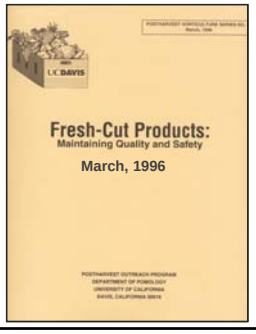
III International Conference on Fresh-cut Produce
UC DAVIS POSTHARVEST TECHNOLOGY
ISHS
September 13-18, 2015

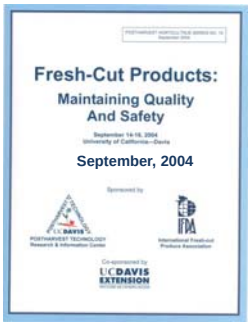
Preharvest Factors and Fresh-cut Quality of Leafy Vegetables

CSIC

Mabel Gil
Research Group on Quality, Safety and Bioactivity of Fruits and Vegetables
CEBAS- CSIC, Murcia Spain

III International Conference on Fresh-cut Produce
UC DAVIS POSTHARVEST TECHNOLOGY
ISHS
September 13-18, 2015





CSIC

Quality characteristics of leafy greens

1. Fresh appearance
2. No discoloration,
3. No decay,
4. Crisp texture,
5. Good flavour (aroma and taste)

Main causes of quality loss

1. Browning
2. Off- flavours
3. Texture loss

CSIC

Pre-harvest factors that affect quality of leafy greens

1. Genotypes
2. Environmental conditions
3. Agricultural practices
4. Maturity at harvest
5. Harvest time

CSIC

1. Genotype

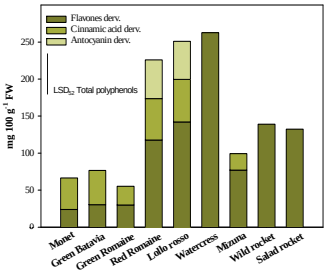
Criteria for the selection

- Yield (high plant density, low core lenght, solid midrib),
- Visual appearance, flavor,
- Resistant to internal defects (tipburn and pinkrib),
- Health promoting constituents

CSIC

1. Genotype: Selection based on health promoting constituents

Individual and total polyphenol content of baby leaves



1. Genotype

Criteria for the selection

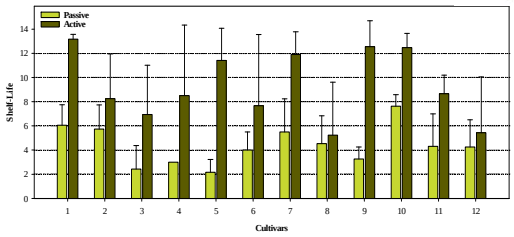
- Yield (high plant density, low core elnght, solid midrib),
- Visual appearance, flavor,
- Resistant to internal defects (tipburn and pinkrib),
- Health promoting constituents
- Selection of cultivars with lower response for cutting

1. Genotype: Selection based on lower response to cutting

1. Genotype: Selection based on browning potential

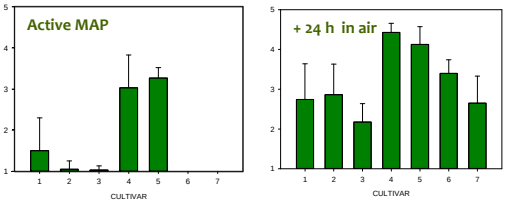
Shelf life of 12 cultivars of fresh-cut Romaine lettuce stored in **passive and active MAP** for 3 days at 4°C plus the rest of storage at 7°C

syngenta SEEDS



1. Genotype: Selection based on browning potential

Cut edge browning of fresh-cut Romaine lettuce cultivars stored for 11 days at 7°C



1 = absence, 3 = moderate and 5 = severe

1. Genotype: Selection based on browning potential

Harvest dates:
- December
- January
- April

Tissues studied:
- Photosynthetic + midrib
- Midrib

Results:
Cultivar 1 vs cv 4: higher susceptibility to browning, lower vitamin C, higher phenolic content and higher enzymatic activities of browning related enzymes (PPO and POD)

Differences between two Romaine cultivars

Quality attributes	1	4
Vitamin C	-	+
Phenolic content	+	-
PAL	+	-
POD	+	-
PPO	+	-
% PPO activation	+	-
Respiration rate	+	-
Browning in air	+	-
Browning in MAP	-	-
Off-flavours in MAP	+	

1. Genotype: Selection based on browning potential

Biomarkers of enzymatic browning (AGL2013-48529-R)

Plant metabolites including phenolic compounds and other primary and secondary metabolites evaluated using a metabolomic approach

Agilent QTOF 6550

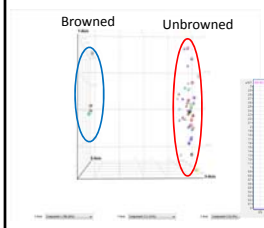
Agilent QQQ 6460

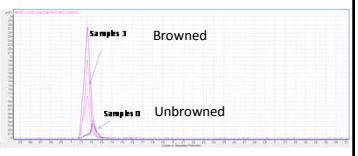
UPLC-MS-QTOF (Quadrupole + Time of flight): Non-targeted metabolomics. Exact molecular weight and chemical formula. High sensitivity. Authentic standards for identification

UPLC-MS-QQQ (Triple Quadrupole): Targeted metabolomics. High sensitivity. Authentic standards for quantitation

1. Genotype: Selection based on browning potential

Biomarkers of enzymatic browning (AGL2013-48529-R)





Selection of specific groups of metabolites by clustering and principal component analysis

1. Genotype: Selection based on browning potential

Biomarkers of enzymatic browning (AGL2013-48529-R)

The untargeted metabolomic study was able to differentiate three groups of metabolites that were able to explain differences in the browning process

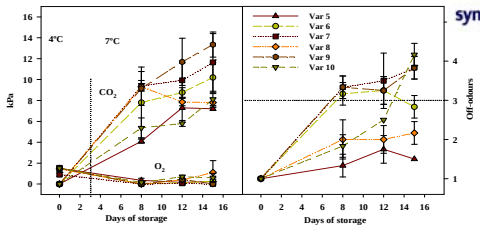
Phospholipids, Fatty acids and Phenylpropanoids

Compound	Camila 0 days of storage	Camila 5 days of storage	tenela 0 days of storage	tenela 5 days of storage	Class
PP(16:1(9Z)/18:0)					Phospholipids
PI(18:2(9Z,12Z)/0:0)					Phospholipids
PS(18:3(6Z,9Z,12Z)/0:0)					Phospholipids
LysoPE(0:0/20:2(11Z,14Z))					Phospholipids
PE(19:0/0:0)					Phospholipids
LysoPE(0:0/20:2(11Z,14Z))					Phospholipids
LysoPE(0:0/20:3(8Z,11Z,14Z))					Phospholipids
LysoPE(0:0/18:0)					Phospholipids
LysoPE(18:3(6Z,9Z,12Z)/0:0)					Phospholipids
LysoPE(0:0/18:3(9Z,12Z,15Z))					Phospholipids
LysoPE(0:0/16:0)					Phospholipids
LPA(18:2(9Z,12Z)/0:0)					Phospholipids
PA(18:3(9Z,12Z,15Z)/0:0)					Phospholipids

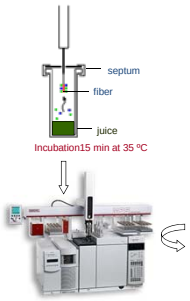
Compound	Barracuda 0 Days of storage	Barracuda 5 Days of storage	Greenforest 0 Days of storage	Greenforest 5 Days of storage	Class
PS(20:5(5Z,8Z,11Z,14Z,17Z)/0:0)					Phospholipids
LysoPE(15:0/0:0)					Phospholipids
PE(18:0/0:0)					Phospholipids
LysoPE(0:0/18:4(6Z,9Z,12Z,15Z))					Phospholipids
PS(18:3(6Z,9Z,12Z)/0:0)					Phospholipids
PE(18:3(6Z,9Z,12Z)/0:0)					Phospholipids
LysoPE(0:0/18:3(9Z,12Z,15Z))					Phospholipids
PS(18:2(9Z,12Z)/0:0)					Phospholipids

1. Genotype: Selection based on low off-flavor potential

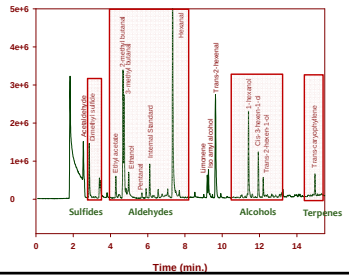
Gas composition (left) and off-flavors (right) of fresh-cut Iceberg cultivars stored in Active MAP for 3 days at 4°C + 13 days at 7°C



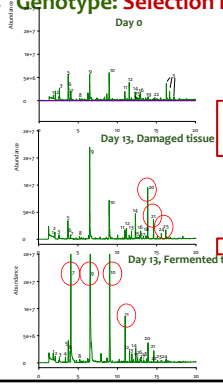
1. Genotype: Selection based on low off-flavor potential



Analysis of volatiles by GC-MS Solid Phase Micro Extraction (SPME)



1. Genotype: Selection based on low off-flavor potential

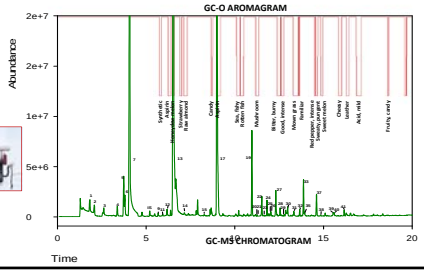


1. Acetaldehyde
2. Dimethyl sulfide
3. 2-Methyl propanal
4. Ethyl acetate
5. 2-Methyl butanal
6. 3-Methyl butanal
7. Ethanol
8. α- Pinene
9. 3-Methyl-pentanal
10. Toluene
11. Butanoic acid 2-methyl ethyl ester
12. Butanoic acid 3-methyl ethyl ester
13. Hexanal
14. 1-Undecane
15. Heptanal
16. Limonene
17. Trans-2-hexenal
18. Styrene
19. 1-hexanol
20. 2-ethyl-1,6-dioxaspiro [4,4]nonane
21. 2-ethyl-1,6-dioxaspiro [4,4]nonane
22. 3-Hexen-1-ol
23. 2-nonanone
24. 2-Hexen-1-ol
25. 2,4-Hexadienal
26. 2,4-Hexadienal
27. 2-Methoxy-3-(1-methylethyl)-pyrazine
28. 1-Octen-3-ol
29. Acetic acid
30. 2-Ethyl-1-hexanol
31. 2-Methoxy-2-(1-methylpropyl)-pyrazine
32. 2-Methoxy-2-(2-methylpropyl)-pyrazine
33. 2-Nonenal
34. 15-(-)Camphor
35. Benzaldehyde
36. β-Elemene
37. 2,6-Nonadienal
38. Caryophyllene
39. Benzeneacetaldehyde
40. 2,4-nonadienal
41. 2,4-nonadienal
42. Siloxanes derivatives

1. Genotype: Selection based on low off-flavor potential

Biomarkers of gaseous and volatile compounds (AGL2013-48529-R)

Combination of gas chromatography-coupled olfactometry techniques (GC-O) and gas chromatography–mass spectrometry (GC–MS)



1. Genotype selection

Conclusions

- Selection of cultivars is critical because of the strong influence in the "freshness" of the fresh-cut product

Advantages

- Consistency of the raw material
- Consistency of the quality of fresh-cut product
- Consistency of the stability of the shelf life

1. Genotype selection

nunhems

Breeding program for lettuce

nunhems

1. Genotype selection

1. Genotype selection

1. Genotype selection: Disadvantages

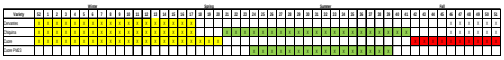
- Other factors such as environmental and cultural practices influence shelf life
- Storage conditions influence shelf life. Experimental design: Active MAP (0.5% O_2 + 8-10% CO_2) + 24 h in air
- Results must be confirmed in different seasons and years
- Seed companies must provide the selected cultivars through several years (confidential agreement)
- Growers must use recommended cultural practices for cultivation

Pre-harvest factors that affect quality of leafy greens

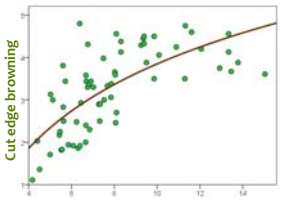
1. Genotypes
2. Environmental conditions
3. Agricultural practices
4. Maturity at harvest
5. Harvest time

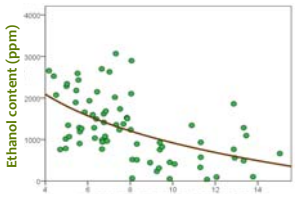
2. Environmental conditions: Temperature, RH, light and rainfall

- Influence cultivation in different production areas and growing cycles



Fresh-cut Romaine lettuce cultivars stored for 11 d at 7°C in Active MAP + 24h 7°C after opening the bags





2. Environmental conditions: Temperature, RH, light and rainfall

- Influence diseases and disorders of lettuces

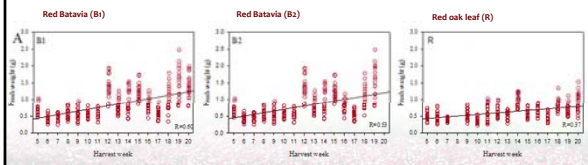
Bacterial soft rot | Frost damage | Excess of water | Pinkrib | Tipburn

Appearance, location and evolution of spots and alterations observed in lettuce leaves (Blancard et al., 2005)

2. Environmental conditions: Temperature, RH, light and rainfall

- Influence differences in the specifications of raw material
  - Maturity indices: size, weight, compactness

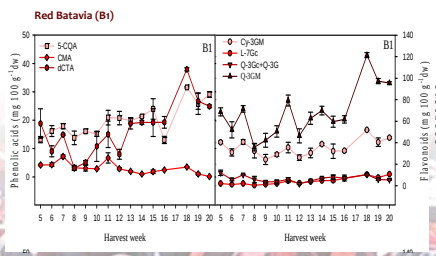
Fresh weight vs harvest week of baby leaf red lettuces



2. Environmental conditions: Temperature, RH, light and rainfall

2. Environmental conditions: Temperature, RH, light and rainfall

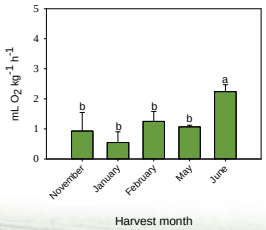
Phenolic compounds vs harvest week vs harvest week of baby leaf red lettuces



Radiation and temperature showed positive correlations with the content of phenolic acids and flavonoids that increased as the season progressed

2. Environmental conditions: Temperature, RH, light and rainfall

Respiration rate of fresh-cut Iceberg lettuce harvested in different months stored for 9 d at 7 °C and 0.5% O2



2. Environmental conditions: Temperature and light intensity

Greenhouse vs field production

Soilless system in greenhouse: lack of lettuce heart and bolting in the case of butterhead and the lack of color in the case of red-leafed genotypes, as well as the presence of tipburn in all genotypes.

2. Environmental conditions

Conclusions

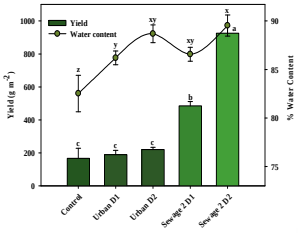
- **Protected culture system:** increases yield, allows off-season production, controls abiotic factors and facilitates pest management.
- **Cultivation in open field:** more resistant crops because of the adverse weather conditions and long growing cycle.

Pre-harvest factors that affect quality of leafy greens

1. Genotypes
2. Environmental conditions
3. Agricultural practices
4. Maturity at harvest
5. Harvest time

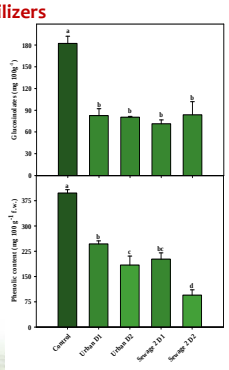
3. Agricultural practices: Fertilizers

Organic soil amendments



3. Agricultural practices: Fertilizers

Organic soil amendments



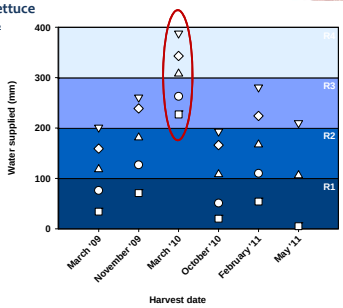
3. Agricultural practices: Irrigation

- Crop: Romaine and iceberg lettuce
- Total cultivated area: 1080 m²
- Number of treatments: 5

T1: Control
T2:FC -50% } Deficit
T3: FC -25%
T4: FC +25 % } Excess
T5: FC +50 %

- Number of harvest: 6

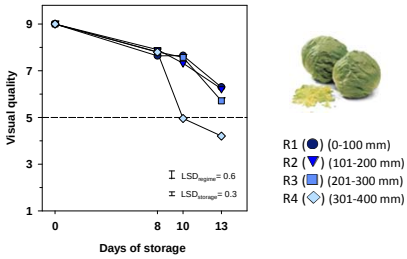
March, November } 2009
March, October } 2010
February, May } 2011



Irrigation regime= Irrigation treatment + Rainfall – Crop evapotranspiration (Etc)

3. Agricultural practices: Irrigation

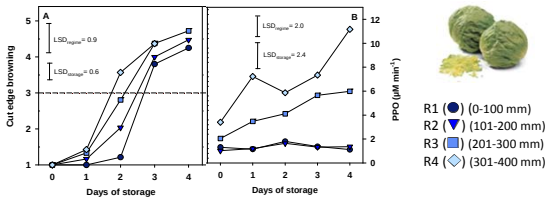
Visual quality of fresh-cut Iceberg lettuce stored during 13 days in MAP (3 d at 4 ºC and 7 ºC for the rest of the storage) as affected by different irrigation regimes



Low irrigation doses are recommended for quality and economic reasons

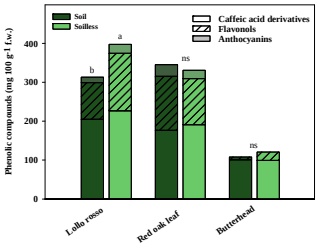
3. Agricultural practices: Irrigation

Cut edge browning (left) and PPO (right) of fresh-cut Iceberg lettuce stored in air 7 ºC as affected by different irrigation regimes



3. Agricultural practices: Soil and soilless systems

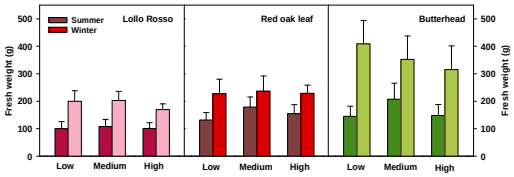
Content of phenolic compounds in different lettuce types



3. Agricultural practices: Nutrient solutions

Macroanions (NO_3^-) and macrocations (K^+, Ca^{2+} and NH_4^+)

Genotype and season strong influence on quality and shelf life of fresh-cut product

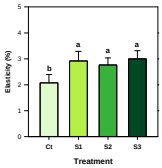


3. Agricultural practices: Salinity

Clarkson et al. (2003) observed that lettuces exposed to an stress increased the plasticity which conferred higher acceptability and shelf life to the product.

Elasticity (reversible extension) and **plasticity** (irreversible extension)

Lettuces cultivated in soilless under different salinity concentrations (mM NaCl)



3. Agricultural practices: Salinity

Analysis of the internal anatomy of the tissue

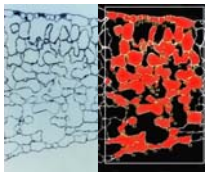


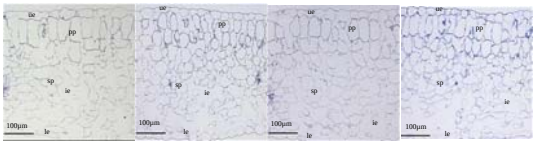
Image captured by the optical microscope (A) and the corresponding cellular vision in the inner part of the area studied (B)

Higher procesability if there is a combination between small cells with a high solute concentration and strong cellular walls (Clarkson et al. 2003; Zhang et al., 2007; Wagstaff et al., 2010).

3. Agricultural practices: Salinity

Analysis of the internal anatomy of the tissue

Lettuces cultivated in soilless under different salinity concentrations (mM NaCl)



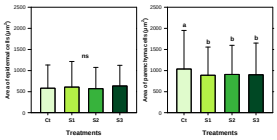
Ct (control) | S1 (50 mM NaCl) | S2 (100 mM NaCl) | S3 (150 mM NaCl)

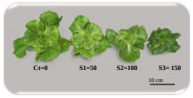
Ue, upper epidermis; le, lower epidermis; pp, palisade parenchyma; sp, spongy parenchyma; ie, intercellular spaces

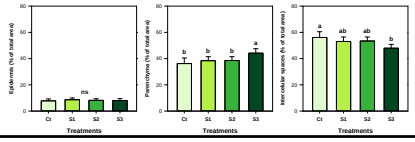
3. Agricultural practices: Salinity

Histology: Analysis of the internal anatomy of the tissue

Lettuces cultivated in soilless under different salinity concentrations (mM NaCl)

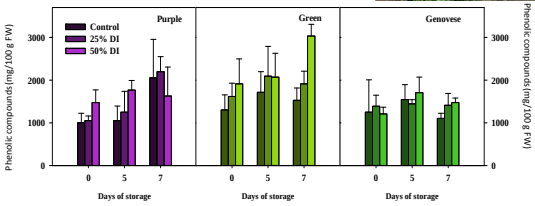






3. Agricultural practices: Drought stress

Content of phenolic compounds with mild and severe deficit irrigation (DI)



3. Agricultural practices

Conclusions

- **Optimization and control of agricultural practices** are of paramount importance, particularly IRRIGATION, to ensure the quality leafy greens.
- The combination of adequate production systems and suitable cultivars is considered essential to ensure the shelf life of leafy greens.

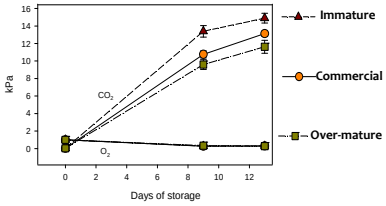
Pre-harvest factors that affect quality of leafy greens

1. Genotypes
2. Environmental conditions
3. Agricultural practices
4. Maturity at harvest
5. Harvest time

4. Maturity at harvest

Influence of maturity stage on quality of fresh-cut Iceberg lettuce

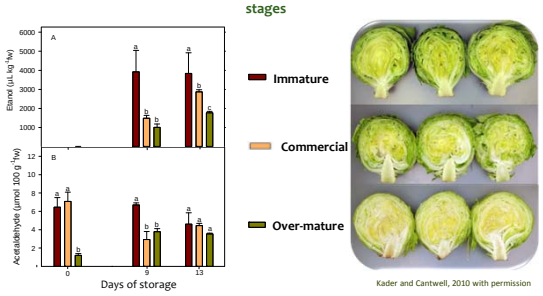
Headspace gas composition of fresh-cut Iceberg lettuce at different maturity stages



Kader and Cantwell, 2010 with permission

4. Maturity at harvest

Ethanol (A) and acetaldehyde (B) of fresh-cut Iceberg lettuce at different maturity stages



Kader and Cantwell, 2010 with permission

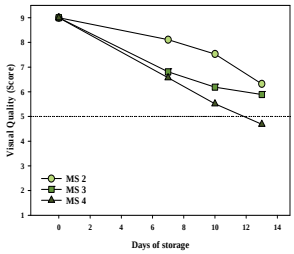
4. Maturity at harvest

Influence of maturity stage on quality of fresh-cut Romaine lettuce

MS 2 > 40 and ≤ 60 mm

MS 3> 60 and ≤ 80 mm

MS 4 > 80 mm



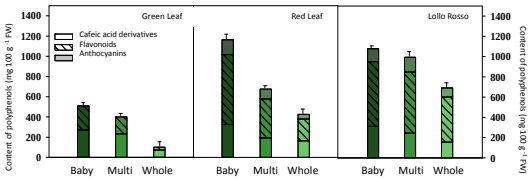
4. Maturity at harvest

Baby Multi Whole heads

	Baby	Multi	Whole
Density of planting (plants/m²)	300	30	7
Cultivation (Days)	66	95	130

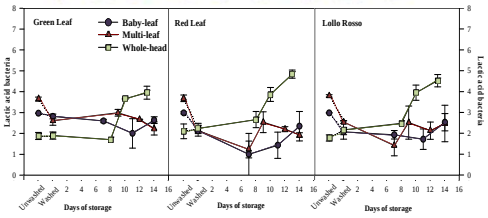
4. Maturity at harvest

Content of individual and total polyphenols



4. Maturity at harvest

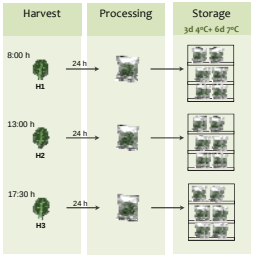
Influence of maturity stage on quality of fresh-cut Iceberg lettuce



Pre-harvest factors that affect quality of leafy greens

1. Genotypes
2. Environmental conditions
3. Agricultural practices
4. Maturity at harvest
5. Harvest time

5. Harvest time: Time of the day for harvest



January

Growing cycle: 54 d
Sun radiation: 7 h/day
T° : 10 ºC
Rain: 0.1 mm

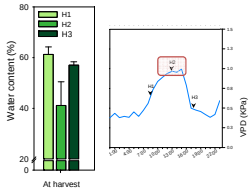
April

Growing cycle: 36 d
Sun radiation: 9 h/day
T° : 13 ºC
Rain: 1.1 mm

5. Harvest time: Time of the day for harvest

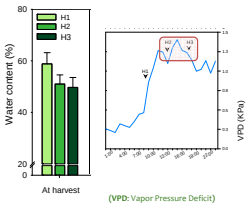
Leaf water content and environmental Vapor Pressure Deficit (VPD)

Winter



- Dehydration of the leaves in the middle of the day but it recovers in the afternoon.

Spring

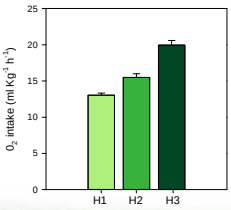


(VPD: Vapor Pressure Deficit)

- Dehydration as the day progresses.

5. Harvest time: Time of the day for harvest

Respiration rate of minimally processed baby spinach cultivated in spring, harvested at different times and stored for 9 d at 7 ºC

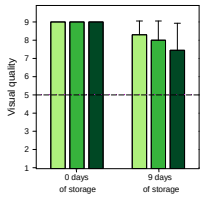


H1: 8:00h
H2: 13:00h
H3: 17:30h

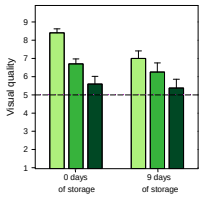
5. Harvest time: Time of the day for harvest

Visual quality of minimally processed baby spinach harvested at different times and stored for 3 d at 4 ºC and 6 d at 4 ºC

Winter



Spring



H1: 8:00h
H2: 13:00h
H3: 17:30h

5. Harvest time: Time of the day for harvest

Conclusions

- In winter, the differences observed among harvest times were reduced after processing during storage, while in spring these differences remained.
- Baby spinach could be harvested at any time of the day in winter and early morning in spring.
- Baby spinach harvested early in the morning had a higher water content, firm texture, lower respiration rate and better visual quality.

Preharvest Factors and Fresh-cut Quality of Leafy Vegetables

Future steps

A greater understanding of the mechanisms, including the metabolites involved in browning, off-odor development and texture loss will led to the identification of the key preharvest factors for each species or cultivar.

References: Preharvest Factors and Fresh-cut Quality of Leafy Vegetables

1 : Genotypes

Martínez-Sánchez, A., Gil-Izquierdo, A., Gil, M.I., Ferreres, F. 2008. A comparative study of flavonoid compounds, vitamin C and antioxidant properties of baby leaf *Brassicaceae* species. J. Agric. Food Chem., 56, 2330-2340.

Tudela, J.A., Marín, A., Martínez-Sánchez, A., Luna, M.C., Gil, M.I. 2013. Preharvest and postharvest factors related to off-odours of fresh-cut iceberg lettuce. Postharvest Biol. Technol., 75, 75-85.

2 : Environmental conditions

Marín, A., Ferreres, F. Barberá, G.G., Gil, M.I.2015. Weather variability influences color and phenolic content of pigmented baby leaf lettuces throughout the season. J. Agric. Food Chem., 63, 1673-1681.

3 : Agricultural practices

Selma, M.V., Luna, C., Martínez-Sánchez, A., Tudela, J.A., Beltrán, D., Baixauli, C., Gil, M.I. 2012. Sensory quality, bioactive constituents and microbiological quality of green and red fresh-cut lettuces (*Lactuca sativa* L.) are influenced by soil and soilless agricultural production systems. Postharvest Biol. Technol., 63, 16-24

Luna, C., Tudela, J.A., Martínez-Sánchez, A., Allende, A., Marín, A., Gil, M.I. 2012. Long term deficit and excess of irrigation influences quality and browning related enzymes and phenolic metabolism of fresh-cut iceberg lettuce (*Lactuca sativa* L.). Postharvest Biol. Technol., 73, 37-45.

References: Preharvest Factors and Fresh-cut Quality of Leafy Vegetables

3 : Agricultural practices

Luna, C., Tudela, J.A., Martínez-Sánchez, A., Allende, A., Gil, M.I. 2013. Optimizing water management to control respiration rate and reduce browning and microbial load of fresh-cut romaine lettuce, Postharvest Biol. Technol., 80, 9–17.

Luna, M.C., Martínez-Sánchez, A., Selma, M.V., Tudela, J.A., Baixaulib, C., Gil, M.I. 2013. Influence of nutrient solutions in open field soilless system on the quality characteristics and shelf life of fresh-cut red and green lettuces (*Lactuca sativa* L.) in different seasons, J. Sci. Food Agric., 93, 415-421.

Gil, M.I., Tudela, J.A., Luna, M.C. 2013. Water management and its effect on the postharvest quality of fresh-cut vegetables. Steward Postharvest Review, September 1:2

Garrido, Y., Tudela, J.A. Marín, A., Mestre, T., Martínez, V., Gil, M.I. 2014. Physiological, phytochemical and structural changes of multi-leaf lettuce caused by salt stress. J. Sci. Food Agric., 94, 1592-1599

Bekhradi, F., Luna, M.C., Delshad, M., Jordan, M.J., Sotomayor, J.A., Martínez-Conesa, C., Gil, M.I. 2015. Effect of deficit irrigation on the postharvest quality of different genotypes of basil including purple and green Iranian cultivars and a Genovese variety. Postharvest Biol. Technol., 100, 127-135.

Bekhradi, F., Delshad, M., Marín, A., Luna, M.C., Garrido, Y., Kashi, A., Babalar, M., Gil, M.I. 2015. Salt stress on physiological and postharvest quality characteristics of different Iranian genotypes of basil. Horticulture Environment and Biotechnology, in press.

References: Preharvest Factors and Fresh-cut Quality of Leafy Vegetables

4 : Maturity at harvest

Martínez-Sánchez, A., Luna, C., Selma, M.V., Tudela, J.A., Abad, J., Gil, M.I. 2012. Baby-leaf and multi-leaf of green and red lettuces are suitable raw materials for the fresh-cut industry. Postharvest Biol. Technol., 63, 1-10.

Gil, M.I., Tudela, J.A., Martínez-Sánchez, A., Luna, M.C. 2012. Harvest maturity indicators of leafy vegetables. Steward Postharvest Review, June 1:2

5 : Harvest time and postha<rvest handling

Medina, M.S., Tudela, J.A., Mar<<<<<<in, A., Allende, A., Gil, M.I. 2012. Short postharvest storage under low relative humidity improves quality and shelf life of minimally processed baby spinach (*Spinacia oleracea* L.). Postharvest Biol. Technol., 67, 1-9.

Garrido, Y., Tudela, J.A., Gil, M.I. 2015. Time of day for harvest and delay to processing affect the quality of minimally processed baby spinach. Postharvest Biol. Technol., 110, 9-17.

III International Conference on Fresh-cut Produce

UC DAVIS POSTHARVEST TECHNOLOGY

ISHS

September 13-18, 2015

Thank you for your attention!

Preharvest Factors and Fresh-cut Quality of Leafy Vegetables