broccoli curd harvested on June 8<sup>th</sup>, 2012 on harvest day (A) and on day 3 of storage at 16 °C and 95 % RH (B).

## 6. Discussion

### 6.1. Sensitivity of non-destructive indexes postharvest

The results presented in chapter 5.1 demonstrated that the storability of different broccoli batches harvested throughout the season 2012 showed a great deal of variation. This variation was detectable through all the optical measuring systems used in the present work. As shown in section 5.1, the correlation coefficients among the optical parameters hue angle, REIP and Fv/Fm increased as quality declined. In contrast, the indexes of broccoli which preserved green color were not related. Similar relationships have been detected among nondestructive indexes (Fm,  $a^*$ , NDVI) in two apple cultivars (Zdunek & Herppich 2012). Indexes of 'Pinova' apples that contained a wide range of chlorophyll contents showed higher correlation coefficients than indexes of 'Topaz' apples, which were more uniform (Zdunek & Herppich 2012).

Apart from a certain background noise, the ranges of uncorrelated values possibly indicated a superior sensitivity of some methods towards quality decline during storage compared to others. In this sense, a perfect correlation between the parameters would indicate that the information provided by different measuring systems was equal. In order to discuss which of the optical quality indexes was most sensitive, significant differences among parameters on successive storage days are considered in more detail in the following sections.

#### 6.1.1. Color measurements

With the help of CIELAB parameters, it was possible to detect very small differences in broccoli color which were hardly visible to the human eye. Particularly, the use of the parameter  $b^*$  made it possible to successfully detect initial changes at the very beginning of DS (Fig. 20). The index  $b^*$  represents the characteristics of colors on a scale ranging from blue to yellow, following the eye's color-receptive perception (Minolta 1996). Thus, an isolated aspect of color is able to rate differences in broccoli color very accurately in absolute amounts and to show differences between storage days and different harvest batches very quickly. Despite the quality differences in the batch harvested on July 18<sup>th</sup>, the initially detected values of  $b^*$  were similar to those of other investigations of broccoli reported in the literature. This held true even when different cultivars were used, e.g. 'Parthenon' and 'Monaco' (Gnanasekharan et al. 1992; Ren et al. 2006; Fernandez-Leon et al. 2012).

However, the single use of  $a^*$  and  $b^*$  has been criticized, as those are "merely coordinates that indirectly reflect hue and chroma but are difficult to interpret separately" (McGuire 1992).

In contrast, the hue angle value was designed to reflect the overall human perception of color, next to lightness ( $L^*$ ) and saturation (chroma) (Minolta 1996). Hue angle is the

parameter most widely used in the quality rating of broccoli (3.2). In its calculation, the hue angle takes the  $a^*$  value into account, which represents the aspect of color on a green to red scale (3.2.1.1). In this work, hue angle values were less sensitive to the differences in the color of single broccoli batches on consecutive storage days compared to the  $b^*$  parameter (Fig. 19 and Fig. 20). Statistical differences between  $b^*$  values occurred from the first to the second day of DS for broccoli harvested in June, whereas hue angle values only changed from the second to third storage day. Moreover,  $b^*$  values in the August harvest batch increased significantly, whereas hue values did not show any differences during DS. The reduced sensitivity of hue angle can be explained by considering the  $a^*$  value, which reacted quite slowly over the progression of storage days and was therefore not displayed in the results chapter.

Hue angle values of fresh broccoli measured with Minolta devices range from  $122^\circ$  to  $138^\circ$  in the literature (Costa et al. 2005b; Zaicovski et al. 2008; Büchert et al. 2011a; Gómez-Lobato et al. 2012c). In the present examination, all hue angle values measured on first storage day were lower. It is assumed by Shewfelt et al. (1984) that the hue angle of broccoli is highly dependent on the cultivar chosen. Initial  $b^*$  values reported in the above-mentioned literature varied less (value difference of 6) when compared to hue values (value difference of  $16^\circ$ ) which were similar to values collected here.

#### 6.1.2. Further spectral analysis, including imagery

The plot of normalized reflection spectra showed that the major difference that took place during storage was an overall increase in reflectance (Fig. 23). Using the original spectra for PCA, no change in the characteristics of spectral regions could be demonstrated. This was highlighted by the first PC, which explained almost the entire variation in all recorded spectra. Depending on the harvest batch, broccoli curds turned yellow to a certain extent during storage, which was indicated by a decline in hue angle values.

From 680 to 750 nm, there was a steep rise in reflectance, as is characteristic for spectra of green plant parts (Knipling 1970). A similar steep slope was observed by Hernández-Hierro et al. (2014) in broccoli spectra. However, the remaining spectral pattern obtained by Hernández-Hierro et al. (2014) in the visible range (450–680 nm) is rather flat. In the present work, a peak was observed at 550 nm, similar to the reflection spectra of lemon or different plant leaves (Lichtenthaler et al. 1996; Merzlyak et al. 1999). This may be due to the use of broccoli powder in the work of Hernández-Hierro et al. (2014) compared to the intact curds used here. Despite an overall increase in reflectance, the pattern of spectra did not change much. Thus, the changes in the diffuse reflection spectra of broccoli florets during storage resembled those reported for leaves with differing chlorophyll content (Lichtenthaler et al. 1996).

In other postharvest processes, e.g. ripening of apples and lemon or advanced senescence of leaves, the switch from green to yellow color is accompanied by distinct changes in the spectral signature (Merzlyak et al. 1999; Zude 2003). Postharvest changes in broccoli are known to show many similarities to leaf senescence on both the molecular

and biochemical levels (Page et al. 2001). Therefore, as long as pronounced color changes occur in broccoli during storage, a change in the character of reflection intensity in the visible range might be expected. There are two reasons for the absence of this change: First, the color impression is a result of visual perception of the human eye, which does not rate the entire visible spectrum in the same way, but according to three color-matching functions (3.2.1.1). Wavelengths below 500 nm are recognized to a higher extent than wavelengths of 500 to 680 (Fig. 6, Ohta & Robertson 2006b). When considering calculations of CIELAB with the help of color matching functions, even a perfect parallel translation of broccoli spectra would actually result in more perception of yellow. Additionally, the color matching function Z (see 3.2.1.1) comprises wavelengths below 450 nm, which could not be considered in the present study due to a high level of background noise. Wavelengths at the limits of the working spectral range are generally known to contain lower signal-to-noise ratios (Qin & Lu 2008; Sanchez et al. 2009; Lorente et al. 2012). Higher background noise might also have resulted from wavelength-dependent scattering effects in the tissue or from an insufficient spectrum of the illuminant.

The second reason why observed color is altered without pronounced changes in the spectral pattern is that mathematical ratios of reflection intensities do actually also change through an increase in overall intensity. Accordingly, in combination with visual perception, an overall increase in reflection within the visible range of spectra results in a color change relatively quickly. However, it should be noted that only uncorrelated information is generally grouped into different PCs. Hence, small changes in the spectral pattern, as well as parallel translation of parts of the spectra, are not described by this method.

PCA results of first and second derivative of spectra demonstrated that the red edge region (VIS/NIR-transition wavelengths) of the reflection spectra is suitable for more elaborate quality analysis of broccoli curds. This was indicated by the variance in the spectral readings, which was split into an increasingly higher number of PCs when derivatives of spectra were used (Table 5). The second derivative of broccoli reflection spectra has also been used by Hosaka et al. (2012) for the setup of a neural network that enabled the correct prediction of color change in broccoli.

During storage, the REIP of broccoli spectra declined significantly in all harvest batches except the one from September 24<sup>th</sup> (Fig. 25). In contrast, the NDVI of broccoli showed a significant decline only in broccoli curds of four batches, while the three other batches remained at the same level of NDVI (Fig. 26). Hence, the broccoli storability of different harvest batches could be judged more sensitively with the analysis of REIP than that of NDVI. So far, neither NDVI nor REIP have been used for postharvest quality assessment of broccoli by other authors. However, regarding their sensitivity, these spectral indexes did not indicate changes during storage before CIELAB parameters did.

In hyperspectral images, the increase of overall reflection intensity could be effectively used as an instrument for quality rating, because it took place on the curd in an

inhomogeneous fashion. This was indicated by the mean and variance of Euclidean distances in the visible range of hyperspectral images. The reasons for this heterogeneity are discussed in a later chapter dealing with generative development (6.2.1). Mean Euclidean distances were highly correlated with mean  $b^*$  values, which were very sensitive towards quality changes, as discussed above. It should be noted that the broccoli curds analyzed with the hyperspectral image system were still visually rated as marketable after four days of DS. Consequently, these findings contradicted the presumption in Hosaka et al. (2012), which was based on observations from Lipton and Harris (1974) that spot measurements cannot adequately rate the character of a whole broccoli curd. Moreover, the high correlation underlines the fact that the increase in yellowing and the increase in the variation of the broccoli curd surface go hand in hand.

### 6.1.3. Chlorophyll fluorescence measurements

The fluorescence indexes  $F_v/F_m$  and  $\Phi_{PSII}$  were both highly sensitive to changes occurring during storage. This was indicated by a significant decline in both parameters already between the first and second day of storage at 16 °C. For  $F_v/F_m$ , this early change was detected in broccoli from three out of seven different harvest batches. Additionally,  $\Phi_{PSII}$  indicated changes in broccoli harvested on September 24<sup>th</sup> (Fig. 33). No other non-destructive method indicated a decline in storability in this batch. This superior sensitivity corresponded to findings from Toivonen (1992) and, regarding the higher sensitivity of  $\Phi_{PSII}$  in comparison to  $F_v/F_m$ , to DeEll and Toivonen (1999).

On the other hand, broccoli harvested in August stayed at the same level of  $F_v/F_m$  values throughout four days of storage, whereas color and reflection indexes ( $b^*$ , REIP) had already shown significant changes over the same time. Based on the present results, the sensitivity of fluorescence measurements cannot be judged fully in comparison to color and reflection indexes.

Moreover, the levels of  $F_v/F_m$  values partly contradicted the rating of quality by means of color and reflection measurements. Broccoli curds harvested on July 18<sup>th</sup> were clearly distinguishable throughout the whole storage period by hue angle. This harvest batch was characterized by insufficient appearance and shape, as described in chapter 4.1.1. Nevertheless, it exhibited values of  $F_v/F_m$  similar to other batches (Fig. 32). The mean of its  $F_v/F_m$  values was still 0.76 on day 4 of storage at 16 °C, and the maximum of healthy plants has been reported to be 0.84 (Björkman & Demmig 1987). In this case, inferior quality of curds was not indicated by fluorescence measurements.

The use of fluorescence imaging led to an underestimation of the determined photosynthetic parameters. Values from the Fluorcam system were significantly lower compared to batches measured by the Minipam or Pam2000 devices (Fig. 32 & Fig. 33). It is possible that the saturation pulse light generated by an electronic shutter-equipped halogen lamp (250 W) was insufficient for closure of PS II. Broccoli curds might also have partly exceeded the optimum sample size, which should be smaller than 10 x 13 cm (PSI 2002). Therefore the value levels were not suitable for overall comparison. However,

fluorescence imaging provided rapid information about infections on the curd surface (Fig. 35). Fluorescence imaging has been widely used for the determination of similar infections in horticultural crops (Gorbe & Calatayud 2012). Yet, as far as the author is aware, no studies on fluorescence images of broccoli have been published so far.

#### 6.1.4. Prediction potential

When sensitivity was rated, the decisive factor in the evaluation was the first day on which statistical changes were observed. Nevertheless, indexes which changed on the same storage day did not end up at the same value level.

Hue and  $b^*$  values of seven broccoli batches harvested in 2012 split up into three and four statistically different subgroups, respectively, after DS (Fig. 21 and Fig. 22). Regarding the  $b^*$  value, four harvest batches from the year 2012 showed a significant increase initially on storage day 3 (Fig. 20). However, the comparison of  $b^*$  values among different batches on day 3 resulted in only two different statistical subgroups (Fig. 22). As discussed in subchapter 6.1.1, hue angle values reacted less sensitively to storage than  $b^*$  values, when values were analyzed within one batch throughout the course of storage. Yet, when compared among different harvest batches, the hue angle values split up into three statistical subgroups on storage day 3 (Fig. 21). Thus, a quick reaction to storage conditions can hardly be the only criterion for an exhaustive prediction method for final storability.

This assumption is also true for the non-destructive indexes REIP, Fv/Fm and  $\Phi_{PSII}$ , because high sensitivity at the beginning of storage did not indicate differences in final value levels (Fig. 25, Fig. 32, Fig. 33, Fig. 34). Regarding NDVI and  $L^*$ , initial values varied significantly, depending on the respective broccoli batch. However, these differences were not related to different storability, but to plant nutrition, which will be further discussed in subchapter 6.2.

In the literature, the storability of broccoli is often rated according to color change models based on Arrhenius' law (Weemaes et al. 1999b; Tijssens et al. 2001; Ren et al. 2006; Schouten et al. 2009). In the proposed model functions, time and temperature are fixed parameters which effectively determine kinetics of color change. Other parameters of Arrhenius' law, namely the activation energy and a preexponential factor, are then estimated, using the input parameters and observed rate constants (3.2.1.2). In the present work, the conditions of storage were constant. Therefore, the deviation in the obtained results of different harvest batches represented the proportion of variance that is not explained by those models. According to Arrhenius' law, the resulting variation in broccoli color after storage can only be interpreted through differences in activation energy and preexponential factors.

## 6.2. Predetermination of the broccoli curd

The prediction mechanisms that use non-destructive, optical devices may be supplemented with other characteristics of the broccoli curd, which can lead to preliminary determination of its storability. The results regarding floral development and levels of chlorophyll catabolites are discussed in the following chapter.

### 6.2.1. Curd morphology and development

A relationship between curd development and storability was indicated by a correlation analysis between broccoli curd diameters and final hue angle values on day 4 of DS. Based on Pearson correlation coefficients, a moderate relationship was observed both in 2011 and 2012, regarding the first four harvest batches (Fig. 45 & Fig. 46). The diameter of broccoli curds is often equated to generative curd development (Wurr et al. 1995; Mourao & Brito 2000; Leja et al. 2002; Zutz et al. 2013). Therefore, a certain level of influence of the developmental stage on storability might be assumed. Broccoli curds with smaller diameter probably contained higher chlorophyll contents at harvest, as shown by Leja et al. (2002). Assuming an equal degradation rate of chlorophyll in curds with different diameter, changes of hue angle would occur faster during storage in bigger curds.

On the other hand, curd diameter in the mature stage (before flowering) may not be related to development stage, but to the prevailing temperature conditions. Chilling during the juvenile phase may cause larger curds (Kalisz et al. 2014) while exposure to heat leads to smaller curd sizes (Heather et al. 1992). Therefore, broccoli of the same curd size does not necessarily have to be in the same developmental stage, especially when comparing different batches that have been exposed to various growing conditions.

Assuming that curd diameter is related to cultivation time on the field (Wurr et al. 1995; Grevsen 2000; Mourao & Brito 2000; Kałużewicz et al. 2012), it is also conceivable that microbial growth played an additional role in broccoli yellowing. Although yellowing is mainly believed to result from physiological processes of the plant, the contribution of microbial flora, mainly *Pseudomonas* species, is also the subject of speculation with regard to the yellowing of broccoli (Padaga et al. 2000). In blanched broccoli, microbial growth accelerates color change by producing acid (Gunawan & Barringer 2000). A longer exposure time of the curds might have resulted in higher microbial loads.

In the last three harvests of 2012, curd diameter and final broccoli color were not correlated. This was, on the one hand, due to a very good storability of all curds in the batches of August and late September, with hue angle values still exceeding 110° on day 4 (Fig. 46). In contrast, the batch harvested on September 11<sup>th</sup> varied greatly in hue angle, which was, however, not related to curd diameter (Fig. 46). The variability may be partly explained by covering with different plastic films, as discussed in the subchapter 6.3.2. However, the harvest batch from July 18<sup>th</sup> that was covered in the same way did show



high correlation coefficients of hue angle and curd diameter (Fig. 46). Thus, the variation of broccoli color within the harvest batch from September 11<sup>th</sup> remains an open question.

In addition to curd diameter, the rating of developmental stages of broccoli curds was conducted by measuring bud diameters, as explained in chapter 4.3.1. The mean bud diameters were in the same range as reported in the literature (Farnham & Björkman 2011; Kalisz et al. 2014). Buds from heat tolerant broccoli cultivars have been reported to be larger in diameter compared to heat sensitive cultivars (Farnham & Björkman 2011). When planting in summer, only two out of nine tested cultivars were considered to be of good quality (Farnham & Björkman 2011). These two experimental hybrids had mean bud sizes of 1.75 and 1.78 mm compared to cultivars with minor quality with bud sizes lower than 1.64 mm in the summer planting (Farnham & Björkman 2011). The 'Ironman' cultivar used in the present investigations exhibited mean bud diameters from 1.60 to 1.76 mm in the batch harvested on August 4<sup>th</sup>, 2011, depending on the field allotment and position on the curd (Fig. 44). Based on the bud sizes measured by Farnham and Björkman (2011), 'Ironman' should be regarded as only medium heat tolerant. However, typical symptoms of heat injury as described by Björkman and Pearson (1998) were not observed in the present investigations. Dufault (1996) considers bud size to be a very stable characteristic which is not affected by temperatures during the growing season, which corresponds to the present findings.

In this work, bud size was originally measured in order to define developmental stages of whole broccoli curds. Based on the findings of Tian et al. (1995) it was assumed that curds with greater flower buds were more advanced in their development and exhibited a shorter shelf life. Tian et al. (1995) determined the maturity stages of single broccoli flowers by grading pollen into three maturity groups. An accelerated rate of chlorophyll loss was noted in the larger buds, which most likely (63 %) contained mature pollen with pollen grains (Tian et al. 1995). Yet, in the present investigation, the correlation of mean bud diameter of broccoli curds and final hue angle values after 8 days of storage at 10 °C was weak (Fig. 45). In contrast, correlation coefficients with curd diameters were higher, as discussed above. Curd and bud diameters were not related at all (Fig. 45).

In addition, no differences were found between bud size distributions in the center and edge of broccoli curds (Fig. 44). Curds from the first allotment showed exactly the same bud size distribution both on their center and edge segments compared to allotments two and three (Fig. 44). Some authors differentiate between the edge and center of broccoli curds in their experiments when measuring shelf life (DeEll & Toivonen 1999; Bakker et al. 2009a; Schouten et al. 2009). Based on the results of bud size presented here, this division is unnecessary. According to Kieffer et al. (1998), different floral branches of broccoli curds evolve consecutively, following a Fibonacci spiral. These chronological differences in evolution were not reflected in different bud size distributions on separate curd sections (Fig. 44).

For the analysis of curd development, 60 buds from each curd were measured. With respect to the great number of flower buds on each curd, this sample size may still not

have represented the development status of whole curds. Tian et al. (1995) state that 70 % of the florets on their broccoli curds were in an intermediate pollen maturity stage. Most likely, the buds measured in the present work were mainly in an intermediate stage and were therefore not clearly affecting storability.

Nevertheless, the different stages of single floret buds support the rating of storability with the help of image analysis. Based on Euclidean distances in the hyperspectral image data of broccoli curds, different quality stages could be distinguished (Table 6). Pixel-wise variation in reflection intensity in the visible wavelength range rose during storage (Fig. 29). Tian et al. (1995) reported accelerated chlorophyll loss in florets that contained mature pollen. The variation observed within broccoli curds after four days of storage was therefore related to differences in the degree of senescence of single florets and the concomitant chlorophyll degradation in the sepals of single buds. However, the observed differences in Euclidean distances could not be distinguished through image classification procedures, because no independent dot clusters were observed (Fig. 30). This emphasizes the difficulty with strict classification of physiological stages (pollen maturity and senescence) on the broccoli curd. Physiological processes predominantly followed a smooth transition over time.

#### 6.2.2. Patterns of photosynthetic pigments

The amounts of chlorophyll *a* and *b* detected in broccoli were within the range reported by other authors who also used freshly harvested curds (Costa et al. 2005b; Kaosamphan et al. 2010; Aiama-or et al. 2012). The results of statistical analysis indicated that chlorophyll *b* was degraded prior to chlorophyll *a*: Significant changes in chlorophyll *b* were observed one day earlier over the progress of storage compared to those of chlorophyll *a* both in the June and August harvest batches (Fig. 47). This may result from the quicker degradation of the LHCs II compared to PS II. The LHC II contains relatively huge amounts of chlorophyll *b* (Schopfer & Brennicke 2006, p. 529). However, in most studies, chlorophyll *a* and *b* are analyzed simultaneously through one HPLC run or through spectroscopy using extinction coefficients, e.g. from Arnon (1949) or Porra et al. (1989). Therefore, the single determination of chlorophyll *b* does not provide any practical advantage. Yet, against this background, the correct determination of the proportions of chlorophyll *a* and *b* gains further importance in senescence determination. In this context, despite its excessive use, Arnon's equation has been criticized for its imprecise estimation (Merchant 2010).

In addition to LHC II breakdown, a fact that might be responsible for a higher chlorophyll *a/b* ratio is the degradation pathway of chlorophyll, where the conversion of chlorophyll *b* into chlorophyll *a* is regarded as a prerequisite (Scheumann et al. 1999; Tanaka & Tanaka 2006). A similar trend of an increasing chlorophyll *a/b* ratio has been observed during storage on barley seedlings (Scheumann et al. 1999). The increase in the chlorophyll *a/b* ratio was most pronounced in the harvest batch from August, as indicated by a steep slope (Fig. 48). Regarding the other batches, ratios can be

interpreted as reaching a plateau within four days of storage at 16 °C. Accordingly, hue angle dropped to the same low value level for the batches from June, July and September, whereas broccoli harvested in August remained high (Fig. 21). A high increase in the chlorophyll *a/b* ratio therefore indicated better storability in this work.

However, only the observations of chlorophyll *a/b* ratios over the course of storage were successful in distinguishing different patterns related to storage quality. The chlorophyll *a/b* ratio on the first storage day was not suitable for prediction. The level of the chlorophyll *a/b* ratio is believed to depend on radiation intensity, water and nitrogen status (Kitajima & Hogan 2003; Maina & Wang 2015). Shaded plants contain a higher number of LHCs with higher chlorophyll *b* concentration in order to exploit sufficient radiation for photosynthesis (Rühle & Wild 1985) and therefore exhibit a lower chlorophyll *a/b* ratio. Contrastingly, Maina and Wang (2015) reported that the chlorophyll *a/b* ratio of the desert plant *Haloxylon ammodendron* increased with seasonal courses in a three year study, regardless of light conditions. Also in the present study, there was rather a trend of increasing chlorophyll *a/b* ratios following the growing season (Fig. 48), which, however, did not correspond to the respective radiation sum (data not shown).

With the exception of Chlide, green chlorophyll derivatives were only found in very small amounts in the broccoli used in the present investigation (Fig. 49). Still, using a fluorescence detector in combination with liquid chromatography, amounts of chlorophyll derivatives were detectable at ppm scale. It has already been stated by Matile et al. (1999) that “[...] these compounds are detectable only in trace amounts (if at all), indicating that removal of phytol and the central Mg-atom is quickly followed by processes that cause the loss of green color.” Nevertheless, the appearance of chlorophyll derivatives has been used in order to rate postharvest treatments (Yamauchi et al. 1997; Costa et al. 2005a; Costa et al. 2006a; Aiamla-or et al. 2010; Kaosamphan et al. 2010; Aiamla-or et al. 2012) based on HPLC analysis with a UV/VIS detector. In comparison to the reported peaks in the respective chromatograms, it is assumed that the fluorescence detector used in the present study can provide far more precise results.

As shown in Fig. 49, Chlide concentrations declined in all harvest batches during storage, which is consistent with observations from Aiamla-or et al. (2010) and Kaosamphan et al. (2010). Chlide concentrations were particularly high on the day of harvest compared to amounts on the following storage days (Fig. 49). This may be due to the specific kinetic function of Chlide, which, in whole brined olives, was observed to exhibit an initial peak at an early stage of storage, followed by its decomposition (Heaton et al. 1996). Reactions in broccoli floret extract have also supported the evolution of Chlide (Yamauchi et al. 1997). It should, however, be taken into consideration that Chlide is also involved in chlorophyll synthesis (Schouten et al. 2002; Schelbert et al. 2009). Its concentration therefore may not be fully reliable for the exclusive rating of chlorophyll degradation.

In broccoli, several authors have detected accumulations of the compounds Phein and Pheide postharvest (Weemaes et al. 1999a; Weemaes et al. 1999b; Costa et al. 2005a; Costa et al. 2006a; Kaosamphan et al. 2010). In the present work, absolute amounts of

these two Mg-free compounds stayed nearly at the same level, which corresponds to the model from Schouten et al. (2009) instead, who assume that a steady state exists between generation from chlorophyll and decay to colorless compounds. Consequently, based on the absolute amounts of chlorophyll derivatives in this work, quality losses during storage cannot effectively be determined.

However, in relation to chlorophyll *a*, the harvest batches from August and early September showed patterns of either accumulation of or decrease in chlorophyll derivatives (Fig. 50). It is clearly indicated that broccoli of the August harvest batch showed increasing ratios of chlorophyll *a* to its derivatives over the course of storage. As described above, broccoli from the August harvest batch was of superior storability regarding both its color and photosynthetic capacity (5.1). There are reports about the accumulation of chlorophyll derivatives during storage as a prevention of senescence and as an outcome of so-called stay-green mutants (Schelbert et al. 2009; Kaewsuksaeng 2011). So far, an accumulation of chlorophyll *a* in relation to green chlorophyll derivatives has not been discussed. In the September harvest batch, chlorophyll *a* concentrations quickly decreased in relation to green chlorophyll derivatives, which might have indicated a fast trickle-down along the chlorophyll degradation pathway. Yet the remaining broccoli batches did not show a comprehensible progression of ratios of chlorophyll *a* and its derivatives (data not shown).

Pheophytinization has been identified as the preferential pathway of chlorophyll breakdown in broccoli (Schelbert et al. 2009; Büchert et al. 2011a). This assumption is supported by a general increase of Pheoin/chlorophyll *a* ratios in all harvested broccoli batches during storage (Fig. 51). In accordance with other results regarding storability ratings, the pheophytinization rate was the lowest in the August harvest batch. Yet, Pheoin/chlorophyll *a* ratios of the quickly degrading broccoli harvested in June varied over storage. Inconsistent patterns of pheophytinization may result from sudden changes in pH during the senescence process, when chlorophylls are transported to the more acidic vacuole, as Costa et al. (2006b) assume, based on observations from Guiamét et al. (1999) in soy beans. Pheophytinization, which is the process of dissolution of the central Mg<sup>2+</sup>-ion of chlorophyll molecules, is highly promoted by an acidic environment (Gunawan & Barringer 2000). Besides, it should be noted that Pheoin is not only a chlorophyll degradation product during senescence, but also the primary electron acceptors in reaction centers of PS II. A certain variation in Pheoin concentrations during storage, as well as different initial levels after harvest, may result from varying amounts of PS II centers in single broccoli curds or entire batches.

Carotenoids were present in considerable amounts in the investigated broccoli batches, with  $\beta$ -Car and Lut as the most prominent substances (Fig. 52). Vx and Nx were also detected, in lower quantities. These proportions adequately reflect the carotenoids present in thylakoid membranes of chloroplasts (Schopfer & Brennicke 2006, p. 175). The results correspond to investigations of Fernandez-Leon et al. (2012), where  $\beta$ -Car and Lut were also the most abundant carotenoids detected in broccoli. Yet, the concentrations of both carotenoids and chlorophylls reported by Fernandez-Leon et al. (2012) were much

lower. They differ by up to one order of magnitude from the concentrations determined in this work. Kurilich et al. (1999) detected 0.4 to 2.4 mg 100 g<sup>-1</sup> FW  $\beta$ -Car in various broccoli accessions, which corresponds to about 50 % of the concentration found in this work. Cogo et al. (2011) detected between 2 and 5 mg 100 g<sup>-1</sup> FW total carotenes in 'Green Star' broccoli, which is also less than in the present work. In contrast, Nath et al. (2011) report detecting about more than 25 mg 100 g<sup>-1</sup> FW  $\beta$ -Car in broccoli. Consequently, it is difficult to judge the meaning of the level of carotenoid concentrations measured in the present work and to thereby draw a conclusion.

During storage, the declining trend in  $\beta$ -Car observed in the present work corresponds to results of Nath et al. (2011) and Cogo et al. (2011) regarding broccoli stored at temperatures of 15 °C and 22 °C, respectively. Barth and Zhuang (1996) report a decline in total carotene concentrations of broccoli stored at 5 °C which also corresponded to decreasing moisture content, caused by different package materials. In the present investigation, the total carotenoid contents decreased only very slightly. The reaction was insensitive and by far incomparable with losses of chlorophyll, as shown in Fig. 53. Additionally, the carotenoids showed higher variation throughout storage than chlorophylls, which parallels observations made by Cogo et al. (2011).

Remarkable, however, was the detection of auroxanthin (Aur). Aur was first detected in petals of *Viola tricolor* (Karrer & Rutschmann 1942). Assuming that the presence of Aur indicates emerging petals of broccoli buds, its concentration is certainly of interest for senescence determination. Investigations discussed in the previous subchapter (6.2.1) could eventually be supplemented with further conclusions about Aur. There are, on the other hand, also reports about the appearance of Aur during the processing and storage of mango, papaya and olives (for a detailed overview, see Yahia and Ornélas-Paz (2009)). Further studies are therefore necessary to investigate patterns of Aur during broccoli storage and their value for senescence indication.

It was clearly shown that carotenoids determined the color of broccoli when chlorophyll diminished (Fig. 53). Therefore, changes in color of broccoli during storage resulted from the unmasking of carotenoids through chlorophyll loss, as reported by Matile et al. (1999).

### 6.3. External factors on the field affecting storability

In this work, the time it took broccoli plants to develop a mature curd ready for harvest closely followed a seasonal trend towards shorter cultivation time in the peak season (Fig. 40). A similar relationship has already been determined by Marshall and Thompson (1987) and Tan (1999). The shorter cultivation periods were probably related to a necessary temperature sum that was achieved earlier in summer (Fig. 40). However, subsequent storability did not follow any seasonal trend, as was demonstrated by the extensive quality measurements throughout the 2012 season. The progress of quality decline could be monitored in great detail with several non-destructive methods and also through chlorophyll degradation. Nevertheless, the prediction of further storability based on both postharvest quality assessment and inner specifications of the broccoli curd still

posed many difficulties. Interactions between broccoli storability with climate and cultivation effects were investigated with the help of a database consisting of both experimental and on-farm measures linked to the climate data from different planting sites.

### 6.3.1. Radiation and temperature during floral initiation

In the literature, both radiation and temperature have been reported to have diverse effects on the quality of broccoli, regarding both climatic conditions on the field as well as treatments during storage (3.1.1 & 3.1.3). In this work, the results indicate that the radiation intensity that broccoli plants received in the field played an important role for subsequent storability. The effect of high radiation intensity (over  $600 \text{ W m}^{-2}$ ) was found to be detrimental to storability (Fig. 42). Regression analysis of hue angle values after DS indicated a moderate negative dependency ( $R^2 = 0.47$ ) on high radiation intensities on the field. This result is supported by the report from Topcu et al. (2015). UV-radiation during vegetative growth reduced storability and promoted color changes as indicated by  $L^*$  and  $C^*$  values after 60 days of storage at  $0 \text{ }^\circ\text{C}$  and 90-95 % RH (Topcu et al. 2015). A particular effect of high radiation quantities on broccoli quality is observed by Leja et al. (2002). Among other parameters, higher chlorophyll contents were found in broccoli curds grown in spring under a higher radiation intensity than in autumn (Leja et al. 2002). In the present work, initial chlorophyll contents of 5 different batches after harvest were within the same range, independent of radiation or temperature (see also chapter 6.2.2). This result parallels observations from Schonhof et al. (2007). Moreover, small differences in chlorophyll content, as might have been indicated by initial  $L^*$  and  $\text{NDVI}^*$  values (5.2.2), did not lead to differences in storability in the end.

The detrimental effect of high radiation intensity in this work was limited to a time period of 16 to 24 days before harvest, which is associated with the approximate phase of early curd development (Heather et al. 1992). During this phase, broccoli plants are known to be susceptible to high temperatures, causing misshapen flower buds (Björkman & Pearson 1998). However, the analogous effect of high temperature on hue angle after storage was not very pronounced (Fig. 41,  $R^2 = 0.29$ ). Yet, the relationship is closer than that in investigations of Kałużewicz et al. (2009), who assumed that temperatures from  $20$  to  $25 \text{ }^\circ\text{C}$  in the last third of the cultivation period caused loose broccoli heads, based on a low correlation coefficient ( $r = 0.32$ ). When using polynomial models, Dufault (1996) reports higher determination coefficients for unacceptable head color, depending on average maximum temperatures during cultivation time. In that case, the head color of some cultivars at harvest was unacceptable when the average of maximum daily temperatures exceeded  $26 \text{ }^\circ\text{C}$ . In the present investigation, hue angle values were in a similar range shortly after harvest (except July 18<sup>th</sup>, 2012) and varied greatly only as storage progressed (Fig. 19). Thus, in the present work, only storability was affected whereas quality at harvest remained relatively similar.

Kałużewicz et al. (2009), Dufault (1996) and partly also Björkman and Pearson (1998) used broccoli from field experiments, where the effect of radiation and temperatures cannot be considered independently. It is therefore possible that quality aspects in those investigations were also influenced by high radiation, in addition to heat.

Nevertheless, with regard to storability, the present work shows that radiation and temperature intensity during early curd growth resulted in detrimental effects on broccoli storability. Björkman and Pearson (1998) state that the impact of heat on floral development resulted from the weakening or disruption of gene expression during the initiation of floral bud enlargement. Based on findings from Carr and Irish (1997) and Shannon and Meeks-Wagner (1993) it was assumed that for this process, the genes *BoiAP3* or *tfl* may be responsible. Hasperué et al. (2013), who studied the influence of the time of day at harvest on the storability of broccoli, assume that the expression of genes was influenced not only during the day, but also postharvest. It is therefore assumed that different gene expression during an earlier period of cultivation may also affect subsequent storability.

### 6.3.2. Light quality during curd growth

Investigations of light quality conducted in this work suggest a specific wavelength effect on the storability of broccoli curds. The quality characteristics of broccoli varied when grown under differently colored plastic films (Fig. 36). As shown in the materials section, the differences were not caused by different temperature or RH when compared to uncovered plants (Fig. 12). Concerning equal temperatures under crop covers, observations parallel those of Francescangeli et al. (2007) who studied the effects of black shading meshes on broccoli plants.

When blue plastic covers were put on the plants, broccoli curds showed inferior quality parameters after four days of storage at 16 °C when compared to at least one of the other cover variants (Fig. 36). The broccoli grown under blue covers were lighter in color than uncovered broccoli and more yellow (higher  $b^*$  values) than all other variants. Spectroscopy indexes REIP and NDVI were lower in broccoli grown under blue compared to green covers. In addition, the photosynthetic activity was lower than under transparent ( $\Phi_{PS II}$ ) and green (Fv/Fm) covers (Fig. 36).

Broccoli illuminated exclusively by blue light for some hours every day from early curd growth until harvest received a relatively higher portion of light through its blue-light photoreceptors, the cryptochroms. Cryptochroms are proteins that enable light reception and are known to be responsible for training the circadian clock of plants and animals (Devlin & Kay 1999). After harvest, cabbage and kale still undergo a circadian rhythm which supports the maintenance of defense mechanisms against herbivory (Goodspeed et al. 2013) and prevents chlorophyll degradation (Liu et al. 2015). In the current study, broccoli storage was conducted in darkness (Fig. 9). Constant darkness in broccoli storage is known to have a negative effect on chlorophyll content retention in comparison to constant light or simulated day/night conditions (Büchert et al. 2011c; Zhan et al. 2012;

Jin et al. 2015; Liu et al. 2015). Based on the present results, it can be speculated that the influence of blue light on the field confounded the circadian rhythm of broccoli curds and therefore led to accelerated chlorophyll degradation during dark storage.

Ma et al. (2014) examined the application of blue LED light on broccoli during storage. LEDs emitted the same wavelength range as the plants received under the blue plastic films used in the present investigations. However, Ma et al. (2014) found no effects of blue light illumination on chlorophyll retention in broccoli when compared to illumination by white light LEDs. In investigations from Hasperué et al. (2016) white-blue LEDs prolonged the shelf life of broccoli stored at 22 °C in terms of color and chlorophyll retention. Jin et al. (2015) state that blue LED light accelerated the yellowing of broccoli during storage, yet data is only presented for other light variants.

In addition to cryptochroms, light in the blue wavelength range is also absorbed by carotenoids for photosynthesis. As discussed in subchapter 6.2.2, broccoli curds turn yellow as chlorophyll degrades and carotenoid components are unmasked. If curds that were grown under blue covers contained higher carotenoid contents, the yellow color could have appeared earlier, indicated by higher  $b^*$  values. This is, however, not very likely because the absolute amount of light was reduced by plastic film covers (Fig. 11), compared to uncovered crops.

Broccoli grown under green plastic film covers retained higher values of spectral indexes NDVI and REIP on the last day of storage when compared to the other variants (Fig. 36). Both spectral indexes target, on the one hand, wavelengths near the second absorption peaks of chlorophylls which are located at 643 nm and 662 nm for chlorophyll *b* and *a*, respectively (Schopfer & Brennicke 2006, p. 176). On the other, wavelengths of the NIR are considered, for which about 50 % of the incoming radiation is reflected due to intercellular ranges (Knipling 1970). In the present work, the differences in REIP and NDVI are related to varying reflection intensity in the (visible) red range, whereas reflection in the NIR range is similar for both green covered and uncovered broccoli curds (data not shown). It is not quite clear why green light on the field in a wavelength range of 450 to 600 nm influenced chlorophyll retention during storage. When selective green light is simply interpreted as shade, lower chlorophyll *a/b* ratios and thinner petal tissues can be expected (Rühle & Wild 1985). These characteristics are, however, not associated with higher absorbance in the red wavelength range. It should be noted that the differences among spectral indexes, although statistically significant, were very narrow and did not lead to differences in yellowing or photosynthetic activity (Fig. 36). In examinations of Jin et al. (2015), the application of green LED light ( $\lambda = 520\text{nm}$ ) preserved the color of broccoli during storage at 25 °C. This was indicated by both lower  $L^*$  and higher hue angle values on the second day of storage when compared to broccoli illuminated by fluorescent light or stored in the dark. The most noticeable finding in the report of Jin et al. (2015) is a much higher content of phenols in broccoli stored under green light. Yet, the further mechanisms and the general impact of green light on broccoli storability remains an open question.



### 6.3.3. Plant nutrition

As described in subchapter 3.1.4, the application of considerable amounts of fertilizer is vital for profitable broccoli production. On the other hand, nitrogen fertilizer may have negative effects on broccoli quality, causing hollow stem disorder or soft rot (Hipp 1974; Bakker et al. 2009a). According to observations from the commercial grower who provided the broccoli curds for the present investigations, high nitrogen levels are likely to have a negative effect on curd storability. Although nitrogen concentrations in the broccoli tissue were not investigated here, a strong influence of N-fertilization on curd parameters was observed: Initial value levels of both NDVI and  $L^*$  were highly dependent on nitrogen fertilization, following a parabolic function (Fig. 38 & Fig. 39). A similar parabolic relation is well known as the typical nitrogen response curve of crop yield (see e.g. DEFRA 2011) and is also reported for yield, ascorbic acid and nitrate content in broccoli (Babik & Elkner 2000; Fortier et al. 2010). Following the function used in the present work, broccoli fertilization with up to 10.4 g nitrogen per plant led to darker color (lower  $L^*$  values) and higher ratios of reflection intensity at wavelengths 675 and 780 nm (higher NDVI values) (Fig. 38 & Fig. 39). It is possible that both low  $L^*$  and high NDVI values represent higher chlorophyll contents in broccoli curds. Nitrogen is essential for chlorophyll formation because it is incorporated into the chlorophyll molecule. Chlorophyll has high light-absorbing properties (Schoefs 2002; Hörtensteiner & Kräutler 2011) which may lead to a darker appearance and therefore to lower  $L^*$  values. The second absorption peak of chlorophyll is located at 643 nm and 662 nm for chlorophyll *b* and *a*, respectively (Schopfer & Brennicke 2006, p. 176), and therefore has a strong influence on the wavelengths that determine NDVI.

However, on day 4 of storage, indexes  $L^*$  and NDVI were no longer related to levels of N-fertilization (Fig. 38 & Fig. 39). The analysis of NDVI and  $L^*$  in the broccoli batch from July 9<sup>th</sup>, 2012, which was cultivated under varying fertilizer application (4.2.1) confirmed the minor role of N-fertilization in storability.

Although  $L^*$  values of low fertilized broccoli curds were significantly higher throughout the whole storage period in comparison to curds from adequate or elevated fertilization levels, all three variants declined in parallel to each other (Fig. 37). There was, therefore, no interaction between the factors storage day and fertilizer (see also Table 7). NDVI values performed similarly, although they showed an additional difference between broccoli which were over-fertilized and optimum supply broccoli (Fig. 37). Therefore, NDVI seems to be better suited for the evaluation of different fertilizer levels than  $L^*$ . The use of NDVI for evaluation of nitrogen status in broccoli has been the subject of much controversy. El-Shikha et al. (2007) conclude that analysis of NDVI measured by remote sensing could not differentiate between effects of insufficient water or nitrogen supply in broccoli plantations. A combination of NDVI and the normalized difference far red index (NDFR) is better suited for separate evaluation of water and nitrogen effects (El-Shikha et al. 2007). It should, however, be noted that El-Shikha et al. (2007) analyze the indexes on field scale, where distinctions of soil and plant canopy played additional roles. Similarly,

NDVI in winter wheat was affected equally by both N-fertilization and irrigation during different growth stages (Gebbers et al. 2013). The investigations of Gebbers et al. (2013) also showed an effect of N-fertilization on REIP in winter wheat, which was not found in broccoli in the present study. NDVI measurements of single broccoli curds have not been published so far. Lightness  $L^*$  has been used by some authors for the evaluation of postharvest treatments of broccoli (Costa et al. 2006a; Büchert et al. 2011c; Fernández-León et al. 2013b). In the present investigations, the sensitivity of  $L^*$  values towards quality changes during storage was lower than that of  $b^*$  values. Thus,  $L^*$  is only displayed as related to the rating of nitrogen fertilizer and not for storability detection *per se*. There are no reports about the use of  $L^*$  for rating of N-status in broccoli so far.

As shown in Table 7,  $b^*$  values were significantly influenced by the interaction of fertilization and storage. Yet, further statistical analysis by means of pair-wise comparisons does not clarify the actual effect on differently fertilized curds. Graeff et al. (2008) estimated the nitrogen status of broccoli plants using  $b^*$  values. Higher fertilizer doses led to lower  $b^*$  values in their investigations (Graeff et al. 2008). However, storability was not evaluated in this context.

Bakker et al. (2009a) report that increasing nitrogen fertilization had a positive effect on the hue angle of broccoli from 0 to 100 or 150 kg N ha<sup>-1</sup>, depending on the cultivar used. When fertilizer doses were further increased, values reached a cultivar-specific plateau (Bakker et al. 2009a). In contrast, no influence of fertilization on hue angle was found in the present investigations (Table 7). This is probably due to a narrower range of different fertilizer doses close to optimum supply when compared to the investigations from Bakker et al. (2009a). The lowest dose in the present investigation was 3.7 g nitrogen per plant, which would correspond to 111 N ha<sup>-1</sup> based on a plant density of 3 plants m<sup>-2</sup>.

The reported differences in NDVI and  $L^*$  values were absolutely indistinguishable by visual rating. Obviously, both indexes are very sensitive in the rating of nitrogen fertilization and concomitant chlorophyll levels. The different chlorophyll levels did, however, not lead to different storability. It is therefore assumed that the level of fertilization may be negligible in the rating of storability, regarding the senescence processes of the broccoli curd.

#### 6.3.4. Water supply preceding harvest

Based on data from different harvest batches grown in 2012 (7 batches) and 2013 (2 batches), a high dependency of broccoli storability on water supply could be confirmed ( $R^2 = 0.71$ , Fig. 43). This relationship refers to water supply during the fortnight preceding harvest, which parallels results from Wurr et al. (2002).

Other authors have also reported a higher retention of broccoli color during storage when water stress was applied throughout the whole cultivation period (Zaicovski et al. 2008; Cogo et al. 2011). Yet, the effect found in the present work is most prominent when the last two weeks of cultivation are considered. In addition, when water stress was applied

during earlier stages, a risk of deleterious effects on curd morphology has been reported (Wurr et al. 2002). Although Chen et al. (2008b) did not observe differences in fresh weight under different irrigation management, a certain decrease in yield may be expected when water is limited throughout cultivation (Wurr et al. 2002; El-Shikha et al. 2007).

Color retention of low watered broccoli may have resulted from higher stem turgor, as reported by Wurr et al. (2002). The preservation of stem turgor is probably the result of a drought acclimatization process. Drought adapted plants are able to retain turgor by actively increasing the osmotic potential of cells through accumulation of ions (Schopfer & Brennicke 2006). Moreover, drought adaptation also leads to a greater tolerance towards negative leaf water potential before the synthesis of abscisic acid is promoted (Schopfer & Brennicke 2006). Abscisic acid is known to play an important, yet complex role in leaf senescence (Liang & Chu 2015). It is therefore conceivable that the storability of broccoli was mediated by different levels of abscisic acid, which resulted from predetermination through different levels of water supply.

The preservation of green color could also have resulted from higher concentrations of endogenous cytokinin, as Zaicovski et al. (2008) observed in broccoli grown under water stress. Cytokinins are known to prevent chlorophyll degradation and to control ethylene perception and biosynthesis (Tian et al. 1995; Costa et al. 2005a; Gapper et al. 2005).

There are conflicting reports about the influence of water stress on polyphenol concentration in broccoli. Lower irrigation resulted in increased polyphenol concentrations in investigations by Fortier et al. (2010). In contrast, Cogo et al. (2011) observed equal initial concentrations of polyphenols in broccoli grown under different irrigation management and, furthermore, higher concentrations were retained in normally irrigated broccoli during 7 days of storage. A significant role of phenols in senescence prevention through accelerated defense mechanisms against active oxygen species is therefore questionable.

Although the particular mechanism of the relations between color preservation and water supply could not be elucidated by the present study, the results have validated assumptions from Wurr et al. (2002) through a practice-oriented approach based on genuine on-farm data on irrigation and precipitation within two growing seasons.

## 7. Conclusions

Beginning with the first objective of the present work,

**“Determination of a non-destructive quality rating method that can give clear evidence about (further) storability of broccoli”**

several optical indexes pointed toward changes very quickly during storage, at a time they could hardly be perceived by visual rating. Changes of optical indexes  $b^*$ , REIP and both photosynthetic parameters ( $Fv/Fm$ ,  $\Phi_{PSII}$ ) were recognized in broccoli curds at a very early stage of storage at 16 °C and 95 % RH.

As far as color measurements are concerned, it can be concluded that  $b^*$  values are quicker to detect differences during storage than hue angle values. From this point of view, the use of  $b^*$  values should be given preference to over hue angle values for the quality assessment of broccoli.

In the comparison of indexes of diffuse reflection spectra, REIP indicated changes prior to NDVI. Yet, calculation of REIP proved to be less convenient than NDVI; because reflection data needed to be normalized first and the second derivation also had to be calculated.

Regarding photosynthetic indexes,  $\Phi_{PSII}$  was more sensitive than  $Fv/Fm$ . However, the number of samples (harvest batches) measured with  $\Phi_{PSII}$  was lower. Therefore, further examinations of  $\Phi_{PSII}$  on broccoli batches with excellent storability are necessary to finally determine which photosynthetic index is more sensitive. Additionally, it should be mentioned that the measurement of  $\Phi_{PSII}$  during storage requires a quite elaborate preparation in order to provide for sufficient light adaptation of the broccoli curd. Only when illumination is established in storage rooms as proposed by Büchert et al. (2011b) may this quality assessment be considered.

In addition to indexes measured by spot measuring devices, early changes within single broccoli curds were effectively indicated by Euclidean distances among pixel-wise reflection intensities recorded by hyperspectral images. The increase in Euclidean distances was based on the differences in senescence of single florets on broccoli curds. To recognize variation, a composite of only three reflection intensities at different wavelengths, placed as RGB channels, was necessary. The variation within the broccoli curd was as sensitive to quality changes as a mean of six spot measurements of  $b^*$  values on one curd. Regarding these facts, a spectral analysis of broccoli reflection spectra in the visible range did not prove to have an advantage over colorimetry. Further examinations at lower temperatures may be required in order to clarify if early quality losses might, in the end, be detected more quickly by Euclidean distances in hyperspectral image data than by spot measurements of  $b^*$  values.

Despite the sensitivity of the methods presented, none of them predicted the eventual level of storability at the beginning of storage. Changes on the same day of DS did not

necessarily indicate equal value levels on day 4. Therefore, the prediction of storability based on color measurements, diffuse reflection spectra or chlorophyll fluorescence does not seem to be feasible.

Furthermore, when comparing among different harvest batches, initial value levels were reasonable only for the indexes hue,  $b^*$  and REIP. The value level of photosynthetic indexes, although highly sensitive to changes, did not show a reasonable pattern of quality when batches were compared. This was especially recognizable in the high  $F_v/F_m$  values of a broccoli batch which already showed inferior quality according to visual appearance at harvest. In conclusion, loss of color and photosynthetic activity are not necessarily inter-related. Fluorescence measurements can therefore only be recommended for measuring the progress of storage of single batches, which is a quite limited field of application. Comparability of values among different harvest batches is a relevant criterion for the transfer of methods into horticultural production.

Color and reflection measurements including imaging, on the other hand, could be implemented in sorting machines for monitoring the produce's current status. This might be reasonable for large vegetable producing or trading companies.

In conclusion, the results of storability prediction by means of optical indexes suggest that the plant's maximum shelf life may be restricted by its inner characteristics, resulting from the previous determination of the broccoli curd. A similar concept is discussed by McRoberts and Lepreux (2003), who define the quality of horticultural produce as deriving from genetic, management and environmental components.

Accordingly, the findings with regard to the second objective

**“Rating of the relative influence of pre-harvest conditions on the post-harvest life of broccoli”**

support the hypothesis that the predetermination of the broccoli curd is affected by climate and cultivation influences.

During the cultivation of broccoli, both the quantity and quality of radiation have been shown to affect quality characteristics in broccoli during storage. A temporary reduction of the visible range to blue light ( $\lambda = 400$  to  $500$  nm) resulted in a decrease in storability, as was indicated by several non-destructive indexes. The effect is possibly related to the circadian rhythm of broccoli curds. Additionally, incidences of high radiation intensity ( $> 600 \text{ W m}^{-1}$ ) during curd initiation and early curd growth, partly accompanied by high temperatures, appeared to cause a trend towards lower hue angle values after four days of storage at  $16 \text{ }^\circ\text{C}$ . A monitoring of both temperature and radiation intensity is therefore recommended in broccoli cultivation. Although the mechanisms involved could not be investigated here, the importance of radiation during cultivation on subsequent storability is highlighted in this work.

The effect of radiation was, however, not as pronounced as the influence of water availability preceding harvest. Irrigation and rainfall during the last two weeks on the field strongly determined the storability of broccoli in terms of  $b^*$  values. The findings in this

work fully support the careful control of irrigation during the fortnight preceding maturity as recommended by Wurr et al. (2002). As far as possible, it is recommended that growers reduce the overall amount of water to less than 40 L m<sup>-2</sup> in the last two weeks by reducing irrigation. Although a similar relationship had already been investigated by other authors (Wurr et al. 2002; Zaicovski et al. 2008; Cogo et al. 2011), the present investigation was the first practical (and partly *on-farm*) approach that demonstrated a relationship between characteristics of arbitrary chosen broccoli and absolute amounts of irrigation and rainfall.

The amount of nitrogen fertilizer applied during cultivation determined the levels of L\* and NDVI on the first day of storage. At this time, the L\* and NDVI in independent harvest batches followed a parabolic function depending on the amount of nitrogen, which was similar to the typical nitrogen response curve of crop yield. The amount of applied nitrogen fertilizer, however, did not provide any information on the further proceeding of both indexes during storage. It also did not show any effect on the further color retention of broccoli during storage. These findings indicate, on the one hand, that fertilization has a negligible effect on storability, when used at common levels. On the other hand, the strong relationship between nitrogen status and the indexes L\* and NDVI might be of great value for broccoli production in general. NDVI in particular, because it does not require normalization of spectra prior to its calculation, can be easily used for monitoring nitrogen demand in broccoli plants in the field.

The work on external field factors represented a reasonable approach towards a better understanding of influences on storability of broccoli. Still, the results obtained have to be considered with some precaution, as the number of sampled batches was still too low for reliable modeling. Additionally, a reliable model should consider all interactions between the factors, which would soon lead to barely comprehensible dependencies. Therefore, the approach represented a guide of topics growers might emphasize in cultivation practice instead. Yet, for effective modeling, “studies of the plant-environment complex and a pursuit of principles rather than limited 'rules of thumb'” (Lakso 1990) are needed. Certainly, the potential in this approach was in the use of database programming, which provided a greater knowledge output regarding climate and cultivation factors than previous examinations on field grown broccoli. In reports of climatic influences in the literature, methods of calculation generally vary. Monthly or daily means and sums of temperature are often used to characterize cultivation conditions (Babik & Elkner 2000; Leja et al. 2002; Pék et al. 2012). Some approaches involving more elaborate processing of climate data have also been presented (Heather et al. 1992; Dufault 1996). However, the potential of climate databases for vegetable production has yet to be fully realized.

Wurr et al. (2002) states that “most of the work on broccoli shelf life has concentrated on the influence of various postharvest treatments and has largely ignored the influence of preharvest factors.” This is still true and might be justified by the presence of easier to measure control mechanisms during the postharvest phase. The knowledge about optimum storage conditions of broccoli is on a very high level nowadays. The latest findings indicate that the use of light in storage rooms has positive effects (Büchert et al.

2011c; Zhan et al. 2012; Jin et al. 2015; Liu et al. 2015; Hasperué et al. 2016). In connection with findings regarding adequate temperature, humidity and gas atmosphere, an excellent guide to storage conditions of broccoli can be established. However, the transfer of recommended techniques from academic research to the grower is often inhibited due to a lack of economic efficiency. Through the use of combined climate and cultivation databases, detailed monitoring might create new possibilities for decision support. Using the proposed approaches, rating and prediction of broccoli storability may be improved.

The inner quality of broccoli curds was investigated in accordance with the third objective

**“Identification of early senescence indicators in curd development and chlorophyll catabolism of broccoli curds”.**

Assuming that factors during cultivation had predetermined the quality of curds, the goal was to find early senescence indicators in broccoli curds. Yet, the search for a particular senescence marker among chlorophyll derivatives was not successful. It is possible that chlorophyll catabolites did not accumulate in broccoli tissue during storage, as levels in general were low. Neither absolute amounts nor ratios of chlorophyll catabolites were unequivocally suitable for advanced indication of senescence.

There was, however, evidence that the chlorophyll *a/b* ratio and relative enrichment of chlorophyll *a* may play a role in broccoli with superior storage quality. According to the results presented above, as long as the chlorophyll *a/b* ratio is rising, broccoli may still be of acceptable storability. In particular, the slope of increasing chlorophyll *a/b* ratios might point to extended storability. With respect to the chlorophyll degradation pathway, a harvest batch of good storability may remain at the stage of chlorophyll *b* to *a* conversion for a longer period of time. In turn it can be assumed that as long as the chlorophyll *a/b* ratio is rising strongly, senescence may still remain at an early stage.

Regarding a relative enrichment of chlorophyll *a* in relation to green chlorophyll derivatives in broccoli which preserved a green color, the focus remained on the first step of chlorophyll degradation. Broccoli which changed color proceeded further along the catabolic pathway. In connection with investigated chlorophyll *a/b* ratios, the results suggested that the relative chlorophyll *a* accumulation was caused by secession of the chlorophyll degradation pathway during its first step.

However, similar to chlorophyll fluorescence indexes, the chlorophyll *a/b* ratio was not reliable when compared among different harvest batches. Lower storage temperatures and immediate sampling after harvest might support further investigations on early senescence compounds, as was shown by high concentrations of Chlide in broccoli on harvest day in one batch.

Concerning the degradation pathway of chlorophyll in general, the results suggested that pheophytinization dominates over dephytylation, as indicated by a general increase in Pheoin/chlorophyll *a* ratios.

The yellowing of broccoli curds during storage was the result of chlorophyll loss, which unmasked the carotenoids present in the tissue. In addition to carotenoids that are generally present in thylakoid membranes of chloroplasts, auroxanthin was also detected, which might be a compound of the yellow petals and therefore has a certain potential value for senescence indication. This would be true if senescence and generative development were closely interrelated. There was, however, only a small amount of evidence for this hypothesis in the results achieved in this work. The use of bud size diameters was not effective for the prediction of further quality rating of broccoli curds. Chlorophyll loss in single buds, however, enabled the early detection of senescence with the help of Euclidean distances in hyperspectral imaging.

In addition, the extent of color change (hue angle) after four days of storage at 16 °C and high RH was shown to be related to diameters of whole broccoli curds in four out of seven harvested batches. This relationship was either a result of the advanced developmental status of larger curds or a consequence of increased microbial loads related to cultivation time. Although the nature of interrelation between curd sizes and yellowing can only be speculated about, growers are advised to harvest smaller curds instead of waiting for a further growth in curd diameters. The current practice, in which producers, retail sale and consumers prefer big curds of 500 g, might also be reconsidered.



## 8. References

- Aiamla-or, S., Kaewsuksaeng, S., Shigyo, M. & Yamauchi, N. 2010. Impact of UV-B irradiation on chlorophyll degradation and chlorophyll-degrading enzyme activities in stored broccoli (*Brassica oleracea* L. *Italica* Group) florets. *Food Chemistry* 120: 645-651.
- Aiamla-or, S., Nakajima, T., Shigyo, M. & Yamauchi, N. 2012. Pheophytinase activity and gene expression of chlorophyll-degrading enzymes relating to UV-B treatment in postharvest broccoli (*Brassica oleracea* L. *Italica* Group) florets. *Postharvest Biology and Technology* 63: 60-66.
- Aiamla-or, S., Shigyo, M., Ito, S. & Yamauchi, N. 2014. Involvement of chloroplast peroxidase on chlorophyll degradation in postharvest broccoli florets and its control by UV-B treatment. *Food Chemistry* 165: 224-231.
- Anonymous 2001. Final project report "Long Life Broccoli". CV35 9EF. UK Ministry of Agriculture, Fisheries and Food, Horticulture Research International, Wellesbourne, Warwick.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24: 1-15.
- Atallah, S.S. & Gomez, M.I. 2013. Eastern broccoli crop budgets. *Extension Bulletin* No. EB-2013-14. Cornell University, Department of Applied Economics and Management.
- Babik, I. & Elkner, K. 2000. The effect of nitrogen fertilization and irrigation on yield and quality of broccoli. In: Booij, R. & Neeteson, J. (eds.): *Workshop Towards and Ecologically Sound Fertilisation in Field Vegetable Production* pp. 33-43. Acta Horticulturae (ISHS) 571.
- Baier, M., Foerster, J., Schnabel, U., Knorr, D., Ehlbeck, J., Herppich, W.B. & Schlüter, O. 2013. Direct non-thermal plasma treatment for the sanitation of fresh corn salad leaves: Evaluation of physical and physiological effects and antimicrobial efficacy. *Postharvest Biology and Technology* 84: 81-87.
- Bakker, C.J., Swanton, C.J. & McKeown, A.W. 2009a. Broccoli growth in response to increasing rates of pre-plant nitrogen. I. Yield and quality. *Canadian Journal of Plant Science* 89: 527-537.
- Bakker, C.J., Swanton, C.J. & McKeown, A.W. 2009b. Broccoli growth in response to increasing rates of pre-plant nitrogen. II. Dry matter and nitrogen accumulation. *Canadian Journal of Plant Science* 89: 539-548.
- Barth, M.M. & Zhuang, H. 1996. Packaging design affects antioxidant vitamin retention and quality of broccoli florets during postharvest storage. *Postharvest Biology and Technology* 9: 141-150.
- Bauriegel, E., Giebel, A., Geyer, M., Schmidt, U. & Herppich, W.B. 2011a. Early detection of *Fusarium* infection in wheat using hyper-spectral imaging. *Computers and Electronics in Agriculture* 75: 304-312.

- Bauriegel, E., Giebel, A. & Herppich, W. 2011b. Hyperspectral and chlorophyll fluorescence imaging to analyse the impact of *Fusarium culmorum* on the photosynthetic integrity of infected wheat ears. *Sensors* 11: 3765-3779.
- Bengtsson, G.B., Schöner, R., Lombardo, E., Schöner, J., Borge, G.I. & Bilger, W. 2006. Chlorophyll fluorescence for non-destructive measurement of flavonoids in broccoli. *Postharvest Biology and Technology* 39: 291-298.
- Björkman, O. & Demmig, B. 1987. Photon yield of O<sub>2</sub> evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* 170: 489-504.
- Björkman, T. & Pearson, K.J. 1998. High temperature arrest of inflorescence development in broccoli (*Brassica oleracea* var. *italica* L.). *Journal of Experimental Botany* 49: 101-106.
- Brennan, P.S. & Shewfelt, R.L. 1989. Effect of cooling delay at harvest on broccoli quality during postharvest storage. *Journal of Food Quality* 12: 13-22.
- Büchert, A.M., Civello, P.M. & Martínez, G.A. 2011a. Chlorophyllase versus pheophytinase as candidates for chlorophyll dephytilation during senescence of broccoli. *Journal of Plant Physiology* 168: 337-343.
- Büchert, A.M., Civello, P.M. & Martínez, G.A. 2011b. Effect of hot air, UV-C, white light and modified atmosphere treatments on expression of chlorophyll degrading genes in postharvest broccoli (*Brassica oleracea* L.) florets. *Scientia Horticulturae* 127: 214-219.
- Büchert, A.M., Gómez Lobato, M.E., Villarreal, N.M., Civello, P.M. & Martínez, G.A. 2011c. Effect of visible light treatments on postharvest senescence of broccoli (*Brassica oleracea* L.). *Journal of the Science of Food and Agriculture* 91: 355-361.
- Cantwell, M.I. & Kasmire, R.F. 2002. Postharvest handling systems: Flower, leafy and stem vegetables. In: Kader, A.A. (ed.): *Postharvest technology of horticultural crops*, pp. 423-433. University of California, Oakland.
- Carr, S.M. & Irish, V.F. 1997. Floral homeotic gene expression defines developmental arrest stages in *Brassica oleracea* L. vars. *botrytis* and *italica*. *Planta* 201: 179-188.
- Chen, L.F., Lin, C.H., Kelkar, S.M., Chang, Y.M. & Shaw, J.F. 2008a. Transgenic broccoli (*Brassica oleracea* var. *italica*) with antisense chlorophyllase (*BoCLH1*) delays postharvest yellowing. *Plant Science* 174: 25-31.
- Chen, Y.T., Chen, L.F. & Shaw, J.F. 2008b. Senescence-associated genes in harvested broccoli florets. *Plant Science* 175: 137-144.
- Cogo, S.L.P., Chaves, F.C., Schirmer, M.A., Zambiasi, R.C., Nora, L., Silva, J.A. & Rombaldi, C.V. 2011. Low soil water content during growth contributes to preservation of green colour and bioactive compounds of cold-stored broccoli (*Brassica oleracea* L.) florets. *Postharvest Biology and Technology* 60: 158-163.

- Costa, L., Vicente, A.R., Civello, P.M., Chaves, A.R. & Martínez, G.A. 2006a. UV-C treatment delays postharvest senescence in broccoli florets. *Postharvest Biology and Technology* 39: 204-210.
- Costa, M.L., Civello, P.M., Chaves, A.R. & Martínez, G.A. 2005a. Effect of ethephon and 6-benzylaminopurine on chlorophyll degrading enzymes and a peroxidase-linked chlorophyll bleaching during post-harvest senescence of broccoli (*Brassica oleracea* L.) at 20 °C. *Postharvest Biology and Technology* 35: 191-199.
- Costa, M.L., Civello, P.M., Chaves, A.R. & Martínez, G.A. 2005b. Effect of hot air treatments on senescence and quality parameters of harvested broccoli (*Brassica oleracea* L var *Italica*) heads. *Journal of the Science of Food and Agriculture* 85: 1154-1160.
- Costa, M.L., Civello, P.M., Chaves, A.R. & Martínez, G.A. 2006b. Hot air treatment decreases chlorophyll catabolism during postharvest senescence of broccoli (*Brassica oleracea* L. var. *italica*) heads. *Journal of the Science of Food and Agriculture* 86: 1125-1131.
- Coupe, S.A., Sinclair, B.K., Watson, L.M., Heyes, J.A. & Eason, J.R. 2003. Identification of dehydration-responsive cysteine proteases during post-harvest senescence of broccoli florets. *Journal of Experimental Botany* 54: 1045-1056.
- Dark, S.O.S. 1938. The development of the flowers from the curd of broccoli (*Brassica oleracea botrytis*). *Annals of Botany* 2: 751-752.
- DeEll, J.R. & Toivonen, P.M.A. 2000. Chlorophyll fluorescence as a nondestructive indicator of broccoli quality during storage in modified-atmosphere packaging. *Hortscience* 35: 256-259.
- DeEll, J.R. & Toivonen, P.M.A. 1999. Chlorophyll fluorescence as an indicator of physiological changes in cold-stored broccoli after transfer to room temperature. *Journal of Food Science* 64: 501-503.
- DEFRA 2011. Nitrogen for field crops. In: Department for Environmental Food and Rural Affairs (ed.): *Fertiliser Manual (RB209)*. Agricultural Document Library, UK.
- Devlin, P.F. & Kay, S.A. 1999. Cryptochromes – bringing the blues to circadian rhythms. *Trends in Cell Biology* 9: 295-298.
- Dixon, G.R. 2007. Vegetable brassicas and related crucifers. CABI, Wallingford.
- Duclos, D.V. & Björkman, T. 2008. Meristem identity gene expression during curd proliferation and flower initiation in *Brassica oleracea*. *Journal of Experimental Botany* 59: 421-433.
- Dufault, R.J. 1996. Dynamic relationships between field temperatures and broccoli head quality. *Journal of the American Society for Horticultural Science* 121: 705-710.
- El-Shikha, D.M., Waller, P., Hunsaker, D., Clarke, T. & Barnes, E. 2007. Ground-based remote sensing for assessing water and nitrogen status of broccoli. *Agricultural Water Management* 92: 183-193.

- FAOSTAT 2014. Cauliflowers and broccoli production (tonnes). In: *FAOSTAT Database on Agriculture* Food and Agriculture Organization of the United Nations, Rome, Italy
- Farnham, M.W. & Björkman, T. 2011. Evaluation of experimental broccoli hybrids developed for summer production in the eastern united states. *Hortscience* 46: 858-863.
- Fernandez-Leon, M.F., Fernandez-Leon, A.M., Lozano, M., Ayuso, M.C. & Gonzalez-Gomez, D. 2012. Identification, quantification and comparison of the principal bioactive compounds and external quality parameters of two broccoli cultivars. *Journal of Functional Foods* 4: 465-473.
- Fernández-León, M.F., Fernández-León, A.M., Lozano, M., Ayuso, M.C. & González-Gómez, D. 2013a. Altered commercial controlled atmosphere storage conditions for 'Parhenon' broccoli plants (*Brassica oleracea* L. var. *italica*). Influence on the outer quality parameters and on the health-promoting compounds. *LWT - Food Science and Technology* 50: 665-672.
- Fernández-León, M.F., Fernández-León, A.M., Lozano, M., Ayuso, M.C. & González-Gómez, D. 2013b. Different postharvest strategies to preserve broccoli quality during storage and shelf life: Controlled atmosphere and 1-MCP. *Food Chemistry* 138: 564-573.
- Forney, C.F. & Rij, R.E. 1991. Temperature of broccoli florets at time of packaging influences package atmosphere and quality. *Hortscience* 26: 1301-1303.
- Fortier, E., Desjardins, Y., Tremblay, N., Bélec, C. & Côté, M. 2010. Influence of irrigation and nitrogen fertilization on broccoli polyphenolics concentration. In: Hongju He, H. & Wei Liu, L. (eds.): *International Symposium on Vegetable Safety and Human Health*, Beijing, China pp. 55-62. Acta Horticulturae (ISHS) 856.
- Francescangeli, N., Sangiacomo, M.A. & Marti, H.R. 2007. Vegetative and reproductive plasticity of broccoli at three levels of incident photosynthetically active radiation. *Spanish Journal of Agricultural Research* 5: 389-401.
- Fujime, Y. & Okuda, N. 1996. The physiology of flowering in brassicas, especially about cauliflower and broccoli. In: Dias, J.S., Crute, I. & Monteiro, A.A. (eds.): *ISHS Brassica Symposium - IX Crucifer Genetics Workshop*, Lisbon, Portugal. pp. 247-254. Acta Horticulturae (ISHS) 407.
- Fujime, Y., Saito, Y. & Nakayama, Y. 1988. Photothermal induction of flower head formation in broccoli plants. *Journal of the Japanese Society for Horticultural Science* 57: 70-77.
- Funamoto, Y., Yamauchi, N., Shigenaga, T. & Shigyo, M. 2002. Effects of heat treatment on chlorophyll degrading enzymes in stored broccoli (*Brassica oleracea* L.). *Postharvest Biology and Technology* 24: 163-170.
- Gapper, N.E., Coupe, S.A., McKenzie, M.J., Sinclair, B.K., Lill, R.E. & Jameson, P.E. 2005. Regulation of harvest-induced senescence in broccoli (*Brassica oleracea* var. *italica*) by cytokinin, ethylene, and sucrose. *Journal of Plant Growth Regulation* 24: 153-165.

- Gauss, J.F. & Taylor, G.A. 1969. Environmental factors influencing reproductive differentiation and subsequent formation of inflorescence of *Brassica oleracea* L. var. *italica*, Plenck, cv. 'Coastal'. *Journal of the American Society for Horticultural Science* 94: 275-280.
- Gebbers, R., Tavakoli, H. & Herbst, R. 2013. Crop sensor readings in winter wheat as affected by nitrogen and water supply. In: Stafford, J.V. (ed.): *Precision agriculture '13*, pp. 79-86. Wageningen Academic Publishers.
- Gliszczyńska-Świągło, A., Kałużewicz, A., Lemańska, K., Knaflewski, M. & Tyrakowska, B. 2007. The effect of solar radiation on the flavonol content in broccoli inflorescence. *Food Chemistry* 100: 241-245.
- Gnanasekharan, V., Shewfelt, R.L. & Chinnan, M.S. 1992. Detection of color changes in green vegetables. *Journal of Food Science* 57: 149-154.
- Gómez-Lobato, M.E., Civello, P.M. & Martínez, G.A. 2012a. Effects of ethylene, cytokinin and physical treatments on BoPaO gene expression of harvested broccoli. *Journal of the Science of Food and Agriculture* 92: 151-158.
- Gómez-Lobato, M.E., Civello, P.M. & Martínez, G.A. 2012b. Expression of a lipoxygenase encoding gene (*BoLOX1*) during postharvest senescence of broccoli. *Postharvest Biology and Technology* 64: 146-153.
- Gómez-Lobato, M.E., Hasperué, J.H., Civello, P.M., Chaves, A.R. & Martínez, G.A. 2012c. Effect of 1-MCP on the expression of chlorophyll degrading genes during senescence of broccoli (*Brassica oleracea* L.). *Scientia Horticulturae* 144: 208-211.
- Goodspeed, D., Liu, J.D., Chehab, W.E., Sheng, Z., Francisco, M., Kliebenstein, D.J. & Braam, J. 2013. Postharvest circadian entrainment enhances crop pest resistance and phytochemical cycling. *Current Biology* 23: 1235-1241.
- Gorbe, E. & Calatayud, A. 2012. Applications of chlorophyll fluorescence imaging technique in horticultural research: A review. *Scientia Horticulturae* 138: 24-35.
- Graeff, S., Judit, P., Claupein, W. & Liebig, H.-P. 2008. Evaluation of image analysis to determine the N-fertilizer demand of broccoli plants (*Brassica oleracea* convar. *botrytis* var. *italica*). *Advances in Optical Technologies* 2008: 8.
- Grevsen, K. 1998. Effects of temperature on head growth of broccoli (*Brassica oleracea* L. var. *italica*): Parameter estimates for a predictive model. *Journal of Horticultural Science & Biotechnology* 73: 235-244.
- Grevsen, K. 2000. Modelling plant development of broccoli. In: Stoffella, P.J., Cantliffe, D.J. & Damato, G. (eds.): *VIII International Symposium on Timing Field Production in Vegetable Crops*, Bari, Italy pp. 567-574. Acta Horticulturae (ISHS) 533.
- Grevsen, K., Olesen, J.E. & Veierskov, B. 2003. The effects of temperature and plant developmental stage on the occurrence of the curd quality defects "bracting" and "riciness" in cauliflower. *Journal of Horticultural Science and Biotechnology* 78: 638-646.
- Guiamét, J.J., Pichersky, E. & Noodén, L.D. 1999. Mass exodus from senescing soybean chloroplasts. *Plant and Cell Physiology* 40: 986-992.

- Gunawan, M.I. & Barringer, S.A. 2000. Green color degradation of blanched broccoli (*Brassica oleracea*) due to acid and microbial growth. *Journal of Food Processing and Preservation* 24: 253-263.
- Hasan, Y. 2014. Vernalisation of cauliflower. 09.04.2014, phone conversation, Hannover.
- Hasperué, J.H., Chaves, A.R. & Martínez, G.A. 2011. End of day harvest delays postharvest senescence of broccoli florets. *Postharvest Biology and Technology* 59: 64-70.
- Hasperué, J.H., Gómez-Lobato, M.E., Chaves, A.R., Civello, P.M. & Martínez, G.A. 2013. Time of day at harvest affects the expression of chlorophyll degrading genes during postharvest storage of broccoli. *Postharvest Biology and Technology* 82: 22-27.
- Hasperué, J.H., Guardianelli, L., Rodoni, L.M., Chaves, A.R. & Martínez, G.A. 2016. Continuous white–blue LED light exposition delays postharvest senescence of broccoli. *LWT - Food Science and Technology* 65: 495-502.
- Hasperué, J.H., Lemoine, M., Chaves, A. & Martínez, G.A. 2014. Effect of time of day for harvest and postharvest treatments on the sugar metabolism of broccoli (*Brassica oleracea* var. *italica*). *Agricultural and Food Science* 23: 48-59.
- Heather, D.W., Sieczka, J.B., Dickson, M.H. & Wolfe, D.W. 1992. Heat tolerance and holding ability in broccoli. *Journal of the American Society for Horticultural Science* 117: 887-892.
- Heaton, J.W., Lencki, R.W. & Marangoni, A.G. 1996. Kinetic model for chlorophyll degradation in green tissue. *Journal of Agricultural and Food Chemistry* 44: 399-402.
- Heaton, J.W. & Marangoni, A.G. 1996. Chlorophyll degradation in processed foods and senescent plant tissues. *Trends in Food Science & Technology* 7: 8-15.
- Hernández-Hierro, J.M., Esquerre, C., Valverde, J., Villacreces, S., Reilly, K., Gaffney, M., González-Miret, M.L., Heredia, F.J., O'Donnell, C.P. & Downey, G. 2014. Preliminary study on the use of near infrared hyperspectral imaging for quantitation and localisation of total glucosinolates in freeze-dried broccoli. *Journal of Food Engineering* 126: 107-112.
- Herppich, W.B., Geyer, M., Kabakeris, T., Schultze, M., Zrenner, R. & Schreiner, M. 2013. Gemüse im Freiland termingerech produziert. In: *Forschungsreport*, pp. 9-11. Senat der Bundesforschungsinstitute des Bundesministeriums für Ernährung und Landwirtschaft.
- Hipp, B.W. 1974. Influence of nitrogen and maturity rate on hollow stem in broccoli. *Hortscience* 9: 68-69.
- Hormes, E. 2008. Blumenkohl und Brokkoli: In jeder Region Chancen herausarbeiten. In: *Gemüse*, pp. 37-39. Verlag Eugen Ulmer, Stuttgart.
- Hörtensteiner, S. 2006. Chlorophyll degradation during senescence. *Annual Review of Plant Biology* 57: 55-77.

- Hörtensteiner, S. 2004. The loss of green color during chlorophyll degradation - a prerequisite to prevent cell death? *Planta* 219: 191-194.
- Hörtensteiner, S. 2013. Update on the biochemistry of chlorophyll breakdown. *Plant Molecular Biology* 82: 1-13.
- Hörtensteiner, S. & Kräutler, B. 2011. Chlorophyll breakdown in higher plants. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1807: 977-988.
- Hosaka, A., Makino, Y., Kawagoe, Y. & Oshita, S. 2012. Prediction of degradation rate of broccoli during storage by hyper spectral imaging In: *International Conference of Agricultural Engineering CIGR-Ageng*, Valencia, Spain.
- Ishikawa, Y. & Hirata, T. 2001. Color change model for broccoli packaged in polymeric films. *Transactions of the ASAE* 44: 923-927.
- Jenni, S., Dutilleul, P., Yamasaki, S. & Tremblay, N. 2001. Brown bead of broccoli. II. Relationships of the physiological disorder with nutritional and meteorological variables. *Hortscience* 36: 1228-1234.
- Jin, P., Yao, D., Xu, F., Wang, H. & Zheng, Y. 2015. Effect of light on quality and bioactive compounds in postharvest broccoli florets. *Food Chemistry* 172: 705-709.
- Johnson, L.F. & Trout, T.J. 2012. Satellite NDVI assisted monitoring of vegetable crop evapotranspiration in california's San Joaquin Valley. *Remote Sensing* 4: 439-455.
- Kaewsuksaeng, S. 2011. Chlorophyll degradation in horticultural crops. *Walailak Journal of Science & Technology* 8: 11.
- Kalisz, A., Cebula, S., Kunicki, E., Gil, J., Sćkara, A. & Grabowska, A. 2014. Effect of chilling stress before transplanting on morphological parameters of broccoli heads. *Acta Scientiarum Polonorum, Hortorum Cultus* 13: 129-139.
- Kałużewicz, A., Krzesinski, W. & Knaflewski, M. 2009. Effect of temperature on the yield and quality of broccoli heads. *Vegetable Crops Research Bulletin* 71: 51-58.
- Kałużewicz, A., Krzesiński, W., Knaflewski, M., Lisiecka, J., Spiżewski, T. & Frąszczak, B. 2012. Effect of temperature on the growth of broccoli (*Brassica oleracea* L. var. *italica* Plenck) cv. fiesta. *Vegetable Crops Research Bulletin* 77: 129-141.
- Kaosamphan, A., Yamauchi, N., Srilaong, V., Aiamla-Or, S., Wongs-Aree, C. & Uthairatanakij, A. 2010. Involvement of chlorophyllase on chlorophyll degradation in stored broccoli florets and its control by UV treatment. In: Kanlayanarat, S., McGlasson, W.B. & Techavuthiporn, C. (eds.): *Southeast Asia Symposium of Quality and Safety of Fresh and Fresh Cut Produce*, Bangkok, Thailand. pp. 111-118. *Acta Horticulturae (ISHS)* 875.
- Karrer, P. & Rutschmann, J. 1942. Auroxanthin, ein kurzweilig absorbierender Carotinfarbstoff. *Helvetica Chimica Acta* 25: 1624-1627.
- Kays, S.J. 1991. *Postharvest physiology of perishable plant products*. Van Nostrand Reinhold, New York.

- Khan, M.A.M., Ulrichs, C. & Mewis, I. 2010. Influence of water stress on the glucosinolate profile of *Brassica oleracea* var. *italica* and the performance of *Brevicoryne brassicae* and *Myzus persicae*. *Entomologia Experimentalis et Applicata* 137: 229-236.
- Khan, M.A.M., Ulrichs, C. & Mewis, I. 2011. Water stress alters aphid-induced glucosinolate response in *Brassica oleracea* var. *italica* differently. 21: 235-242.
- Khudairi, A.K. 1972. The ripening of tomatoes. *American Scientist* 60: 696-707.
- Kidmose, U.L.L.A. & Hansen, M.E.R.E. 1999. The influence of postharvest storage, temperature and duration on quality of cooked broccoli florets. *Journal of Food Quality* 22: 135-146.
- Kieffer, M., Fuller, M.P. & Jellings, A.J. 1998. Explaining curd and spear geometry in broccoli, cauliflower and 'romanesco': quantitative variation in activity of primary meristems. *Planta* 206: 34-43.
- King, G.A. & Morris, S.C. 1994a. Early compositional changes during postharvest senescence of broccoli. *Journal of the American Society for Horticultural Science* 119: 1000-1005.
- King, G.A. & Morris, S.C. 1994b. Physiological changes of broccoli during early postharvest senescence and through the preharvest-postharvest continuum. *Journal of the American Society for Horticultural Science* 119(2): 270-275.
- Kitajima, K. & Hogan, K.P. 2003. Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant, Cell & Environment* 26: 857-865.
- Klieber, A. & Wills, R.B.H. 1991. Optimisation of storage conditions for 'Shogun' broccoli. *Scientia Horticulturae* 47: 201-208.
- Klinzmann, M. & Voigt, J. 2013. Kulturschutznetze im Feldgemüsebau. Auswirkungen auf Wachstum, Entwicklung und Qualität von Brokkoli. Master thesis, Gottfried Wilhelm Leibniz Universität Hannover.
- Knipling, E.B. 1970. Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. *Remote Sensing of Environment* 1: 155-159.
- Kurilich, A.C., Tsau, G.J., Brown, A., Howard, L., Klein, B.P., Jeffery, E.H., Kushad, M., Wallig, M.A. & Juvik, J.A. 1999. Carotene, Tocopherol, and Ascorbate Contents in Subspecies of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry* 47: 1576-1581.
- Labate, J.A., Robertson, L.D., Baldo, A.M. & Björkman, T. 2006. Inflorescence identity gene alleles are poor predictors of inflorescence type in broccoli and cauliflower. *Journal of the American Society for Horticultural Science* 131: 667-673.
- Lakso, A.N. 1990. Interactions of physiology with multiple environmental stresses in horticultural crops. *Hortscience* 25: 1365-1369.
- Latté, K.P., Appel, K.-E. & Lampen, A. 2011. Health benefits and possible risks of broccoli – An overview. *Food and Chemical Toxicology* 49: 3287-3309.



- Leja, M., Mareczek, A. & Starzyńska, A. 2002. Some antioxidant and senescence parameters of broccoli as related to its developmental stages. *Acta Physiologiae Plantarum* 24: 237-241.
- Lentz, W. 1998. Model applications in horticulture: a review. *Scientia Horticulturae* 74: 151-174.
- Li, H., Jiang, H. & Li, T. 2011. Broccoli plant nitrogen, phosphorus, and water relations at field scale and in various growth media. *International Journal of Vegetable Science* 17: 190-210.
- Liang, C. & Chu, C. 2015. Towards understanding abscisic acid-mediated leaf senescence. *Science China Life Sciences* 58: 506-508.
- Lichtenthaler, H.K., Gitelson, A. & Lang, M. 1996. Non-destructive determination of chlorophyll content of leaves of a green and an aurea mutant of tobacco by reflectance measurements. *Journal of Plant Physiology* 148: 483-493.
- Lindemann-Zutz, K., Fricke, A. & Stützel, H. 2016. Prediction of time to harvest and its variability in broccoli (*Brassica oleracea* var. *italica*) Part I. Plant developmental variation and forecast of time to head induction. *Scientia Horticulturae* 198: 424-433.
- Lipton, W.J. & Harris, C.M. 1974. Controlled atmosphere effects on the market quality of stored broccoli (*Brassica oleracea* L., *Italica* Group). *Journal of the American Society for Horticultural Science* 99: 200-205.
- Lisiewska, Z. & Kmiecik, W. 1996. Effects of level of nitrogen fertilizer, processing conditions and period of storage of frozen broccoli and cauliflower on vitamin C retention. *Food Chemistry* 57: 267-270.
- Liu, J.D., Goodspeed, D., Sheng, Z., Li, B., Yang, Y., Kliebenstein, D.J. & Braam, J. 2015. Keeping the rhythm: light/dark cycles during postharvest storage preserve the tissue integrity and nutritional content of leafy plants. *BMC Plant Biology* 15: 92.
- Lorente, D., Aleixos, N., Gómez-Sanchis, J., Cubero, S., García-Navarrete, O. & Blasco, J. 2012. Recent advances and applications of hyperspectral imaging for fruit and vegetable quality assessment. *Food and Bioprocess Technology* 5: 1121-1142.
- Ma, G., Zhang, L., Setiawan, C.K., Yamawaki, K., Asai, T., Nishikawa, F., Maezawa, S., Sato, H., Kanemitsu, N. & Kato, M. 2014. Effect of red and blue LED light irradiation on ascorbate content and expression of genes related to ascorbate metabolism in postharvest broccoli. *Postharvest Biology and Technology* 94: 97-103.
- Maina, J.N. & Wang, Q. 2015. Seasonal response of chlorophyll *a/b* ratio to stress in a typical desert species: *Haloxylon ammodendron*. *Arid Land Research and Management* 29: 321-334.
- Manchali, S., Chidambara Murthy, K.N. & Patil, B.S. 2012. Crucial facts about health benefits of popular cruciferous vegetables. *Journal of Functional Foods* 4: 94-106.
- Marshall, B. & Thompson, J.E. 1987. A model of the influence of air-temperature and solar-radiation on the time to maturity of calabrese *Brassica oleracea* var. *italica*. *Annals of Botany* 60: 513-519.

- Martinez-Hernandez, G.B., Gomez, P.A., Pradas, I., Artes, F. & Artes-Hernandez, F. 2011. Moderate UV-C pretreatment as a quality enhancement tool in fresh-cut Bimi-® broccoli. *Postharvest Biology and Technology* 62: 327-337.
- Matile, P., Hörtensteiner, S. & Thomas, H. 1999. Chlorophyll degradation. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 67-95.
- McGuire, R.G. 1992. Reporting of objective color measurements. *Hortscience* 27: 1254-1255.
- McRoberts, N. & Lepreux, O. 2003. The challenge of connecting pre-harvest and post-harvest sector concepts of quality in food production. In: Tijssens, L.M.M. & Vollebregt, H.M. (eds.): *International Conference on Quality in Chains. An Integrated View on Fruit and Vegetable Quality*, Wageningen, Netherlands. pp. 105-113. Acta Horticulturae (ISHS) 604.
- Merchant, S.S. 2010. Green with envy: on citation of the chlorophyll assay—commentary. In: *Plant Physiology Classics Collection*, pp. 1. American Society of Plant Biologists.
- Merzlyak, M.N., Gitelson, A.A., Chivkunova, O.B. & Rakitin, V.Y. 1999. Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening. *Physiologia Plantarum* 106: 135-141.
- Minolta 1996. Exakte Farbkommunikation - Vom Farbgefühl bis zur objektiven Messung. In: CIE (ed.). KONICA MINOLTA SENSING INC, Vienna, Austria.
- Moniruzzaman, M., Rahman, S.M.L., Kibria, M.G., Rahman, M.A. & Hossain, M.M. 2007. Effect of boron and nitrogen on yield and hollowstem of broccoli. *Journal of Soil and Nature* 1: 24-29.
- Moretti, C.L., Mattos, L.M., Calbo, A.G. & Sargent, S.A. 2010. Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. *Food Research International* 43: 1824-1832.
- Mourao, I. & Brito, L. 2000. Empirical models for harvest date prediction in broccoli (*Brassica oleracea* L. var. *italica* Plenck). In: King, G.J. (ed.): *Third International Symposium on Brassicas and Twelfth Crucifer Genetics Workshop*, Wellesbourne, U.K. pp. 47-53 Acta Horticulturae (ISHS) 539.
- Mühlecker, W., Ongania, K.-H., Kräutler, B., Matile, P. & Hörtensteiner, S. 1997. Tracking down chlorophyll breakdown in plants: Elucidation of the constitution of a “fluorescent” chlorophyll catabolite. *Angewandte Chemie. International Edition in English* 36: 401-404.
- Nath, A., Bagchi, B., Misra, L.K. & C. Deka, B. 2011. Changes in post-harvest phytochemical qualities of broccoli florets during ambient and refrigerated storage. *Food Chemistry* 127: 1510-1514.
- Nebdal, L., Trtilek, M. & Herppich, W.B. 2000. Methods and equipment for fluorescence imaging on plant material. In: Zude, M. (ed.): *Computer-Bildanalyse in der Landwirtschaft - Workshop 1999 und 2000*. pp. 127-135. Bornimer Agrartechnische Berichte.

- Niinemets, Ü., Bilger, W., Kull, O. & Tenhunen, J.D. 1998. Acclimation to high irradiance in temperate deciduous trees in the field: changes in xanthophyll cycle pool size and in photosynthetic capacity along a canopy light gradient. *Plant, Cell & Environment* 21: 1205-1218.
- Ohta, N. & Robertson, A.R. 2006a. CIE standard colorimetric system. In: *Colorimetry: Fundamentals and Applications*, pp. 63-114. John Wiley & Sons, Ltd.
- Ohta, N. & Robertson, A.R. 2006b. Measurement and calculation of colorimetric values. In: *Colorimetry: Fundamentals and Applications*, pp. 153-174. John Wiley & Sons, Ltd.
- Ohta, N. & Robertson, A.R. 2006c. Uniform Color Spaces. In: *Colorimetry: Fundamentals and Applications*, pp. 115-151. John Wiley & Sons, Ltd.
- Olarte, C., Sanz, S., Federico Echávarri, J. & Ayala, F. 2009. Effect of plastic permeability and exposure to light during storage on the quality of minimally processed broccoli and cauliflower. *LWT - Food Science and Technology* 42: 402-411.
- Ouda, B.A. & Mahadeen, A.Y. 2008. Effect of fertilizers on growth, yield, yield components, quality and certain nutrient contents in broccoli (*Brassica oleracea*). *International Journal of Agriculture and Biology* 10: 627-632.
- Padaga, M., Heard, G.M., Paton, J.E. & Fleet, G.H. 2000. Microbial species associated with different sections of broccoli harvested from three regions in Australia. *International Journal of Food Microbiology* 60: 15-24.
- Page, T., Griffiths, G. & Buchanan-Wollaston, V. 2001. Molecular and biochemical characterization of postharvest senescence in broccoli. *Plant Physiology* 125: 718-727.
- Pék, Z., Daood, H., Gasztonyi Nagyné, M., Berki, M., Tóthné, M.M., Neményi, A. & Helyes, L. 2012. yield and phytochemical compounds of broccoli as affected by temperature, irrigation, and foliar sulfur supplementation. *Hortscience* 47: 1646-1652.
- Piepho, H.-P. & Kalka, E. 2003. Threshold models with fixed and random effects for ordered categorical data. *Food Quality and Preference* 14: 343-357.
- Pink, D., Buchanan-Wollaston, V., Griffiths, G., Paterson, C. & Wurr, D.C.E. 2000. Long life' broccoli: Factors affecting the post-harvest performance of broccoli heads. In: King, G.J. (ed.): *3rd ISHS International Symposium on Brassicas, 12th Crucifer Genetics Workshop*, Wellesbourne. Horticulture Research International, Horticulture Research International Wellesbourne, CV35 9EF, UK.
- Pliakoni, E.D., Deltsidis, A.I., Huber, D.J., Sargent, S.A. & Brecht, J.K. 2015. Physical and biochemical changes in broccoli that may assist in decision-making related to international marine transport in air or CA/MA. In: Amodio, M.L. & Colelli, G. (eds.): *XI International Controlled and Modified Atmosphere Research Conference*, Trani, Italy pp. 951-957. Acta Horticulturae (ISHS) 1071.

- Porra, R.J., Thompson, W.A. & Kriedemann, P.E. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 975: 384-394.
- PSI 2002. User's guide: Kinetic fluorescence CCD camera for photosynthesis research. In. Photon Systems Instruments, s.r.o., Brno, Czech Republic.
- Qin, J. & Lu, R. 2008. Measurement of the optical properties of fruits and vegetables using spatially resolved hyperspectral diffuse reflectance imaging technique. *Postharvest Biology and Technology* 49: 355-365.
- Ren, K., Tu, K., Pan, L. & Chen, Y. 2006. Kinetic modelings of broccoli color changes during chilled storage. *Journal of Food Processing and Preservation* 30: 180-193.
- Richards, J.A. & Jia, X. 2006. *Remote sensing digital image analysis*. 4 ed. Springer-Verlag, Berlin Heidelberg New York.
- Robbins, R.J., Keck, A.-S., Banuelos, G. & Finley, J.W. 2005. Cultivation Conditions and Selenium Fertilization Alter the Phenolic Profile, Glucosinolate, and Sulforaphane Content of Broccoli. *Journal of Medicinal Food* 8: 10.
- Roiser, M.H., Müller, T. & Kräutler, B. 2015. Colorless chlorophyll catabolites in senescent florets of broccoli (*Brassica oleracea* var. *italica*). *Journal of Agricultural and Food Chemistry* 63: 1385-1392.
- Rouse, J.W., Jr., Haas, R.H., Deering, D.W., Schell, J.A. & Harlan, J. 1973. Monitoring the vernal advancement and retrogradation (green wave effect) of natural vegetation. MD: NASA/GSFC & Texas A&M University, Remote Sensing Center, College Station, Texas, Greenbelt.
- Rühle, W. & Wild, A. 1985. Die Anpassung des Photosyntheseapparates höherer Pflanzen an die Lichtbedingungen. *Naturwissenschaften* 72: 10-16.
- Saga, Y. & Tamiaki, H. 2012. Demetalation of chlorophyll pigments. *Chemistry & Biodiversity* 9: 1659-1683.
- Sanchez, M.T., Perez-Marin, D., Flores-Rojas, K., Guerrero, J.E. & Garrido-Varo, A. 2009. Use of near-infrared reflectance spectroscopy for shelf-life discrimination of green asparagus stored in a cool room under controlled atmosphere. *Talanta* 78: 530-536.
- Scheer, H. 2013. Chlorophylls and carotenoids. In: William, J.L. & Lane, M.D. (eds.): *Encyclopedia of Biological Chemistry*, pp. 498-505. Academic Press, Waltham.
- Schelbert, S., Aubry, S., Burla, B., Agne, B., Kessler, F., Krupinska, K. & Hörtensteiner, S. 2009. Pheophytin pheophorbide hydrolase (pheophytinase) is involved in chlorophyll breakdown during leaf senescence in *Arabidopsis*. *The Plant Cell*.
- Scheumann, V., Schoch, S. & Rüdiger, W. 1999. Chlorophyll *b* reduction during senescence of barley seedlings. *Planta* 209: 364-370.

- Schoefs, B. 2002. Chlorophyll and carotenoid analysis in food products. Properties of the pigments and methods of analysis. *Trends in Food Science & Technology* 13: 361-371.
- Schonhof, I., Kläring, H.P., Krumbein, A., Claußen, W. & Schreiner, M. 2007. Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. *Agriculture, Ecosystems & Environment* 119: 103-111.
- Schopfer, P. & Brennicke, A. 2006. *Pflanzenphysiologie*. 6 ed. Elsevier GmbH, Spektrum Akademischer Verlag, Munich.
- Schouten, R.E., Tijskens, L.M.M. & van Kooten, O. 2002. Predicting keeping quality of batches of cucumber fruit based on a physiological mechanism. *Postharvest Biology and Technology* 26: 209-220.
- Schouten, R.E., Zhang, X., Verschoor, J.A., Otma, E.C., Tijskens, L.M.M. & van Kooten, O. 2009. Development of colour of broccoli heads as affected by controlled atmosphere storage and temperature. *Postharvest Biology and Technology* 51: 27-35.
- Serrano, M., Martinez-Romero, D., Guillén, F., Castillo, S. & Valero, D. 2006. Maintenance of broccoli quality and functional properties during cold storage as affected by modified atmosphere packaging. *Postharvest Biology and Technology* 39: 61-68.
- Shannon, S. & Meeks-Wagner, D.R. 1993. Genetic interactions that regulate inflorescence development in *Arabidopsis*. *The Plant Cell* 5: 639-655.
- Shelp, B.J., Penner, R. & Zhu, Z. 1992. Broccoli (*Brassica oleracea* var. *italica*) cultivar response to boron deficiency. *Canadian Journal of Plant Science* 72: 883-888.
- Shewfelt, R.L. 1993. Measuring quality and maturity. In: *Postharvest handling: a systems approach*, pp. 99-124. Academic Press, Inc., California, USA.
- Shewfelt, R.L., Batal, K.M. & Heaton, E.K. 1983. Broccoli storage: Effect of N<sup>6</sup>-benzyladenine, packaging, and icing on color of fresh broccoli. *Journal of Food Science* 48: 1594-1597.
- Shewfelt, R.L., Heaton, E.K. & Batal, K.M. 1984. Nondestructive color measurement of fresh broccoli. *Journal of Food Science* 49: 1612-1613.
- Sørensen, L. & Grevsen, K. 1994. Effects of plant spacing on uniformity in broccoli for once-over harvest. *European Journal of Horticultural Science* 59: 102-105.
- Suzuki, Y., Kimura, T., Takahashi, D. & Terai, H. 2005. Ultrastructural evidence for the inhibition of chloroplast-to-chromoplast conversion in broccoli floret sepals by ethanol vapor. *Postharvest Biology and Technology* 35: 237-243.
- Tan, D.K.Y. 1999. Effect of temperature and photoperiod on broccoli development, yield and quality in south-east Queensland Doctor of Philosophy, The University of Queensland

- Tan, D.K.Y., Birch, C.J., Wearing, A.H. & Rickert, K.G. 2000. Predicting broccoli development: I. Development is predominantly determined by temperature rather than photoperiod. *Scientia Horticulturae* 84: 227-243.
- Tanaka, A. & Tanaka, R. 2006. Chlorophyll metabolism. *Current Opinion in Plant Biology* 9: 248-255.
- Thiel, M., Rath, T. & Ruckelshausen, A. 2011 + 2012. Messung von Feuchtigkeitsvariationen bei Pflanzenblättern am Beispiel von Brokkoli auf Basis eines NIR Hyperspectral Imaging Systems. In: Zude, M., Weis, M. & Ruckelshausen, A. (eds.): *Computerised Image Analysis in Agriculture*, Stuttgart and Osnabrück. pp. 48-63. Bornimer Agrartechnische Berichte, Leibniz Institute for Agricultural Engineering, Potsdam-Bornim.
- Thompson, T.L., Doerge, T.A. & Godin, R.E. 2002. Subsurface drip irrigation and fertigation of broccoli: I. Yield, quality, and nitrogen uptake. *Soil Science Society of America Journal* 66: 186–192.
- Tian, M.S., Davies, L., Downs, C.G., Liu, X.F. & Lill, R.E. 1995. Effects of floret maturity, cytokinin and ethylene on broccoli yellowing after harvest. *Postharvest Biology and Technology* 6: 29-40.
- Tian, M.S., Downs, C.G., Lill, R.E. & King, G.A. 1994. A role for ethylene in the yellowing of broccoli after harvest. *Journal of the American Society for Horticultural Science* 119: 276-281.
- Tian, M.S., Woolf, A.B., Bowen, J.H. & Ferguson, I.B. 1996. Changes in color and chlorophyll fluorescence of broccoli florets following hot water treatment. *Journal of the American Society for Horticultural Science* 121: 310-313.
- Tijskens, L.M.M., Schijvens, E.P.H.M. & Biekman, E.S.A. 2001. Modelling the change in colour of broccoli and green beans during blanching. *Innovative Food Science & Emerging Technologies* 2: 303-313.
- Toivonen, P.M.A. 1992. Chlorophyll fluorescence as a nondestructive indicator of freshness in harvested broccoli. *Hortscience* 27: 1014-1015.
- Toivonen, P.M.A. & DeEll, J.R. 2001. Chlorophyll fluorescence, fermentation product accumulation, and quality of stored broccoli in modified atmosphere packages and subsequent air storage. *Postharvest Biology and Technology* 23: 61-69.
- Toivonen, P.M.A. & DeEll, J.R. 1998. Differences in chlorophyll fluorescence and chlorophyll content of broccoli associated with maturity and sampling section. *Postharvest Biology and Technology* 14: 61-64.
- Toivonen, P.M.A. & Sweeney, M. 1998. Differences in chlorophyll loss at 13 degrees C for two broccoli (*Brassica oleracea* L.) cultivars associated with antioxidant enzyme activities. *Journal of Agricultural and Food Chemistry* 46: 20-24.
- Toivonen, P.M.A., Zebarth, B.J. & Bowen, P.A. 1994. Effect of nitrogen fertilization on head size, vitamin C content and storage life of broccoli (*Brassica oleracea* var. *Italica*). *Canadian Journal of Plant Science* 74: 607-610.

- Topcu, Y., Dogan, A., Kasimoglu, Z., Sahin-Nadeem, H., Polat, E. & Erkan, M. 2015. The effects of UV radiation during the vegetative period on antioxidant compounds and postharvest quality of broccoli (*Brassica oleracea* L.). *Plant Physiology and Biochemistry* 93: 56-65.
- UNECE 2010. UNECE-STANDARDS FFV-48 concerning the marketing and commercial quality control of broccoli. In: UNECE (ed.). *Agricultural Quality Standards of the United Nations Economic Commission for Europe*, New York and Geneva.
- Vagen, I.M., Skjelvag, A.O. & Bonesmo, H. 2004. Growth analysis of broccoli in relation to fertilizer nitrogen application. *Journal of Horticultural Science & Biotechnology* 79: 484-492.
- Vallejo, F., Tomás-Barberán, F.A., Benavente-García, A.G. & García-Viguera, C. 2003. Total and individual glucosinolate contents in inflorescences of eight broccoli cultivars grown under various climatic and fertilisation conditions. *Journal of the Science of Food and Agriculture* 83: 307-313.
- von Willert, D.J., Matyssek, R. & Herppich, W. 1995. *Experimentelle Pflanzenökologie: Grundlagen und Anwendungen*. Thieme, Stuttgart.
- Wang, C.Y. 1979. Effect of short-term high CO<sub>2</sub> treatment on the market quality of stored broccoli. *Journal of Food Science* 44: 1478-1482.
- Weemaes, C., Ooms, V., Indrawati, Ludikhuyze, L., Van den Broeck, I., Van Loey, A. & Hendrickx, M. 1999a. Pressure-temperature degradation of green color in broccoli juice. *Journal of Food Science* 64: 504-508.
- Weemaes, C.A., Ooms, V., Van Loey, A.M. & Hendrickx, M.E. 1999b. Kinetics of chlorophyll degradation and color loss in heated broccoli juice. *Journal of Agriculture and Food Chemistry* 47: 2404-2409.
- Weston, L.A. & Barth, M.M. 1997. Preharvest factors affecting postharvest quality of vegetables. *Hortscience* 32: 812-816.
- Wurr, D.C.E., Fellows, J.R., Phelps, K. & Reader, R.J. 1995. Vernalization in calabrese (*Brassica oleracea* var. *italica*) - a model for apex development. *Journal of Experimental Botany* 46: 1487-1496.
- Wurr, D.C.E., Hambidge, A.J., Fellows, J.R., Lynn, J.R. & Pink, D.A.C. 2002. The influence of water stress during crop growth on the postharvest quality of broccoli. *Postharvest Biology and Technology* 25: 193-198.
- Yahia, E.M. & Ornélas-Paz, J.d.J. 2009. Chemistry, stability, and biological actions of carotenoids. In: de la Rosa, L.A., Alvarez-Parilla, E. & Gonzalez-Aguilar, G.A. (eds.): *Fruit and vegetable phytochemicals: Chemistry, nutritional value and stability*, pp. 177-222.
- Yamauchi, N., Harada, K. & Watada, A.E. 1997. In vitro chlorophyll degradation in stored broccoli (*Brassica oleracea* L. var. *italica* Plen.) florets. *Postharvest Biology and Technology* 12: 239-245.
- Yoldas, F., Ceylan, S., Yagmur, B. & Mordogan, N. 2008. Effects of nitrogen fertilizer on yield quality and nutrient content in broccoli. *Journal of Plant Nutrition* 31: 1333-1343.

- Zaicovski, C.B., Zimmerman, T., Nora, L., Nora, F.R., Silva, J.A. & Rombaldi, C.V. 2008. Water stress increases cytokinin biosynthesis and delays postharvest yellowing of broccoli florets. *Postharvest Biology and Technology* 49: 436-439.
- Zdunek, A. & Herppich, W.B. 2012. Relation of biospeckle activity with chlorophyll content in apples. *Postharvest Biology and Technology* 64: 58-63.
- Zebarth, B.J., Bowen, P.A. & Toivonen, P.M.A. 1995. Influence of nitrogen fertilization on broccoli yield, nitrogen accumulation and apparent fertilizer-nitrogen recovery. *Canadian Journal of Plant Science* 75: 717-725.
- Zhan, L., Hu, J., Li, Y. & Pang, L. 2012. Combination of light exposure and low temperature in preserving quality and extending shelf-life of fresh-cut broccoli (*Brassica oleracea* L.). *Postharvest Biology and Technology* 72: 76-81.
- Zhuang, H., Barth, M.M. & Hildebrand, D.F. 1994. Packaging influenced total chlorophyll, soluble protein, fatty acid composition and lipoxygenase activity in broccoli florets. *Journal of Food Science* 59: 1171-1174.
- Zude, M. 2003. Comparison of indices and multivariate models to non-destructively predict the fruit chlorophyll by means of visible spectrometry in apple fruit. *Analytica Chimica Acta* 481: 119-126.
- Zutz, K., Fricke, A. & Stützel, H. 2013. Vorhersage von Ernteterminen zur Terminproduktion bei Brokkoli (*Brassica oleracea* var. *italica*). In: 48. *Gartenbauwissenschaftliche Jahrestagung*, pp. 36. BHGL & DGG, Bonn.



## List of abbreviations

1-MCP	1-Methylcyclopropene
a*	coordinate of CIELAB, negative values indicate green, positive values indicate magenta
Aur	auroxanthin
b*	coordinate of CIELAB, negative values indicate blue, positive values indicate yellow
Chlide	chlorophyllide a
CIE	Commission Internationale de l'Eclairage
CIELAB	color space of CIE 1976 (L*, a*, b*)
DS	“defined storage” – storage under 16 °C and high RH (~95 %) in order to accelerate postharvest processes
F <sub>0</sub>	minimum fluorescence
F <sub>m</sub>	maximum fluorescence (dark)
F <sub>m</sub> '	maximum fluorescence (light)
F <sub>t</sub>	steady-state fluorescence
F <sub>v</sub> /F <sub>m</sub>	(F <sub>m</sub> -F <sub>0</sub> ) /F <sub>m</sub> ; maximum photosynthetic efficiency (dark)
FW	fresh weight
HPLC	high performance liquid chromatography
Hunter lab	Hunter 1948 color space
IGZ	Leibniz Institute of Vegetable and Ornamental Crops at Großbeeren
L*	lightness coordinate of CIELAB, L* = 0 yields black and L* = 100 indicates diffuse white
LHC	light harvesting complex
LUH	Gottfried Wilhelm Leibniz Universität Hannover
Lut	lutein
MAP	modified atmosphere packaging
N	nitrogen
NDVI	normalized difference vegetation index
NIR	near infrared part of electromagnetic spectrum

Nx	neoxanthin
PAR	photosynthetic active radiation
PCA	principal component analysis
Pheoide	pheophorbide <i>a</i>
Pheoin	pheophytin <i>a</i>
PLS	partial least squares
PS II	photosystem II, protein complex of photosynthesis
REIP	red edge inflection point
RGB	colour model of red, green and blue
β-Car	β carotene
stdev	standard deviation
UNECE	United Nations Economic Commission for Europe
USDA	United States Department for Agriculture
UV	ultraviolet part of electromagnetic spectrum
VIS	visible part of electromagnetic spectrum
Vx	violaxanthin
$\Phi_{\text{PSII}}, (F_m' - F_t) / F_m'$	Effective quantum yield of photochemical energy conversion in PSII

### **Acknowledgements (in German)**

Ich bedanke mich beim Bundesministerium für Bildung und Forschung, das die vorliegenden Untersuchungen im Rahmen des Programms „AgroClustEr: Kompetenznetze in der Agrar- und Ernährungsforschung“ über 3,5 Jahre gefördert hat. Bei meinen Betreuern Martin Geyer und Prof. Uwe Schmidt möchte ich mich vor allem für das große Vertrauen bedanken, das sie mir bei der Bearbeitung des Themas entgegenbrachten. Mein besonderer Dank gilt meinem Mentor Andreas Poth, der mir den Bereich der Bildanalyse sowie den der Datenbankanwendungen auf anschauliche Weise näherbringen konnte. Bei Amin Ghadiri Khozroughi und Christian Ammon bedanke ich mich für die großartige fachliche Unterstützung. Bei den Projektpartnern, insbesondere bei Frank Weinhold, Georg Mevenkamp, Miguel Aguila, Matthias Schultze, Karsten Lindemann-Zutz, Andreas Fricke und Rita Zrenner möchte ich mich für die gute Zusammenarbeit bedanken. Ich möchte mich weiterhin bei den Mitarbeitern und Mitgliedern der Graduiertenschule WeGa PhD für die hervorragenden Möglichkeiten der Weiterbildung und ihr Feedback bedanken.

An dieser Arbeit haben mehrere Studierende im Rahmen ihrer Ausbildung mitgewirkt, bei denen ich mich ganz besonders herzlich bedanken möchte. Allen voran ist Anne Keiser zu nennen, die im Bereich der Analytik erfolgreich ihre Masterarbeit anfertigte. Mein Dank gilt außerdem Nelya Mosig, Stephan Rietschel und Christian Mayer.

Für kritische Anregungen bedanke ich mich bei Werner Herppich und Susanne Huyskens-Keil. Wilfried Ficht danke ich für die schematische Darstellung der Spektrometereinheit.

Bei Michael Pflanz, Jelena Surdilovic und Hanna Brabandt möchte ich mich vor allem für die schöne Zeit am ATB bedanken.

Ich danke meinem Mann Tobias Mörbitz und meiner Familie für die Unterstützung.

**Selbstständigkeitserklärung (in German)**

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe sowie alle Textstellen, die ich dem Wortlaut oder dem Sinne nach aus Quellen übernommen habe, entsprechend kenntlich gemacht habe.

**In der Reihe****Bornimer Agrartechnische Berichte**

sind bisher erschienen:

Heft 1	Technik und Verfahren der Landschaftspflege	1992
Heft 2	Beiträge zur Lagerung und Verarbeitung pflanzenbaulicher Produkte	1993
Heft 3	Technik und Verfahren in der Tierhaltung	1993
Heft 4	Technik und Verfahren der Landschaftspflege und für die Verwendung der anfallenden Materialien	1994
Heft 5	Verfahrenstechnik der Aufbereitung, Lagerung und Qualitätserhaltung pflanzlicher Produkte	1994
Heft 6	Biokonversion nachwachsender Rohstoffe und Verfahren für Reststoffbehandlung	1994
Heft 7	Preußische Versuchs- und Forschungsanstalt für Landarbeit und Schlepperprüffeld in Bornim 1927 bis 1945	1995
Heft 8	Qualitätssicherung und Direktvermarktung	1996
Heft 9	Konservierende Bodenbearbeitung auf Sandböden	1996
Heft 10	Anwendung wärme- und strömungstechnischer Grundlagen in der Landwirtschaft	1996
Heft 11	Computer-Bildanalyse in der Landwirtschaft Workshop 1996	1996
Heft 12	Aufbereitung und Verwertung organischer Reststoffe im ländlichen Raum	1996
Heft 13	Wege zur Verbesserung der Kartoffelqualität durch Verminderung der mechanischen Beanspruchung	1997
Heft 14	Computer-Bildanalyse in der Landwirtschaft Workshop 1997	1997
Heft 15	Technische und ökonomische Aspekte der Nutztierhaltung in großen Beständen	1997
Heft 16	11. Arbeitswissenschaftliches Seminar	1997
Heft 17	Nachwachsende Rohstoffe im Land Brandenburg Stand Aktivitäten und Perspektiven einer zukunftsfähigen und umweltgerechten Entwicklung	1998
Heft 18	Qualität von Agrarprodukten	1998
Heft 19	Computer-Bildanalyse in der Landwirtschaft Workshop 1998	1998
Heft 20	Beiträge zur teilflächenspezifischen Bewirtschaftung	1998

---

Heft 21	Landnutzung im Spiegel der Technikbewertung – Methoden Indikatoren, Fallbeispiele	1998
Heft 22	Kriterien der Nachhaltigkeit in der Verfahrensentwicklung für die Nutztierhaltung	1999
Heft 23	Situation und Trends in der Landtechnik / Erneuerbare Energien in der Landwirtschaft	1999
Heft 24	Institut für Landtechnik der Deutschen Akademie der Landwirtschaftswissenschaften zu Berlin 1951 bis 1965	1999
Heft 25	Computer-Bildanalyse in der Landwirtschaft Workshop 1999 / 2000	2000
Heft 26	Computer-Bildanalyse in der Landwirtschaft Workshop 2001	2001
Heft 27	Approaching Agricultural technology and Economic Development of Central and Eastern Europe	2001
Heft 28	6 <sup>th</sup> International Symposium on Fruit, Nut, and Vegetable Production Engineering	2001
Heft 29	Measurement Systems for Animal Data and their Importance for Herd Management on Dairy Cow Farms	2002
Heft 30	Produktion, Verarbeitung und Anwendung von Naturfasern	2002
Heft 31	Computer-Bildanalyse in der Landwirtschaft Workshop 2002	2002
Heft 32	Biogas und Energielandwirtschaft - Potenzial, Nutzung, Grünes Gas™, Ökologie und Ökonomie	2003
Heft 33	Sozioökonomische Aspekte zu Perspektiven des Offenlandmanagements	2003
Heft 34	Computer-Bildanalyse in der Landwirtschaft Workshop 2003	2003
Heft 35	Energieholzproduktion in der Landwirtschaft Potenzial, Anbau, Technologie, Ökologie und Ökonomie	2004
Heft 36	High-Tech Innovationen für Verfahrensketten der Agrarproduktion. Statusseminar 2003	2004
Heft 37	Computer-Bildanalyse in der Landwirtschaft Workshop 2004	2004
Heft 38	Die Landmaschinenprüfung in der DDR 1951-1991 und ihre Vorgeschichte	2004
Heft 39	Energieverlust und Schimmelpilzentwicklung bei der Lagerung von Feldholz-Hackgut	2005
Heft 40	Computer-Bildanalyse in der Landwirtschaft Workshop 2005	2005
Heft 41	Demonstration der Langzeitwirkung bedarfsorientierter Fungizidbehandlung mit dem CROP-Meter	2005

Heft 42	Biochemicals and Energy from Sustainable Utilization of herbaceous Biomass (BESUB)	2005
Heft 43	Ozontes Waschwasser zur Qualitätssicherung leichtverderblicher Produkte - Entwicklung einer <i>Fuzzy-Logic</i> -Steuerung des Waschprozesses	2005
Heft 44	Messsystem zur Bewertung des Unkrautvorkommens	2005
Heft 45	Anwendung der Thermographie zur Optimierung der Belüftungssteuerung bei der Lagerhaltung landwirtschaftlicher Produkte	2005
Heft 46	Membranbioreaktor zur Aufbereitung von Schlachthofabwässern Prozesssteuerung von Biogasanlagen mit Kofermentation	2005
Heft 47	Verschleißeinfluss auf das Förderverhalten von Drehkolbenpumpen	2005
Heft 48	Qualitätserhaltung und Qualitätssicherung von Bioobst und Biogemüse in der Nachernte	2005
Heft 49	Miniaturisiertes Datenerfassungs-System zum Implantieren in Früchte und zur Messung ihrer mechanischen Belastung durch Ernte- und Nachernteverfahren	2005
Heft 50	Prozesskontrolle der Qualität von frischem Obst und Gemüse mit Hilfe eines Multigas-Sensors	2005
Heft 51	Entwicklung eines Echtzeitsensors für die Stärkebestimmung bei Kartoffeln als funktionaler Bestandteil eines optoelektronischen Verleseautomaten	2005
Heft 52	Optimierte Steuerung von Getreide-Schachttrocknern	2005
Heft 53	Möglichkeiten und Grenzen der energetischen Nutzung von Rizinusöl	2005
Heft 54	Non-Destructive Methods for Detecting Health-Promoting Compounds COST Action 924 Working Group Meeting	2005
Heft 55	4 <sup>th</sup> IFAC / CIGR Workshop Control Applications in Post - Harvest and Processing Technology (CAPPT 2006) 26th - 29th March 2006, Potsdam, GERMANY	2006
Heft 56	Computer-Bildanalyse in der Landwirtschaft Workshop 2006	2006
Heft 57	Kontrolle der Frische in der Nacherntekette von Ökogemüse	2006
Heft 58	Entwicklung eines innovativen Dekontaminationsverfahrens als Technologieantwort auf zukünftiges Qualitätsmanagement im Nacherntebereich	2006
Heft 59	Experimental Studies and Mathematical Modelling of Solar Drying System for Production of High Quality Dried Tomato	2007

Heft 60	13. Workshop Computer-Bildanalyse in der Landwirtschaft & 4. Workshop Precision Farming	2007
Heft 61	Energiepflanzen im Aufwind Wissenschaftliche Ergebnisse und praktische Erfahrungen zur Produktion von Biogaspflanzen und Feldholz	2007
Heft 62	14. Workshop Computer-Bildanalyse in der Landwirtschaft	2008
Heft 63	Experten-Workshop Lagerung von Holzhackschnitzeln	2008
Heft 64	Postharvest unlimited 2008	2008
Heft 65	Vom Agrarrohstoff zu neuen Produkten – Verfahrens- technische Forschung im Nacherntebereich	2009
Heft 66	16. Arbeitswissenschaftliches Kolloquium des VDI-MEG Arbeitskreises Arbeitswissenschaften im Landbau	2009
Heft 67	Monitoring der methanbildenden Mikroflora in Praxis- Biogasanlagen im ländlichen Raum: Analyse des Ist- Zustandes und Entwicklung eines quantitativen Nachweissystems	2009
Heft 68	Wieviel Biogas steckt in Pflanzen? Abschluss- Symposium des "Biogas-Crops-Network" (BCN) 7. Mai 2009 Potsdam	2009
Heft 69	Image Analysis for Agricultural Products and Processes 27 to 28. Aug. 2009 Potsdam	2009
Heft 70	5th International Technical Symposium on Food Processing, Monitoring Technology in Bioprocesses and Food Quality Management 31. Aug. to 02. Sept. 2009 Potsdam	2009
Heft 71	Einsatz von Biogas in PEM-Brennstoffzellen	2009
Heft 72	Teilflächenspezifische Grunddüngung	2009
Heft 73	16. Workshop Computer-Bildanalyse in der Landwirtschaft 04. Mai 2010 Braunschweig	2010
Heft 74	Erschließung von Nachhaltigkeitspotenzialen durch Nutzung innovativer Sensortechnologien <i>-Prozesskette Getreide-</i>	2010
Heft 75	Erschließung von Nachhaltigkeitspotenzialen durch Nutzung innovativer Sensortechnologien <i>-Prozesskette pflanzliche Frischeprodukte-</i>	2010
Heft 76	International Workshop The future of the quarter individual milking 14. – 15. September 2010 Potsdam	2010
Heft 77	A flow cytometric approach to monitor the effects of gentle preservation techniques in the postharvest chain	2011



Heft 78	17. und 18. Workshop Computer-Bildanalyse in der Landwirtschaft 05. Mai 2011 Stuttgart und 09. Mai 2012 Osnabrück	2012
Heft 79	2. Öffentliches Symposium des „BCN“ BiogasPOTENZIALE Erkennen, Erforschen, Erwirtschaften	2012
Heft 80	Mechanisms of Bacillus spore germination and inactivation during high pressure processing	2013
Heft 81	19. Workshop Computer-Bildanalyse in der Landwirtschaft 2. Workshop Unbemannte autonom fliegende Systeme in der Landwirtschaft 06. – 07. Mai 2013 Berlin	2013
Heft 82	3rd Global Workshop on Proximal Soil Sensing	2013
Heft 83	19. Arbeitswissenschaftliches Kolloquium des VDI-MEG Arbeitskreises Arbeitswissenschaften im Landbau 11. – 12. März 2014 Dresden	2014
Heft 84	Prozessmikrobiologie in landwirtschaftlichen Biogasanlagen Schlussbericht zum Forschungsverbund BIOGAS-BIOCOENOSIS	2014
Heft 85	Sensoren.Modelle.Erntetechnik Kolloquium zur Verabschiedung von Dr. Ehlert 27. Mai 2014, Potsdam-Bornim	2014
Heft 86	Phosphor für die Landwirtschaft – Strategien für eine endliche Ressource 11. Juni 2014, Potsdam-Bornim	2014
Heft 87	Biofilme in Biogasanlagen - Struktur, Einfluss auf die Biogasausbeute und Optimierung technischer Systeme zur Rückhaltung der mikrobiellen Biomasse BIOGAS-BIOFILM	2015
Heft 88	20. und 21. Workshop Computer-Bildanalyse in der Landwirtschaft 3. Workshop Unbemannte autonom fliegende Systeme (UAS) in der Landwirtschaft 26. Mai 2014, Osnabrück und 06. und 07. Mai 2015, Braunschweig	2015
Heft 89	International Biochar Symposium: Biochar Contribution to Sustainable Agriculture 28 <sup>th</sup> – 29 <sup>th</sup> May 2015, Potsdam	2015
Heft 90	ISHS Symposium 2016 “Sensing Plant Water Status” Methods and Applications in Horticultural Science 05 <sup>th</sup> – 07 <sup>th</sup> October 2016 Potsdam	2016
Heft 91	10 <sup>th</sup> International FRUTIC Symposium Quality and Safety of Fresh Horticultural Commodities February 07, 2017	2017

Heft 92	Etablierung eines <i>core</i> -Mikrobioms für Biogasanlagen Genom-Sequenzierung von Isolaten aus Biogasanlagen und Mapping von Metagenom-Datensätzen BIOGAS-CORE	2017
Heft 93	22. Workshop Computer-Bildanalyse und Unbemannte autonom fliegende Systeme in der Landwirtschaft 23. Workshop Computer-Bildanalyse in der Landwirtschaft 21. April 2016, Wernigerode und 27. April 2017, Potsdam- Marquardt	2017
Heft 94	Leitfaden für die Trocknung von Arznei- und Gewürzpflanzen	2017
Heft 95	Entwicklung von molekularen Markern und Nachweisverfahren auf Basis der quantitativen (realtime) PCR zum Monitoring von prozessrelevanten Mikroorganismen als Frühwarnsysteme für Prozessstörungen	2017
Heft 96	Cold atmospheric pressure plasma treatment of food matrices: Tailored modification of product properties along value-added chains of plant and animal related products	2017
Heft 97	INSECTA Conference 2017 07 <sup>th</sup> – 08 <sup>th</sup> September 2017, Berlin, Germany	2017
Heft 98	Storability of broccoli – investigations of optical monitoring, chlorophyll degradation and predetermination in the field	2018

Interessenten wenden sich an:

Leibniz-Institut für Agrartechnik und Bioökonomie e.V.  
Max-Eyth-Allee 100  
14469 Potsdam

Tel.: (0331) 5699-820  
Fax.: (0331) 5699-849  
E-Mail: [atb@atb-potsdam.de](mailto:atb@atb-potsdam.de)

Schutzgebühr Print: 20,00 Euro

CD-Rom: 10,00 Euro