

Nitrate Content in Minimally Processed Lettuce (*Lactuca sativa L.*) as Affected by Fluorescent Light Exposure During Storage

Yewubnesh Wendimu Seifu*

Department of Plant Sciences, College of Agriculture and Environmental Sciences, Arsi University, Assela, Ethiopia

*Corresponding author: Yewubnesh Wendimu Seifu, Department of Plant Sciences, College of Agriculture and Environmental Sciences, Arsi University, Assela, Ethiopia, Tel: +251966740770; E-mail: yewub27@gmail.com

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Abstract

Butter head lettuce (*Lactuca sativa L.*), a leafy vegetable commonly used in salads is characterized by its ability to accumulate high level of nitrate. Accumulation of nitrate in the edible part of the plant causes nitrate toxicity. Indeed, use of artificial light during storage, exploring new approach to prevent accumulation of nitrate. The objective of this study was to determine how light exposure during storage prevents accumulation of nitrate in butter head lettuce. Minimally processed lettuce in the form of leaf discs were stored in the dark and under fluorescent light at 5, 10 and 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at 10°C (95% RH) for 14 days. The light treatments were provided for 12 hour's photo period. Light exposure during storage significantly prevented nitrate accumulation, delayed degradation of chlorophylls and extends shelf life. In contrast in the darkness, nitrate content was accumulated while chlorophyll level was degraded. The delayed accumulation of nitrate under light can be because of the higher accumulation of soluble sugars, as changes in nitrate level and soluble sugar were found reverse. In conclusion, light exposure in the level applied successfully prevented nitrate accumulation, increasing the level of soluble sugars and delayed loss of chlorophyll pigment.

Keywords: Storage; Soluble sugar; Nitrate; Total chlorophyll; Butter head lettuce

Introduction

Lettuce (*Lactuca sativa L.*) is one of leafy vegetable which is commonly used in salads. In lettuce production, nitrogen fertilization is commonly used for proper green pigment retention and leaf growth. However, lettuce is characterized by its ability to accumulate high level of nitrate [1]. Indeed, the higher amount of nitrate accumulation is noticed in butter head lettuce [2]. The extent of nitrate accumulation in lettuce is affected by different factors. An external factor such as the dosage of N-fertilizer application is the major factor determining the amount of nitrate accumulation in the leaves tissue [3]. Therefore, accumulation of nitrates in the leaves increased with nitrogen fertilizer supply [4]. Accumulation of nitrates in lettuce also has been observed to be affected by light intensity. Lettuce grown in the lower light intensity accumulates the higher level of nitrate [1,5,6]. The increased nitrate at low light intensity is due to reduced activity of nitrate reductase [7]. Hence, increasing light intensity enhances nitrate metabolism by increasing nitrate reductase activity and decreasing the accumulation in leaves [8,9]. Moreover, nitrate assimilation can be determined by photosynthesis since photosynthetic processes provide sugars (carbon skeleton) which are vital to integrate the ammonium mobilized from nitrate reduction and electrons required for reduction of nitrates into nitrites [8]. The level of nitrate accumulation in lettuce can be differed in various plant parts. In butter head lettuce the higher content of nitrate is reported in the leaves [2,3]. Lettuce is commonly consumed raw as fresh cut, high accumulation of nitrate in the edible part of the plant causes nitrate toxicity leading to health problem. Nitrate toxicity is related to meta hemoglobinaemia, when nitrate is converted into nitrites that oxidize hemoglobin and resulted shortage of oxygen transport through blood [10]. In addition, the higher level of

nitrate concentration decreases the level of ascorbic acid and reduces nutritional quality. Therefore storage techniques that prevent accumulation of nitrate and increases nutritional quality of fresh produces needs to be developed. Different strategies can be used to prevent accumulation of nitrate in lettuce. However use of artificial light during storage, exploring new approach to prevent accumulation of nitrate [11]. Furthermore post-harvest light exposure of leafy vegetables is getting more popular and suppresses quality losses [12-15]. Similarly in fresh cut lettuce species, beneficial effects of light provision during storage has been reported by several studies [15-18]. The objective of this study was to determine how light exposure during storage affects nitrate content in minimally processed lettuce.

Materials and Methods

Plant material and storage conditions

Butter head lettuce (*Lactuca sativa L.*) heads were purchased from supermarket. The lettuce heads were selected for uniformity of head size and leaf color. The outer old, damaged and core leaves were removed using sharp knife. Leaves from the middle layer of lettuce head excluding the major vein and midrib were cut into uniform size pieces. Cellular liquids produced during cutting were carefully washed by tap water. After leaf shreds were air dried at room temperature (20°C) for 10 minutes, leaf shreds were minimally processed in the form of leaf discs (1.5 cm diameter). The leaf discs were randomly put in 12 glass petri-dishes (12 cm diameter) with seven leaf discs per petri-dish. The bottoms of the petri-dishes were lined with wetted what man paper, to prevent desiccation of water from the leaf discs. The petri- dishes were sealed with a transparent plastic contained 2 holes of 0.05 cm diameter each. The 12 petri-dishes were randomly stored in the fridge installed with fluorescent light at 3 levels of light intensities and in the dark (control). Three petri- dishes with lettuce leaf discs

were stored under each light treatment and in the dark. The light treatments were provided at 5, 10 and 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and for 12 hours photoperiod during storage of 14 days. Relative humidity and storage temperature were maintained at 95% and 10°C respectively. During each moment of sample collection: at pre-storage (day 0), day 7 and day 14 after storage, sampling was done from three replicates (petri-dish) per each treatment. From each petri-dish 2 leaf discs were randomly picked, frozen using liquid nitrogen and stored in the deep freezer at -80 °C for later analysis of carbohydrate, nitrate and chlorophyll content.

Determination of shelf life

Shelf life was determined based on overall visual quality (OVQ) of leaf discs as determined by characteristics of leaf such as brightness, color, texture as well as presence or absence of leaf defect. For the accurate estimation of the damaged or discolored area of leaf discs, Image J a Java based image processing tool was used. Leaf disc was photographed within two days interval and total area of green colour and chlorotic lesion or yellowish area was measured using leaf image (photograph) analyses with Image J a Java based image processing tool. The yellowed and dis- colored leaf area from Image J analyses was then used to evaluate OVQ using scoring scale ranging from 1 to 9. Excellent OVQ was rated 9 and unusable OVQ was rated 1. Shelf life was determined as the time when OVQ value was rated 6 (OVQ=6). The scored OVQ value lower than 6 was considered as poor OVQ, so that the product is not accepted by consumers [19].

Measurements of soluble sugars and nitrate content

Leaf discs that previously stored at -80°C were dried in freeze dryer (Modulyo, LA00964, Edwards, UK). The dried leaf discs were powdered with ball mill (Retsch, Germany). Powdered samples were extracted in 5 ml of 75% ethanol (10mM HEPES, pH 7.1) incubated at 80°C for 20 minutes. The extracted solution was centrifuged for 5 minutes at 8000 rpm and at 4°C. One milliliter of the supernatant was vacuum dried (SpeedVac, AES200, USA) for 2 hours. The dried residue was re-extracted in 1 ml of MilliQ water at 4°C for 10 minutes. The eluents were analyzed on HPLC (Dionex, ICS 5000) for determination of soluble sugars (glucose, fructose and sucrose). Ten micro liters of the eluents were injected to CarboPac1 (250 × 2mm) column eluted with 100 to 500 mM NaOH. Column and sampler temperature were kept at 25°C and 5°C respectively. Sample flow rate was 0.250 ml min⁻¹. Four milliliter of the supernatant left after extraction of soluble sugar was used to determine nitrate content. The residue left after discarding the supernatant was washed four times with 3 ml of 80% ethanol. The pellets were vacuum dried for 45 minutes and re-extracted at 90°C for 30 minutes. Nitrate content was analyzed on HPLC (Chromleon (c) Dionex, Version 6.40 SP4 Build 770) equipped with 2 mm column eluted with 10 mM to 100 mM NaOH.

Determination of chlorophyll content

Pigments were extracted from leaf discs in 2.5 ml of dimethylformamide (DMF) in dark at -20°C for two weeks. Two week later 1.25 ml of the extract was centrifuged for 5 minutes at 8000 rpm and 4°C. The centrifuged extract was diluted with 1.25 ml of DMF. The supernatant was used to determine chlorophyll a and chlorophyll b spectrophotometrically at wavelength of 663.8 nm and 646.8 nm respectively. The content of these pigments were calculated according to Wellbum with the following formulae. Total chlorophyll was determined from the sum of chlorophyll a and chlorophyll b.

$$\text{Chlorophyll a (Chl a)} = 12A_{663.8} - 3.11A_{646.8}$$

$$\text{Chlorophyll b (Chl b)} = 20.78A_{646.8} - 4.88A_{663.8}$$

Statistical analysis

Statistical evaluations of the results were performed with analysis of variance (ANOVA) using SPSS statistical package (SPSS Inc. and IBM Company, Chicago, IL, USA, 2010). The effect of light was considered as significant if $P < 0.05$. Significant mean comparison was done using Duncan's test ($\alpha = 0.05$).

Results and discussion

Shelf life

Evaluation of OVQ was done to determine the shelf life in butter head lettuce leaf discs. Loss of overall visual quality was determined based on leaf characteristics such as yellowing, browning, wilting and leaf defects such as presence of dark brown area. Light exposure has showed a significant effect on storability of OVQ ($P < 0.05$). The shelf life of leaf discs stored under all light treatment was longer in comparison to those stored in the dark. Leaf discs stored in dark reach limit of consumer's acceptability 6 days earlier than those stored under fluorescent light at 15 and 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$ intensities, while 2 days earlier than leaf discs stored at 5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Figure 1). It was noticed that dark brownish symptom and sunken area which later changed to yellowish color has resulted losses of visual quality. In green leafy vegetables postharvest yellowing is caused by losses of green pigments [21,22]. Similarly in the current study butter head lettuce leaf discs was de-greened gradually due to losses of pigments (Fig.4) leading to losses of overall visual quality and shortens shelf life. However, compared to darkness provision of fluorescent light at all levels have improved shelf life. Similar results were reported for broccoli in which postharvest light exposure has preserved visual appearance and prolong shelf life for extra days [14,22]. Moreover the positive effect of light in delaying leaf yellowing has been reported in brussels sprout [23].

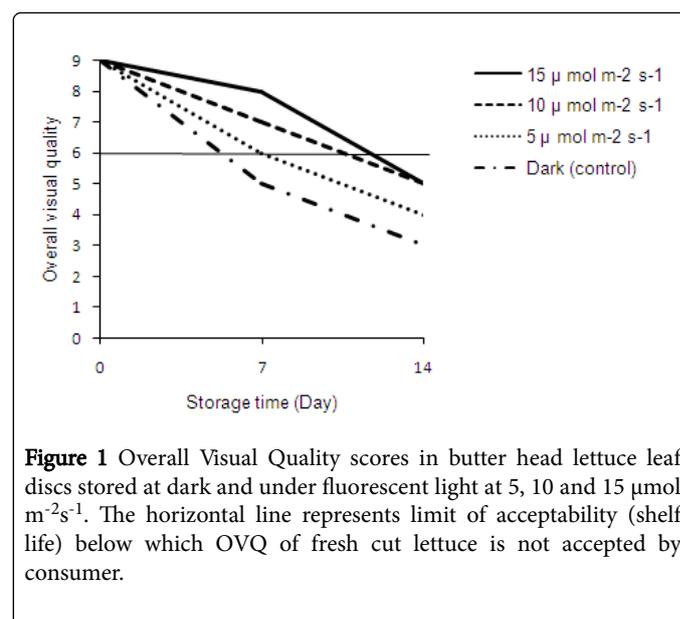


Figure 1 Overall Visual Quality scores in butter head lettuce leaf discs stored at dark and under fluorescent light at 5, 10 and 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$. The horizontal line represents limit of acceptability (shelf life) below which OVQ of fresh cut lettuce is not accepted by consumer.

Nitrate content

Butter head lettuce leaf discs were stored in the dark and at 12 hours photoperiod of fluorescent light at 5, 10 and 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$ intensity. Light exposure during storage has significantly ($P < 0.05$) delayed nitrate accumulation. In the leaf discs stored at 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$, nitrate content was significantly deteriorated over time and decreased by 8.9 mg/g DW and 2.6 mg/g DW, from pre-storage to day 7 and from day 7 to day 14 respectively. Interestingly in leaf discs exposed to 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$, the level of nitrate was significantly decreased by 8.6 mg/g DW from pre-storage level to day 14. However in leaf discs stored at 5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ significant deterioration was not noticed both at day 7 and day 14, but accumulation of nitrate was prevented. In contrast in dark stored leaf discs, nitrate content was significantly increased by 9.6 mg/g DW from pre-storage to day 7, after which no significant change was noticed (Figure 2). These showed that storage of minimally processed lettuce under light condition is beneficial to prevent accumulation of nitrate. These results are in accordance with findings reported in rocket leaves, suggesting that leaves stored under light contained less nitrate level than those stored at dark [11]. According to different studies plants accumulate nitrate in the dark to maintain osmo-regulation process because of shortage of carbohydrate supply in the cells [24], indicating that excessive accumulation of nitrate is not necessary in the light as there is adequate carbohydrate supply to keep cell osmotic pressure. In addition the activity of nitrate reductase is decreased in the dark and therefore enhances nitrate accumulation [25]. Degradation of nitrate in the leaf discs stored under light can be because of the higher accumulation of soluble sugars (data not shown). The level of nitrate content was decreased as total soluble sugar is increasing (Figure 3). Hence, changes in nitrate level and soluble sugar were found reverse. Carbohydrates can be a source of energy and carbon skeleton to maintain nitrogen metabolism in the cell [26]. Therefore, dramatic increase in total soluble sugar may be the key support to enhance nitrogen metabolism that may prevent accumulation of nitrate. The previous studies reported in lettuce suggesting that, pre-harvest application of red plus blue light is best to decreases nitrate accumulation [27]. Similarly, according to the current results, fluorescent light was also conducive to prevent nitrate accumulation in lettuce.

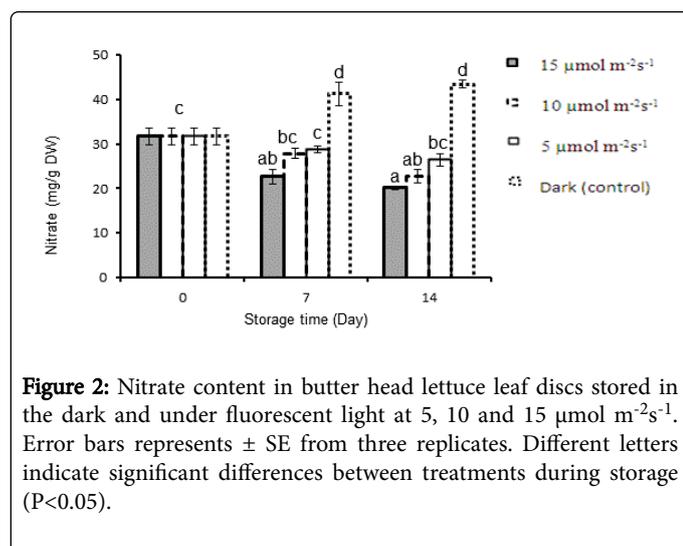


Figure 2: Nitrate content in butter head lettuce leaf discs stored in the dark and under fluorescent light at 5, 10 and 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Error bars represents \pm SE from three replicates. Different letters indicate significant differences between treatments during storage ($P < 0.05$).

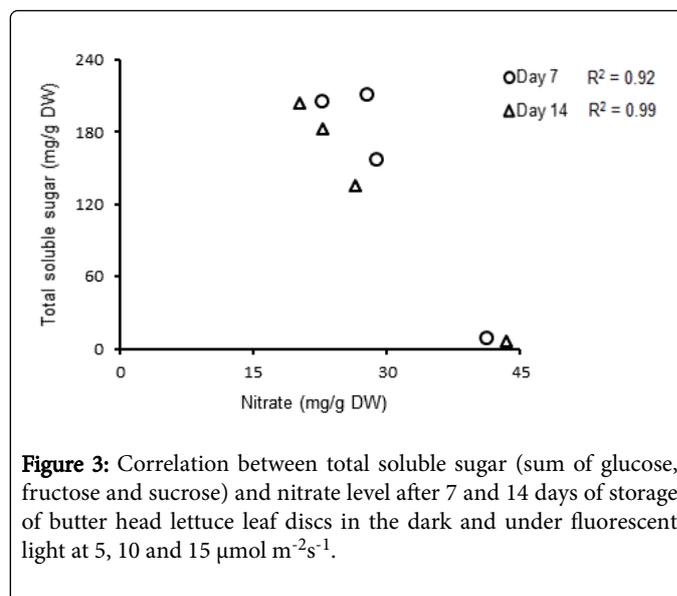


Figure 3: Correlation between total soluble sugar (sum of glucose, fructose and sucrose) and nitrate level after 7 and 14 days of storage of butter head lettuce leaf discs in the dark and under fluorescent light at 5, 10 and 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

Pigment content

Storage of butter head leaf discs in the light has significantly ($P < 0.05$) affected total chlorophyll (sum of chl a and chl b), which showed gradual degradation over time. Total chlorophyll content showed significant degradation in all leaf discs. However the decreased trend was higher in leaf discs stored in the dark than those stored in the light (Figure 4). Leaf discs stored at 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$ contained more of 3.7 mg/g FW and 2.8 mg/g FW total chlorophyll than those stored in the dark at day 7 and day 14 of storage time respectively. In leaf discs exposed to 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$, total chlorophyll content was 2.7 mg/g FW and 1.8 mg/g FW higher than those stored in the dark at day 7 and day 14 respectively. However, in leaf discs stored at 5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ the level of total chlorophyll was 1.2 mg/g FW and 0.4 mg/g FW higher than those stored in the dark at day 7 and day 14 respectively. In green leafy vegetables yellowing is consequence of loss of chlorophyll as an indication of senescence process [21]. In the current study leaf disc was gradually yellowed throughout storage. However light exposure during storage decreases loss of chlorophylls. These results are in accordance with findings reported in Chinese kale and romain lettuce [13,28]. In addition, post-harvest light exposure delaying yellowing has been reported in cabbage, brussels sprout and broccoli [14,23,29]. The higher content of total chlorophylls in light stored leaf discs might be because of the increased level of total soluble sugars delay senescence process probably by inhibiting production of reactive oxygen species [30]. Similarly, Hasperue et al. have been suggested that the higher starch content in broccoli at harvest and exogenous sugar preserves postharvest quality of the product as a result of delaying impact on senescence process [31].

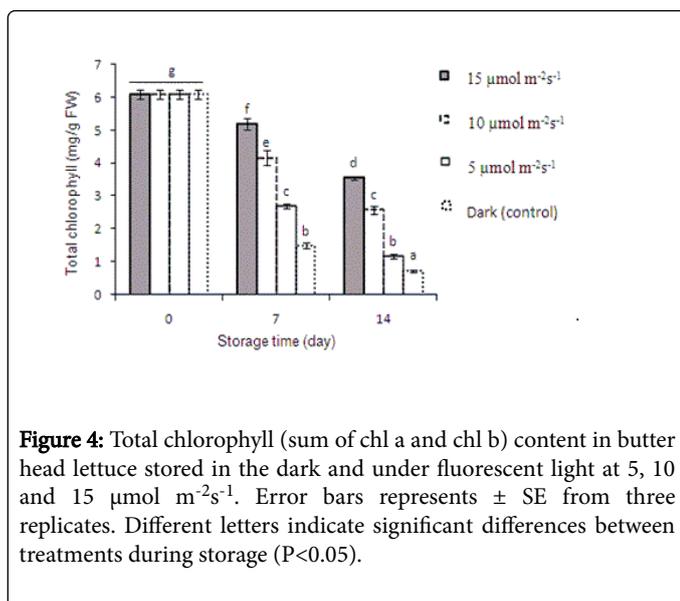


Figure 4: Total chlorophyll (sum of chl a and chl b) content in butter head lettuce stored in the dark and under fluorescent light at 5, 10 and 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Error bars represents \pm SE from three replicates. Different letters indicate significant differences between treatments during storage ($P < 0.05$).

Conclusions

Light exposure during storage successfully prevents accumulation of nitrate, delay loss of chlorophylls and extends shelf life in minimally processed butter head lettuce. The delayed accumulation of nitrate under light condition was because of the higher level of total soluble sugar accumulation. Among the three levels of fluorescent light intensities application of 15 $\mu\text{mol m}^{-2}\text{s}^{-1}$ was found efficient to delay nitrate accumulation and followed by 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and 5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ respectively.

References

- Cometti NN, Martins MQ, Bremenkamp CA, Nunes JA (2011) Nitrate concentration in lettuce leaves depending on photosynthetic photon flux and nitrate concentration in the nutrient solution. Horticultura Brasileira 29: 548-553.
- Abu-Rayyan A, Kharawish BH, Al-Ismail K (2004) Nitrate content in lettuce (*Lactuca sativa* L.) heads in relation to plant spacing, nitrogen form and irrigation level. J.Sci. Food Agric 84: 931-936.
- Yosoff SF, Mohamed MTM, Parvez A, Ahmad SH, Ghazali FM, et al. (2015) Production system and harvesting stage influence on nitrate content and quality of butterhead lettuce. Bragantia 74: 322-330.
- Liu CW, Sung Y, Chen BC, Lai HY (2014) Effects of Nitrogen Fertilizers on the Growth and Nitrate Content of Lettuce (*Lactuca sativa* L.). International Journal of Environmental Research and Public Health 11: 4427-4440.
- Stefanelli D, Winkler S, Jones R (2011) Reduced nitrogen availability during growth improves quality in red oak lettuce leaves by minimizing nitrate content, and increasing antioxidant capacity and leaf mineral content. Agricultural Sciences 2: 477-486.
- Kosma C, Triantafyllidis V, Papasavvas A, Salahas G, Patakas A (2013) Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season. Emir J Food Agric 25: 974-979.
- Appenroth KJ, Meco R, Jourdan V, Lillo K (2000) Phytochrome and post-translational regulation of nitrate reductase in higher plants. Plant Sci 159: 51-56.
- Serio F, Elia A, Signore A, Santamaria P (2004) Influence of nitrogen form on yield and nitrate content of subirrigated early potato. J Sci Food Agric 84: 1428-1432.
- Bonasia A, Conversa G, Gonnella M, Serio F, Santamaria P (2008) Effects of ammonium and nitrate nutrition on yield and quality in endive. J Hort Sci Biotechnol 83: 64-70.
- Addiscott T (2006) Is it nitrate that threatens life or the scare about nitrate? Journal of Science and Food Agriculture 86: 2005-2009.
- Ferrante A, Incrocci L, Maggini R, Tognoni F, Serra G (2003) Preharvest and postharvest strategies for reducing nitrate content in rocket (*Eruca sativa*). ActaHortic 628: 153-159.
- Toledo ME, Ueda Y, Imahori Y, Ayaki M (2003) L-Ascorbic acid metabolism in spinach (*Spinaciaoleracea* L.) during postharvest storage in light and dark. Postharvest Biology and Technology 28: 47-57.
- Noichinda S, Bodhipadma K, Mahamontri C, Narogruck T, Ketsa S (2007) Light during storage prevents loss of ascorbic acid, and increases glucose and fructose levels in Chinese kale (*Brassica oleracea* var. alboglabra). Postharvest Biology and Technology 44: 312-315.
- Büchert AM, Gómez Lobato ME, Villarreal NM, Civello PM, MartIneza GA (2011) Effect of visible light treatments on postharvest senescence of broccoli (*Brassica oleracea* L.). Journal of the Science of Food and Agriculture 91: 355-361.
- Zhan L, Li Y, Hu J, Pang L, Fan H (2012) Browning inhibition and quality preservation of fresh-cut romaine lettuce exposed to high intensity light. Innovative Food Science & Emerging Technologies 14: 70-76.
- Martínez-Sánchez A, Tudela JA, Luna C, Allende A, Gill MI (2011) Low oxygen levels and light exposure affect quality of fresh-cut Romaine lettuce. Postharvest Biology Technology 59: 34-42.
- Braidot E, Petrusa E, Peresson C, Patui S, Bertolini A, et al. (2014) Low-intensity light cycles improve the quality of lamb's lettuce (*Valerianellaolitoria* [L.] Pollich) during storage at low temperature. Postharvest Biology and Technology 90: 15-23.
- Woltering EJ, Seifu YW (2015) Low intensity monochromatic red, blue or green light increases the carbohydrate levels and substantially extends the shelf life of fresh-cut lettuce. ActaHortic 1079: 257-264.
- Agüero MV, Barg MV, Yommi A, Camelo A, Roura SI (2008) Postharvest changes in water status and chlorophyll content of lettuce (*Lactuca sativa* L.) and their relationship with overall visual quality. J Food Sci 73: 47-55.
- Wellburn AR (1994) The Spectral determination of chlorophylls a and b, as well as total carotenoids using various solvents with spectrophotometers of different resolution. Plant Physiol 144: 307-313.
- Siomos AS, Papadopoulou PP, Dogras CC, Vasiliadis E, Dosas A, et al. (2002) Lettuce Composition as Affected by Genotype and Leaf Position. ActaHort 579: 635-639.
- 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.
- Kasim R, Kasim MU (2007) Inhibition of yellowing in brussels sprouts (*B. oleracea* var. gemmifera) and broccoli (*B. oleracea* var. italica) using light during storage. J Food Agric Environ 5: 126-130.
- Blom-Zandstra M, Lampe JEM, Ammerlaan HM (1988) C and N utilization of two lettuce genotypes during growth under non-varying light conditions and after changing the light intensity. Physiol Plant 74: 147-152.
- Huber JL, Huber SC, Campbell WH, Redinbaugh MG (1992) Reversible light/dark modulation of spinach leaf nitrate reductase activity involves protein phosphorylation. Archives of Biochemistry and Biophysics 296: 58-65.
- Champigny ML (1995) Integration of photosynthetic carbon and nitrogen metabolism in higher plants. Photosynthesis Research 46: 117-127.
- Urbonaviciut A, Pinho P, Samuolien G, Duchovskis P, Vitta P, et al. (2007) Effect of short-wavelength light on lettuce growth and nutritional quality. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture 26: 157-165.
- Zhan L, Hu J, Ai Z, Pang L, Li Y, et al. (2013) Light exposure during storage preserving soluble sugar and l-ascorbic acid content of minimally

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- processed romaine lettuce (*Lactuca sativa* L. var. *longifolia*). Food Chemistry 136: 273-278.
29. Heaton JW, Yada RY, Marangoni AG (1996) Discoloration of Coleslaw Is Caused by Chlorophyll Degradation. Journal of Agricultural and Food Chemistry 44: 395-398.
30. Couee I, Sulmon C, Gouesbet G, Amrani A (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. Journal of Experimental Botany 57: 449-459.
31. Hasperue JH, Chaves AR, Martinez G (2011) End of day harvest delays postharvest senescence of broccoli florets. Postharvest Biol Technol 59: 64-70.