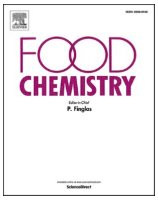
Food Chemistry 504 (2026) 147867

Contents lists available at ScienceDirect

Food Chemistry

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



Chemical composition of lettuce following root-application of osmolytes in hydroponic cultivation

Jenni Uusitupa[a,*], Minnamari Edelmann[b], Kari Jokinen[a], Ilkka Simpura[a], Alexey Shapiguzov[c,d], Pirjo S.A. Mäkelä[a,e]

[a] *Department of Agricultural Sciences, P.O. Box 27, 00014 University of Helsinki, Finland*
[b] *Department of Food and Nutrition, P.O. Box 56, 00014 University of Helsinki, Finland*
[c] *Natural Resources Institute Finland, Toivonlinnantie 518, 21500 Piikkiö, Finland*
[d] *Organismal and Evolutionary Biology Research Program, Faculty of Biological and Environmental Sciences, Viikki Plant Science Centre, Viikinkaari 1, 00014 University of Helsinki, Finland*
[e] *Helsinki Institute of Sustainable Science (HELSUS), Yliopistonkatu 3, 00014 University of Helsinki, Finland*

ARTICLE INFO

Keywords:
Leafy vegetables
NFT-cultivation
Sugars
Amino acids
Nitrate
Minerals

ABSTRACT

Hydroponically produced lettuce (*Lactuca sativa* L.,) naturally accumulates high amounts of nitrate in its leaves, and this compound is harmful for human health. Osmolytes affect the osmotic potential of plant cells and can potentially reduce the accumulation of nitrate. The potential of osmolytes mannitol and glycerol was compared to glycinebetaine —which is known to decrease the nitrate content of lettuce— in two hydroponic experiments. Plant samples were collected at various time points and analyzed for minerals, free- and hydrolyzed amino acids, soluble sugars, osmolality, and nitrate. All three osmolytes reduced the nitrate content in lettuce leaves and increased the contents of dry matter, soluble sugars, and some amino acids. The effect of mannitol and glycerol on lettuce nitrate content and quality were similar to those induced by glycinebetaine. Thus, the quality of hydroponically grown lettuce can be controlled with diverse types of root-applied osmolytes.

1. Introduction

The quality of lettuce (*Lactuca sativa* L.) is influenced by traits affecting the physical appearance of the plant, and the chemical composition that affects both the shelf-life of the product and consumer health. In hydroponic cultivation, lettuce may accumulate a high amount of leaf nitrate (NO_3^-). This chemical in lettuce has important implications for consumers, as a high amount of NO_3^- is known to be harmful to human health. Up to 90 % of NO_3^- in food originates from fresh leafy vegetables (Hambridge, 2003), hence if lettuce is consumed in large quantities, the recommended acceptable daily intake (ADI) of NO_3^- may be exceeded (European Food Safety Authority, 2008). In hydroponic cultivation, lettuce may accumulate a high amount of NO_3^- in its leaves since NO_3^- is a commonly used fertilizer, making it easily available for the plant. However, NO_3^- accumulation in lettuce can be modified by alternative cultivation strategies. For example, various amounts and forms of N-fertilizer can be used (Konstantopoulou et al., 2010; Santamaria et al., 1998), the light intensity and spectrum can be regulated (Liu et al., 2016; Zhang et al., 2018; Yi et al., 2021) or glycinebetaine (GB) can be added to the nutrient solution (Jokinen et al., 2022).

Lettuce regulates the cell osmotic potential with organic and inorganic compounds – osmolytes –which are either synthesized endogenously or taken up by the roots (Blom-Zandstra & Lampe, 1985; Burns et al., 2010). Endogenous osmolytes can be characterized into three groups: osmolytes containing sugar and sugar alcohols (e.g., glycerol and mannitol), osmolytes containing amino acids (e.g., glutamine and proline), and osmolytes containing ammonium compounds (e.g., polyamines and GB) (Singh et al., 2015). Plants accumulate organic osmolytes and low weight molecules, for example, GB, proline and sugars, as a response to abiotic stresses (Chen & Jiang, 2010; Singh et al., 2015). Plants also take up exogenous osmolytes through roots and foliage. Accordingly, exogenous osmolytes for alleviating salt-, drought-, and chilling-induced stresses have been introduced for many vegetable crops (Franzoni et al., 2021; Kalisz et al., 2024; Shahbaz et al., 2012; Yildirim et al., 2015).

NO_3^- is known to serve as an inorganic osmolyte that interacts with

---

* Corresponding author.
E-mail address: jenni.uusitupa@helsinki.fi (J. Uusitupa).

https://doi.org/10.1016/j.foodchem.2026.147867
Received 2 October 2025; Received in revised form 29 December 2025; Accepted 4 January 2026

organic osmolytes. According to Burns et al. (2010), NO_3^- accumulation is related to the total number of solutes in vacuolar sap. As such, it is likely that methods aimed at increasing the content of organic osmolytes in lettuce leaves contribute to the leaf NO_3^- content. In greenhouse production, increasing the content of organic osmolytes can be done either by creating conditions favorable to endogenous accumulation, or by direct exogenous application. For example, it has been shown that light intensity negatively correlates with leaf NO_3^-content (Blom-Zandstra & Lampe, 1985; Shen et al., 2024). Similarly, Ma et al. (2018) showed that root-applied glucose and sucrose decreased NO_3^- uptake in pak choi (*Brassica rapa* var. *chinensis* L.). Although lettuce does not endogenously synthesize GB, root-applied GB was shown to decrease lettuce NO_3^- content in a hydroponic cultivation system (Jokinen et al., 2022). This indicates that various root-applied osmolytes can be used to regulate the NO_3^- content in lettuce. However, their effect on lettuce yield and quality is not known.

Our aim was to investigate the chemical composition of lettuce following the application of three organic osmolytes – GB (EINECS 203-490-6), glycerol (food additive code E422), and mannitol (food additive code E421) – to fertigation solution using a nutrient film technique (NFT) system. The NFT system is commonly used in commercial lettuce greenhouse production. In this system, plants grow in small plugs of a growth substrate that are placed in narrow channels in which the nutrient solution flows continuously. Mannitol and glycerol are sugar alcohols (organic osmolytes) naturally synthesized in lettuce (Roughan & Batt, 1969; Trip et al., 1964). We hypothesized that application of these osmolytes would result in lower leaf NO_3^- content, similar to the root-applied GB, which is a zwitterionic quaternary ammonium compound (Rhodes & Hanson, 1993). According to Jokinen et al. (2022), root-applied GB increases the content of dry matter (DM) and amino acids in lettuce in a dose-dependent manner. Considering the inverse relation between NO_3^- and organic osmolytes, we also hypothesized that GB, mannitol, and glycerol impact the composition of amino acids and sugars. To test these novel hypotheses, we conducted two greenhouse experiments in which we applied GB, glycerol, or mannitol into the fertigation solution in an NFT system and compared the responses of osmolyte-treated plants to plants grown in fertigation solution without organic amendments. In the two experiments, lettuce fresh weight (FW) and DM content were measured, and chemical composition analyses for detecting NO_3^- content, mineral contents, amino acid contents, and sugar contents were employed to quantify the osmolyte effects on lettuce pre-harvest quality.

2. Materials and methods

2.1. Plant material and experimental conditions

Two experiments were conducted under controlled greenhouse conditions at the University of Helsinki, Finland, with a day/night temperature of 18 °C and relative humidity of 55–65 %. High-pressure sodium lamps (Masterson-t; Philips Lightning N.V., Eindhoven, the Netherlands) provided a 22 h photoperiod with PPFD of 150 μmol m^{-2} s^{-1} at the top of the canopy. Lettuce seedlings (cv. 'Exact' in the first experiment and 'Multiblond' in the second experiment) grown in Ellepot® plugs were provided by a commercial greenhouse company. Seedlings were transferred to a Nutrient Film Technique (NFT) system at the four-leaf stage. There were four separate watering systems, each consisting of four channels split into four tables organized in a completely randomized block design with four replicates, exactly as described earlier by Jokinen et al. (2022). Each channel contained 25–27 plants, for a total of 460 plants. The fertigation solution used in all four systems was prepared from commercial fertilizers by mixing a 10 % solution of Vihannes Superex (N-P-K:8-5-28, Kekkilä Professional, Kekkilä Oy, Vantaa, Finland) and 3.2 % solution of YaraTera Calcinit (N:15.5, Yara Suomi Oy, Finland) at a ratio of 1:1 to stock solution. It was then applied to the water tanks until the set electric conductivity (EC 2.0 mS) and content of nitrate nitrogen ($NO_3^- - N$; 200 ppm) were reached. The pH of the final fertigation solution was 6.0. The levels of $NO_3^- - N$ and EC were monitored daily and adjusted by adding fertigation stock solution or fresh tap water as needed.

In the first experiment, 30 days after seeding (DAS) either mannitol (D-Mannitol; Sigma-Aldrich Chemie GmbH, Steinheim, Germany), glycerol (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) or GB (Nutristim pure; Finnfeeds Finland Oy, Naantali, Finland) was applied to the fertigation solution to reach a concentration of 4 mM; no osmolytes were applied to the nutrient solution of untreated plants. In the second experiment, mannitol was applied to the fertigation solution at 27 DAS at final concentrations of 0 mM, 2 mM, 4 mM, or 6 mM. In both experiments, plants were exposed to treatments for 4 days, after which all four watering systems were re-filled with fresh fertigation solution containing no osmolytes.

2.2. Sampling and measurements

Chlorophyll-, flavonol-, and anthocyanin indexes were measured in both experiments at 0, 3, 6, and 10 days after application (DAA) (Dualex Force-A, Orsay, France), and the stomatal conductance in the second experiment at 1, 3, 8, and 10 DAA (Porometer Delta-T-Devices, Cambridge U.K). Plant samples were harvested six times: at 0, 2, 4, 7, 9 and 11 DAA in both experiments. Sampling was conducted at the same time at each time point. At each sampling point, three plants from each channel were cut from the root collar and weighed. The number of leaves of harvested plants was counted, and the leaves were divided into two subsamples. Samples were stored at −20 °C until further analysis.

The subsamples used for the chemical analyses were freeze-dried (CHRIST Beta 2-8 LD plus, CHRIST Gamma 2–16 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) under 57 Pa until dry, and then ground into a fine powder (1 mm sieve; Retsch Grindomix GM 200, Retsch GmbH, Germany). Ground samples were stored at −20 °C until further analysis. The subsamples were weighed before and after the freeze-drying process to determine dry matter (DM) content.

2.3. Chemical analyses

Leaf NO_3^- content was measured with a Horiba LAQUA Twin 741 crops meter (Horiba Advanced Techno Co. Ltd., Kyoto, Japan) according to Uusitupa et al. (2023). Freeze-dried and ground (0.5 mm sieve, Retsch Grindomix GM 200; Retsch GmbH, Haan, Germany) samples were analyzed for mineral content (Ca, Fe, K, Mg, Mn, Na, P, S, Zn) with inductively coupled plasma-optical emission spectrometry (ICP-OES) (iCAP 6200, Thermo Fisher Scientific, Cambridge, UK), and free and hydrolyzed amino acids were analyzed with an ACQUITY UPLC system (Waters Milford, MA, USA) consisting of an Acquity photodiode array (PDA) optical detection system, according to Jokinen et al. (2022). The analysis of mineral contents and amino acids was conducted at 7 DAA, which is the optimal time point to detect differences based on our previous studies (Jokinen et al., 2022; Uusitupa et al., 2023). Soluble sugar content was analyzed with a commercial Assay Kit (Sucrose/D-Fructose/D-Glucose assay kit (K-SUFRG), Megazyme, Wicklow, Ireland) with a modification to the sugar extraction. In brief, 10 mg of freeze-dried ground sample was incubated with 4 mL ultra-pure water (Milli-Q, Merck KgaA, Darmstadt, Germany) in a 70 °C water bath (Julabo SW 20 GWB, G.W.Berg & Co AB. Espoo, Finland) for 30 min, then centrifuged at 1600 G for 10 min at room temperature (RT), after which the supernatant was collected. The pellet was resuspended two times with the same protocol using 3 mL of Milli-Q water each time, and the supernatants were combined. The content of free sucrose, fructose, and glucose were determined spectrophotometrically (Shimadzu UV-1800, Shimadzu, Kyoto, Japan) according to the manufacturer's protocol. For NO_3^- and sugar analyses, samples were collected at two-day intervals in both experiments. Osmolality was measured from squeezed lettuce leaves and fertigation solutions with a freezing point depression osmometer (Micro-Osmometer 3300 M, Advanced Instruments, Norwood, MA, USA), according to Mäkelä et al. (1998).

2.4. Statistical analysis

All data was tested for normality, and the homogeneity of variance was tested with Levene's test. The data on growth, stomatal conductance, osmolality, NO_3^- content, pigment indices, and contents of soluble sugars were subjected to two-way analysis of variance using IBM SPSS Statistics software (Version 29.0.2.0 (20)) to test the effects and length of treatment (DAA). Pairwise comparisons were made using Tukey's multiple range test, and significant differences between treatment means were considered when the *p*-values were < 0.05. Analysis of variance was performed to test the effect of the treatment at the selected timepoint (7 DAA) on the amino acid and mineral content data. Data collected from both experiments were combined to explore the correlations between the variables (NO_3^- content, chlorophyll index, and sugar content).

3. Results

3.1. Lettuce growth and stomatal conductance

In the first experiment, all osmolyte treatments resulted in lower fresh weight (FW) in comparison to untreated plants (Fig. 1, Supplementary materials Table S1). The leaf DM content was also affected by treatment (Treatment $p < 0.001$) and time (DAA $p < 0.001$), but no interaction between the two factors was found; mannitol plants contained significantly higher content of DM after 4 DAA, and all three osmolytes resulted in higher DM content in comparison to untreated plants at 7 and 9 DAA (Fig. 1). In the second experiment, mannitol significantly decreased the FW (Treatment*DAA $p < 0.001$, Table S1). Lower FW was observed after 4 DAA, with mannitol concentrations of 4 mM and 6 mM compared to untreated plants (Fig. 2). Mannitol increased the lettuce dry matter content significantly (Treatment*DAA $p < 0.001$) in a concentration-dependent manner (Fig. 2).

In the second experiment, mannitol-treated plants had significantly lower stomatal conductance in comparison to untreated plants at 3 DAA (Fig. S2). The stomatal conductance was affected by treatment (Treatment $p = 0.001$) and time (DAA $p = 0.001$), but no interaction between the two factors was found.

3.2. Chemical composition of lettuce leaves

In the first experiment, osmolytes decreased the leaf NO_3^- content significantly (Treatment*DAA $p < 0.001$; S1). Mannitol and GB plants showed lower NO_3^- content after 2 DAA, and glycerol treatment after 4 DAA compared to untreated plants (Fig. 3). Also in the second experiment, the NO_3^- content of lettuce leaves decreased significantly (Treatment*DAA $p < 0.001$) following the application of mannitol (Fig. 3). Leaf NO_3^- content decreased further even after osmolyte applications stopped until 9 DAA in the first experiment, and 7 DAA in the second experiment, when the NO_3^- content started to increase.

In the first experiment, osmolytes increased the contents of glucose and fructose in lettuce leaves (Table 1). The content of D-glucose was significantly higher ($p = 0.016$) in GB plants (139 g kg^{-1} DM) than in untreated plants (75 g kg^{-1} DM) at 4 DAA. In mannitol plants, significantly higher ($p = 0.018$) content of D- glucose was observed at 7 DAA in comparison to control plants. In addition, the content of fructose was significantly ($p = 0.034$) higher in GB plants than in untreated plants at 4 DAA. Mannitol plants contained significantly ($p = 0.003$) higher fructose content in comparison to untreated plants at 9 DAA. The highest content of D-fructose, 160 g kg^{-1} DM, was detected in GB plants at 4 DAA when the content in untreated plants was 98 g kg^{-1} DM. The content of sucrose also showed an increasing trend following the application of osmolytes, but the difference observed was not significant. In the second experiment, mannitol significantly increased the content of D-glucose, fructose, and sucrose in a concentration-dependent manner (Table 2). At 7 DAA, the plants treated with 6 mM of mannitol contained almost three times more D-glucose than untreated plants. A negative correlation between the NO_3^- content and the total content of soluble sugars in fresh lettuce was observed (r (83) = −0.308, $p = 0.005$).

In the first experiment, all three osmolytes increased the total content of hydrolyzed amino acids in lettuce leaves compared to untreated plants (Table 3). The most noted increase was observed in the contents of hydrolyzed glutamic acid and tyrosine. In addition, root-applied GB and mannitol affected the contents of free glutamine, aspartic acid, alanine, and proline (Table 4). GB increased the content of free glutamine, and mannitol increased the content of free proline. Both mannitol and GB decreased the content of free aspartic acid. Mannitol also decreased the content of alanine. In general, the contents of minerals (S, P, Zn, Fe, Mn, Mg, Ca, Na, K, N, C) decreased, except for C, which increased with all osmolyte treatments (Table 5).

Only higher (4 and 6 mM) concentrations of mannitol increased the osmolality in the leaves (Fig. S3). After 4 DAA, the osmolality in mannitol-treated plants was significantly higher than in untreated plants, and the difference observed was concentration-dependent. No differences were observed in plants treated with GB or glycerol in comparison to untreated plants.

3.3. Pigment indices in leaves

An increasing trend was observed in chlorophyll index following root-application of osmolytes. In the first experiment, the chlorophyll

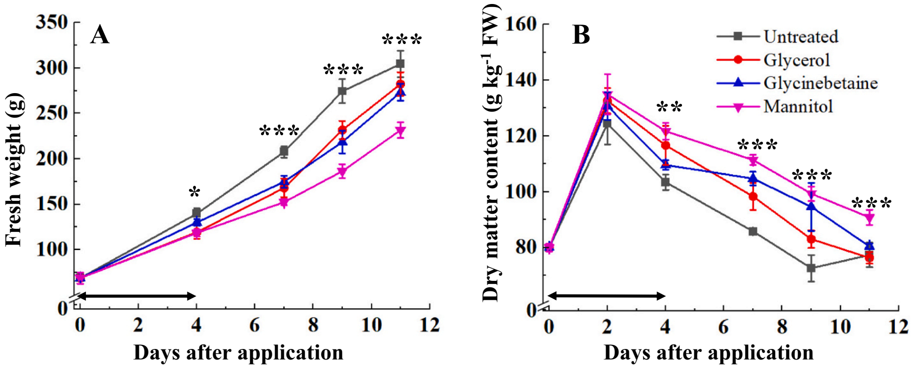


Fig. 1. Lettuce (cv. 'Exact') fresh weight accumulation (A) and dry matter content (B) following application of osmolytes (glycerol, glycinebetaine, or mannitol). Data shown are means ± standard error ($N = 4$). The application period is marked above the x-axis by a horizontal arrow. FW = fresh weight; DM = dry matter. * Indicates significant difference between the osmolyte treatment means at each separate timepoint: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

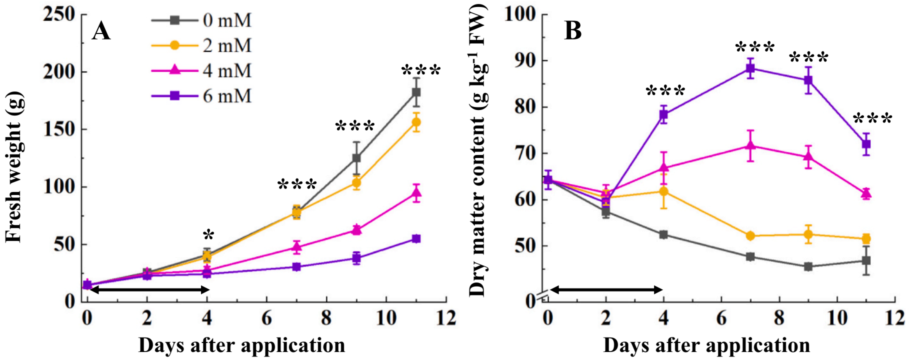


Fig. 2. Lettuce (cv. ‘Multiblond’) fresh weight accumulation (A) and dry matter content (B) following application of different mannitol concentrations. Data shown are means ± standard error (N = 4). The application period is marked above the x-axis by a horizontal arrow. FW = fresh weight; DM = dry matter. * Indicates significant difference between the osmolyte treatment means at each separate timepoint: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

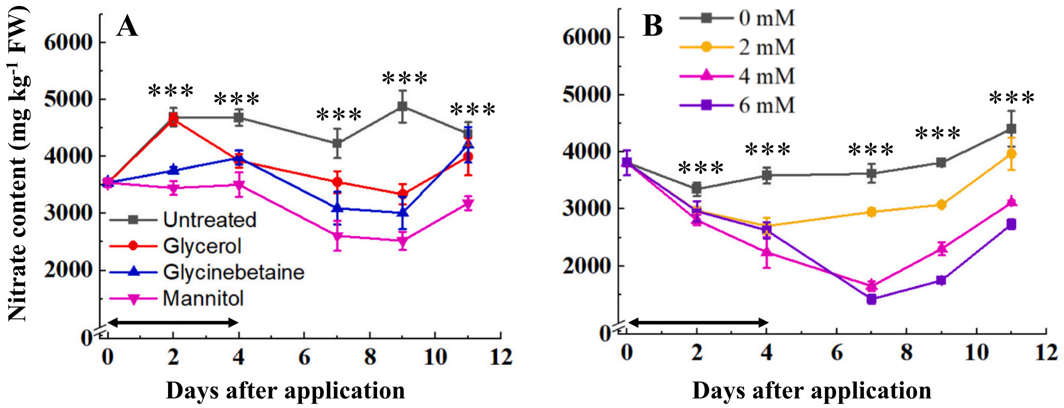


Fig. 3. Lettuce (cv. ‘Exact’) nitrate content (A) following application of 4 mM glycerol, glycinebetaine, or mannitol. Lettuce (cv. ‘Multiblond’) nitrate content (B) following applications of different mannitol concentrations. The application period is marked above the x-axis by a horizontal arrow. Data shown are means ± standard error ($N = 4$). FW = fresh weight. * Indicates significant difference between the osmolyte treatment means at each separate timepoint: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

index was significantly ($p = 0.004$) higher in GB-treated plants compared to untreated plants at 10 DAA (Fig. S4). In the second experiment, 6 mM of mannitol resulted in significantly higher chlorophyll index compared to untreated plants at 3 and 10 DAA. Moreover, a weak negative correlation (r (67) = −0.422, $p \leq 0.00$) was observed between the chlorophyll index and NO_3^-, while there was a positive correlation (r (67) = 0.513, $p < 0.001$) between the total sugar content and chlorophyll index. In the first experiment, the osmolytes did not significantly affect the anthocyanin index (Fig. S4). However, in the second experiment, the anthocyanin index was affected by treatment (Treatment $p = 0.003$), being significantly lower at 3 and 10 DAA in plants treated with 6 mM of mannitol compared to untreated plants. The flavonol index was not affected by osmolytes in the first experiment (Fig. S4), but 4 and 6 mM of mannitol significantly increased (Treatment $p \leq 0.001$) the flavonol index compared to untreated plants in the second experiment.

4. Discussion

To the best of our knowledge, this was the first study to compare the responses of lettuce to different types of root-applied osmolytes. All three tested osmolytes (mannitol, glycerol, and GB) decreased the NO_3^- content in lettuce leaves, which has a positive effect on the edible quality of lettuce. High NO_3^- content in lettuce is a challenge, as this compound is known to have potentially harmful effects on consumer health when ingested, since it is readily converted into nitrite in the gastrointestinal tract (Pannala et al., 2003). Nitrite is considered toxic, especially for infants, because it can combine with hemoglobin to form methemoglobin, which impairs the delivery of oxygen to human tissues (Mensinga et al., 2003). Certainly, the benefits of eating lettuce and other vegetables outweigh any perceived risk of excessive NO_3^-. Moreover, moderate amounts of NO_3^- and nitrites of plant origins play essential physiological roles in supporting cardiovascular health and gastrointestinal immune function (Apte et al., 2024). However, since NO_3^- is classified as a harmful food additive in the EU, the European Commission has set a maximum NO_3^- content for fresh lettuce grown under cover (5000 mg kg^{-1} during wintertime and 4000 mg kg^{-1} during summer), under regulation (EU) 2023/915. Once the set maximum is exceeded, lettuce is rejected from trade, which leads to food waste and losses for the growers. Indeed, this increases the need for methods to control the NO_3^- of lettuce. Accordingly, numerous studies have introduced practical methods to regulate the NO_3^- content of lettuce. For example, regulation of growth temperature (Santamaria et al., 2001), fertigation (Konstantopoulou et al., 2010; Santamaria et al., 1998), and optimizing light intensity (Liu et al., 2016; Zhang et al., 2018; Yi et al., 2021) were shown to decrease lettuce NO_3^- content. As NO_3^- plays an important role in lettuce cultivation, decreased nitrate concentrations may limit yields and deteriorate quality in commercial lettuce production (Stagnari et al., 2015). We suggest that root-applied osmolytes work as a competitive alternative or a valuable supplemental method for controlling lettuce quality in hydroponic production.

Our data indicated that various root-applied osmolytes could be used to regulate leaf NO_3^- with different lettuce varieties. Four-day treatment with all tested osmolytes reduced the NO_3^- content significantly in

Table 1

Soluble sugar content (g kg $^{-1}$ DM) following pre-harvest treatment with 4 mM root-applied osmolytes at 2, 4, 7, 11 days after osmolyte application (DAA).

DAA	Treatment	Fructose	Glucose	Sucrose
2	Untreated	56.59^{a}	46.17^{a}	14.32^{a}
	Glycerol	65.79^{a}	55.29^{a}	20.27^{a}
	Glycinebetaine	80.72^{a}	69.32^{a}	25.47^{a}
	Mannitol	75.22^{a}	61.70^{a}	24.19^{a}
4	Untreated	97.55^{a}	74.78^{a}	22.53^{a}
	Glycerol	121.29^{ab}	103.70^{ab}	34.51^{a}
	Glycinebetaine	160.38^{b}	139.10^{b}	43.54^{a}
	Mannitol	145.65^{ab}	127.33^{ab}	46.27^{a}
7	Untreated	57.87^{a}	37.49^{a}	32.10^{a}
	Glycerol	69.90^{a}	48.03^{ab}	42.74^{a}
	Glycinebetaine	75.35^{a}	62.32^{ab}	36.03^{a}
	Mannitol	108.26^{a}	93.52^{b}	58.20^{a}
9	Untreated	52.25^{a}	41.06^{a}	26.54^{a}
	Glycerol	57.80^{a}	47.92^{a}	24.20^{a}
	Glycinebetaine	69.89^{ab}	57.68^{ab}	23.51^{a}
	Mannitol	90.53^{b}	76.96^{b}	29.16^{a}
11	Untreated	84.95^{a}	66.75^{a}	32.56^{a}
	Glycerol	114.73^{a}	94.99^{a}	38.71^{a}
	Glycinebetaine	101.89^{a}	71.63^{a}	42.49^{a}
	Mannitol	109.67^{a}	92.81^{a}	35.18^{a}
		p-value		
	Time	<0.001	<0.001	<0.001
	Treatment	<0.001	<0.001	0.037
	Time x Treatment	0.442	0.065	0.673

In rows, values followed by the same letter are not significantly different at $p =$ 0.05; N = 4; DM = dry matter.

Table 2

Soluble sugar content (g kg^{-1} DM) following pre-harvest treatment with root-applied mannitol at different concentrations at 2, 4, 7, 11 days after osmolyte application (DAA).

DAA	Mannitol treatment	Fructose	Glucose	Sucrose
2	0 mM	39.94^{a}	31.81^{a}	29.03^{a}
	2 mM	42.77^{a}	34.99^{a}	36.14^{a}
	4 mM	47.44^{a}	41.37^{a}	36.17^{a}
	6 mM	43.41^{a}	35.73^{a}	35.81^{a}
4	0 mM	39.01^{a}	35.33^{a}	25.11^{a}
	2 mM	57.09^{b}	48.45^{b}	38.17^{a}
	4 mM	69.33^{c}	66.45^{c}	69.52^{b}
	6 mM	74.55^{c}	68.23^{c}	53.44^{c}
7	0 mM	44.06^{a}	32.51^{a}	31.04^{a}
	2 mM	69.07^{b}	57.39^{b}	34.63^{a}
	4 mM	92.26^{c}	83.02^{c}	58.23^{b}
	6 mM	93.70^{c}	95.82^{d}	82.97^{c}
9	0 mM	45.64^{a}	36.11^{a}	28.68^{a}
	2 mM	71.38^{b}	58.51^{b}	36.71^{ab}
	4 mM	100.49^{c}	84.19^{c}	55.40^{b}
	6 mM	102.37^{c}	95.90^{c}	78.31^{c}
11	0 mM	50.10^{a}	37.73^{a}	24.87^{a}
	2 mM	68.79^{b}	56.42^{b}	30.83^{a}
	4 mM	84.93^{c}	68.90^{c}	47.75^{b}
	6 mM	101.49^{d}	83.56^{d}	47.18^{b}
		p-value		
	Time	<0.001	<0.001	<0.001
	Treatment	<0.001	<0.001	<0.001
	Time x Treatment	<0.001	<0.001	<0.001

In rows, values followed by the same letter are not significantly different at p = 0.05; N = 4; DM = dry matter.

comparison to untreated plants, and the NO_3^- content remained at a lower level until at least 7 DAA, after which it started to rise. The responses observed align with our earlier studies of GB (Jokinen et al., 2022; Uusitupa et al., 2023). Similar to GB, mannitol also reduced the leaf NO_3^- content in a dose-dependent manner. Thus, we conclude that the level of leaf NO_3^- can be precisely adjusted with various osmolytes by adjusting the osmolyte concentration accordingly. In the first experiment, the NO_3^- content in plants treated with 4 mM of mannitol was significantly lower than that of plants treated with 4 mM of glycerol and GB, indicating that the strength of the response varies with different osmolytes.

The plants treated with 4 mM of mannitol showed significantly lower FW compared to the other treatments. As shown in the second experiment, 2 mM of mannitol decreased the content of NO_3^- without notably suppressing the FW accumulation. Since suppressed FW accumulation is usually an unwanted effect in commercial lettuce production from the growers' perspective, the suppressed FW poses a central challenge related to osmolyte treatments. However, the difference observed in FW was significant only after 7 DAA, whereas the nitrate content was significantly lower in treated plants than in untreated plants after 4 DAA. This means that osmolyte treatment up to six days before harvest can decrease the content of nitrate without negatively impacting the FW. Although the FW accumulation was slowed by osmolytes, the FW accumulation continued throughout the treatment, also with higher concentrations of osmolytes. Thus, with higher concentrations of root-applied osmolytes – if needed to be used – the target FW can be achieved by extending the growth period. Based on the presented data (i.e., plant FW and DM content), we concluded that the osmolyte treatments decreased DM accumulation relatively less than FW accumulation over the cultivation period in both experiments. For consumers, this indicates enhanced nutritional value in lettuce, as most of the nutritive compounds are in the DM.

We observed a significant increase in the contents of soluble sugars, glucose, fructose, and sucrose in comparison to untreated plants. Lettuce flavor is tailored as a ratio of bitterness and sweetness, with most consumers preferring sweeter and less bitter lettuce (Chadwick et al., 2016). The total content of sugars correlates highly with sweetness, hence our data indicates that osmolyte treatments may affect lettuce flavor. However, it remains unclear how the osmolytes affect the content of bitter compounds such as sesquiterpene lactones. Furthermore, the content of soluble sugars is known to correlate with lettuce post-harvest performance and the length of the shelf-life (Min et al., 2021). Jokinen et al. (2022) pointed out that GB reduces the senescence of old lettuce leaves. Considering these findings, we hypothesize that the osmolyte treatments may have a positive impact on commercial lettuce production by extending its shelf-life and promoting improved flavor. We also found that osmolytes lowered the content of all minerals except C in comparison to untreated plants, which aligns with our earlier findings (Uusitupa et al., 2023). However, the increased dry matter content compensates for the change in the mineral contents of the fresh lettuce.

In our study, the osmolality was only slightly increased by the high concentration of mannitol in comparison to untreated plants, but no effect on the osmolality was observed with GB and glycerol. The increased content of soluble sugars likely compensated for the lowered content of NO_3^- and potassium, which was decreased by the osmolytes by as much as 42 % in comparison to untreated plants. As demonstrated by Burns et al. (2010), lettuce endogenously adjusts the osmotic potential of the sap as a sum of all anions, cations, and neutral solutes such as soluble sugars. The accumulation of soluble sugars increases as a response to nitrogen-limited conditions and lower leaf NO_3^- content. For instance, Ciriello et al. (2021) showed that short-term nutrient

Table 3

Content of hydrolyzed amino acids (g kg^{-1} DM) in the leaves of hydroponically grown lettuce (cv. 'Exact') seven days after application of glycinebetaine (GB), glycerol, and mannitol, and in the untreated control plants.

Amino acid	Osmolyte treatment				SE	P-value Trmt (df = 3)
	Untreated	Glycerol 4 mM	GB 4 mM	Mannitol 4 mM		
Cysteine	1.65^{a}	1.97^{b}	1.97^{b}	1.87^{ab}	0.072	0.003
Histidine	3.71^{a}	3.69^{a}	3.59^{a}	3.63^{a}	0.137	0.815
Serine	7.23^{a}	8.98^{b}	8.57^{ab}	8.40^{ab}	0.511	0.028
Arginine	8.70^{a}	9.81^{b}	9.42^{ab}	9.45^{ab}	0.281	0.016
Glycine	8.93^{a}	9.29^{a}	8.69^{a}	8.71^{a}	0.245	0.101
Aspartic acid	15.08^{a}	17.07^{b}	16.70^{ab}	16.55^{ab}	0.582	0.024
Glutamic acid	17.96^{a}	40.43^{b}	43.07^{b}	39.99^{b}	1.286	<0.001
Threonine	5.86^{a}	8.33^{b}	7.97^{b}	8.07^{b}	0.558	0.003
Alanine	9.55^{a}	10.39^{a}	9.66^{a}	9.62^{a}	0.285	0.038
Proline	8.13^{bc}	8.23^{c}	7.67^{a}	7.92^{ab}	0.099	<0.001
Lysine	10.98^{a}	11.71^{b}	10.95^{a}	10.92^{a}	0.220	0.01
Tyrosine	1.55^{a}	3.97^{b}	4.33^{b}	4.63^{b}	0.284	<0.001
Methionine	2.83^{a}	3.24^{a}	3.07^{a}	2.90^{a}	0.170	0.126
Valine	9.64^{a}	10.45^{b}	9.88^{ab}	9.71^{ab}	0.253	0.031
Isoleucine	8.89^{c}	8.06^{b}	7.62^{a}	7.51^{a}	0.087	<0.001
Leucine	13.84^{bc}	14.15^{c}	13.28^{ab}	13.26^{a}	0.190	0.001
Phenylalanine	8.62^{ab}	8.96^{b}	8.47^{a}	8.46^{a}	0.155	0.024
Tryptophan	2.44^{a}	2.32^{a}	2.36^{a}	2.34^{a}	0.079	0.442
Total	145.54^{a}	181.03^{b}	177.25^{b}	173.92^{b}	6.815	<0.001

In rows, values followed by the same letter are not significantly different at $p = 0.05$. N = 4; DM = dry matter; Trmt = treatment; SE = standard error.

Table 4

Content of free amino acids (g kg^{-1} DM) in the leaves of hydroponically grown lettuce (cv. 'Exact') seven days after application of glycinebetaine (GB), glycerol, and mannitol, and in the untreated control plants.

Amino acid	Osmolyte treatment				SE	P-value Trmt (df = 3)
	Untreated	Glycerol 4 mM	GB 4 mM	Mannitol 4 mM		
Histidine	0.18^{a}	0.20^{a}	0.20^{a}	0.19^{a}	0.012	0.379
Asparagine	1.76^{a}	1.80^{a}	2.04^{a}	1.97^{a}	0.133	0.16
Serine	1.54^{a}	1.71^{a}	1.58^{a}	1.47^{a}	0.102	0.166
Glutamine	16.16^{a}	19.30^{ab}	21.65^{b}	19.39^{ab}	1.319	0.011
Glycine	0.10^{a}	0.11^{a}	0.11^{a}	0.11^{a}	0.005	0.104
Aspartic acid	0.72^{b}	0.63^{ab}	0.54^{a}	0.54^{a}	0.047	0.006
Glutamic acid	2.52^{a}	2.50^{a}	2.14^{a}	2.20^{a}	0.183	0.123
Threonine	0.84^{a}	0.89^{a}	0.83^{a}	0.84^{a}	0.040	0.397
Alanine	1.27^{b}	1.21^{b}	1.00^{ab}	0.89^{a}	0.101	0.008
GABA	1.73^{a}	1.83^{a}	1.60^{a}	1.83^{a}	0.159	0.429
Proline	0.38^{a}	0.45^{a}	0.37^{a}	0.62^{b}	0.034	<0.001
Lysine	0.28^{a}	0.27^{a}	0.25^{a}	0.24^{a}	0.032	0.671
Tyrosine	0.24^{a}	0.23^{a}	0.20^{a}	0.19^{a}	0.032	0.492
Methionine	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.003	0.081
Valine	0.98^{a}	1.03^{a}	0.96^{a}	0.91^{a}	0.057	0.238
Isoleucine	0.34^{a}	0.33^{a}	0.30^{a}	0.28^{a}	0.029	0.211
Leucine	0.27^{a}	0.27^{a}	0.26^{a}	0.25^{a}	0.025	0.754
Phenylalanine	0.26^{a}	0.25^{a}	0.24^{a}	0.23^{a}	0.019	0.481
Total	29.61^{a}	33.22^{a}	34.31^{a}	32.18^{a}	1.780	0.104

In rows, values followed by the same letter are not significantly different at p = 0.05; DM = dry matter; Trmt = treatment; SE = standard error.

Table 5

Content of minerals (g kg^{-1} DM) in the leaves of hydroponically grown lettuce (cv.'Exact') seven days after application of glycerol, glycinebetaine (GB), mannitol, and in the untreated control plants.

Minerals	Osmolyte treatment				SE	P-value Trmt (df = 3)
	Untreated	Glycerol 4 mM	GB 4 mM	Mannitol 4 mM		
Carbon (C)	335.8^{a}	352.5^{b}	362.6^{c}	369.0^{c}	0.233	<0.001
Potassium (K)	89.23^{c}	69.51^{b}	54.04^{a}	51.82^{a}	2.533	<0.001
Nitrogen (N)	51.8^{d}	46.2^{c}	43.0^{b}	38.6^{a}	0.098	<0.001
Phosphorus (P)	6.32^{c}	5.12^{b}	3.97^{a}	3.56^{a}	0.208	<0.001
Sulfur (S)	2.39^{b}	2.13^{b}	1.73^{a}	1.79^{a}	0.087	<0.001
Calcium (Ca)	9.24^{c}	7.50^{b}	5.81^{a}	5.61^{a}	0.187	<0.001
Magnesium (Mg)	2.31^{c}	1.90^{b}	1.55^{a}	1.49^{a}	0.061	<0.001
Zinc (Zn)	0.043^{c}	0.031^{b}	0.025^{a}	0.022^{a}	0.002	<0.001
Iron (Fe)	0.071^{c}	0.056^{b}	0.044^{a}	0.039^{a}	0.003	<0.001
Manganese (Mn)	0.154^{c}	0.103^{b}	0.064^{a}	0.073^{a}	0.005	<0.001
Natrium (Na)	0.586^{b}	0.509^{ab}	0.461^{a}	0.433^{a}	0.032	0.002

In rows, values followed by the same letter are not significantly different at p = 0.05. N = 4; DM = dry matter; Trmt = treatment; SE = standard error.

deficiency reduces the content of NO_3^- and potassium and increases the content of soluble sugars in lettuce.

The increased levels of sucrose observed in our study indicate whole-plant physiological adjustments of osmotic potential and carbohydrate metabolism. The exact mechanisms of these responses are yet to be determined, but we hypothesize that the responses observed after osmolyte treatments are linked to adaptation in enzymes. In parallel to osmolality adjustment, changes in primary carbon metabolism leading to increased content of soluble sugars are described as a response to abiotic stress and senescence (Wingler & Roitsch, 2008). Glucose and sucrose play specific roles in plant metabolism, with sucrose being primarily involved in carbohydrate transport to the sink tissues including roots (Rosa et al., 2009). According to Roitsch and González (2004), sugars function in plants not only as nutrients and a source of energy, but also as metabolic signals. The biotic mechanism of the regulation involves invertase enzymes, which are key enzymes in a variety of metabolic processes and links to hormone responses and gene expression. For example, invertases interact with cytokinin, a plant hormone that controls plant growth. Cytokinins also mediate the responses to abiotic stresses (Werner & Schmülling, 2009). Furthermore, we observed an increasing trend in the contents of free glutamine and proline, which are considered as stress indicators in higher plants (Ozturk et al., 2021). However, only mannitol increased the content of free proline, while GB increased the content of free glutamine significantly.

Osmolytes increased the chlorophyll index of lettuce leaves, and mannitol decreased their stomatal conductance. Reduced tissue water potential caused by osmotic treatments may affect CO_2 uptake through stomata and thus photosynthetic carbon assimilation, which, in turn, can impact whole-plant osmotic balance. According to Munns and Tester (2008), plants exposed to salt stress have thicker leaves and a higher chloroplast density per unit leaf area, which explains the paradox of how salt stress reduces stomatal conductance without affecting the CO_2 uptake per unit leaf area. Indeed, we observed that plants treated with osmolytes were more compact and had thicker leaves compared to the untreated plants. Although our results indicate that root-applied osmolytes cause abiotic stress to the plants, many of the responses are positive regarding the edible quality of lettuce. For instance, the increased DM content and chlorophyll index indicate that osmolyte treatment positively affects lettuce color and texture, which are known to be key factors that influence food purchase behavior with minimally processed vegetables (Ragaert et al., 2004).

It is important to note that the quality indicators studied here – changes in nitrate accumulation, amino acids, minerals, sugars, chlorophyll, anthocyanins, and flavonols – induced by root-applied osmolytes cannot fully explain the changes in the quality of lettuce. Comprehensive analyses related to underlying physiological mechanisms, including transcriptomics, metabolomics, and enzyme activity analyses represent a critical direction for future studies aimed at enabling the method's commercial application. In addition, using osmolytes in practical applications poses a potential risk for the microbiological quality of lettuce. Although the osmolytes applied in this study are natural compounds that have been approved for food, they may also serve as sources of energy for several bacteria in the fertigation solution, potentially leading to unexpected technical or biological side effects. Such risks should be carefully studied before adopting the method for wider use. Thus far, all methods for controlling nitrate content by regulating growth conditions or fertigation include potential trade-offs related to growth and quality; the use of osmolytes is no exception. Future studies should include a comprehensive cost-benefit analyses comparing different methods for controlling the quality of lettuce. Based on our current knowledge, we assume that the advantage of osmolytes over other methods is its adjustability and precision. Furthermore, GB is considered a compound with possible health benefits for humans (Craig, 2004), indicating that GB would be the most recommended osmolyte to use in lettuce production. In our previous studies, we demonstrated that lettuce accumulates the root-applied GB in its leaves (Jokinen et al., 2022; Uusitupa et al., 2023). In addition, our data indicates that root-applied osmolytes can have a positive effect on lettuce's post-harvest quality, but this remains to be studied.

5. Conclusions

One of the major factors affecting the quality and yield of lettuce is the composition of fertigation solution. We demonstrated that diverse types and varied concentrations of osmolytes applied to the fertigation solution provide a novel precise method to control the chemical composition of lettuce. Firstly, all osmolytes studied reduced the lettuce NO_3^- content, making it healthier for consumers. Additionally, osmolytes increased the contents of dry matter and soluble sugars, thus improving the texture and nutrient content, and possibly also the flavor and shelf-life of lettuce. However, the chemical composition during the post-harvest life remains to be studied. All osmolytes induced similar trends in plant responses over the continuous four-day treatment period, although the strength of the responses varied between osmolytes. We conclude that timing of the treatment as well as the concentration of the applied osmolyte, are critical, and the appropriate concentration is osmolyte-specific. Based on our current knowledge the treatment period of six days immediately before harvest could be recommended for all osmolytes.

CRediT authorship contribution statement

Jenni Uusitupa: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Minnamari Edelmann:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Kari Jokinen:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **Ilkka Simpura:** Writing – review & editing, Methodology. **Alexey Shapiguzov:** Writing – review & editing, Supervision. **Pirjo S.A. Mäkelä:** Writing – review & editing, Resources, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pirjo Makela has patent #patent ##US20200352115A1 and patent #FI128830 issued to Luonnonvarakeskus. Kari Jokinen has patent #patent ##US20200352115A1 and patent #FI128830 issued to Luonnonvarakeskus. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Jenni Uusitupa thanks Finnish Cultural Foundation, Henrik and Ellen Tornberg Foundation, August Johannes and Aino Tiura Foundation, Kauppapuutarhaliiton puutarhasäätiö sr., and Maiju and Yrjö Rikala Foundation, for awarding her a scholarship to conduct this work. Pirjo Mäkelä, Minnamari Edelmann and Kari Jokinen thank Niemi Säätiö, Finnish Cultural Foundation, and Maiju and Yrjö Rikala Foundation for the research grants awarded. Alexey Shapiguzov thanks Research Council of Finland (grant 346140). We would like to thank Marjo Kilpinen and Eero Kuisma for their technical assistance, Dr. Zhiuyang Teng for help with formal analysis, and Oksasen Puutarha Oy for collaboration.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.147867.

Data availability

Data will be made available on request.

References

Apte, M., Nadavade, N., & Sheikh, S. S. (2024). A review on nitrates' health benefits and disease prevention. *Nitric Oxide, 142*, 1–15. https://doi.org/10.1016/j.niox.2023.11.003

Blom-Zandstra, M., & Lampe, J. E. (1985). The role of nitrate in the osmoregulation of lettuce (*Lactuca sativa* L.) grown at different light intensities. *Journal of Experimental Botany, 36*, 1043–1052. https://www.jstor.org/stable/23688514.

Burns, I. G., Zhang, K., Turner, M. K., & Edmondson, R. (2010). Iso-osmotic regulation of nitrate accumulation in lettuce. *Journal of Plant Nutrition, 34*, 283–313. https://doi.org/10.1080/01904167.2011.533328

Chadwick, M., Gawthrop, F., Michelmore, R. W., Wagstaff, C., & Methven, L. (2016). Perception of bitterness, sweetness and liking of different genotypes of lettuce. *Food Chemistry, 197*, 66–74. https://doi.org/10.1016/j.foodchem.2015.10.105

Chen, H., & Jiang, J. G. (2010). Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. *Environmental Reviews, 18*, 309–319. https://doi.org/10.1139/A10-014

Ciriello, M., Formisano, L., Pannico, A., El-Nakhel, C., Fascella, G., Duri, L. G., & Carillo, P. (2021). Nutrient solution deprivation as a tool to improve hydroponics sustainability: Yield, physiological, and qualitative response of lettuce. *Agronomy, 11*, 1469. https://doi.org/10.3390/agronomy11081469

Craig, S. A. (2004). Betaine in human nutrition. *The American Journal of Clinical Nutrition, 80*(3), 539–549. https://doi.org/10.1093/ajcn/80.3.539

European Food Safety Authority (EFSA). (2008). Nitrate in vegetables-Scientific Opinion of the Panel on Contaminants in the Food chain. *EFSA Journal, 6*(689), 1–79. https://doi.org/10.2903/j.efsa.2008.689

Franzoni, G., Cocetta, G., & Ferrante, A. (2021). Effect of glutamic acid foliar applications on lettuce under water stress. *Physiology and Molecular Biology of Plants, 27*, 1059–1072. https://doi.org/10.1007/s12298-021-00984-6

Hambridge, T. (2003). Nitrate and nitrite: Intake assessment. WHO Food Additives Series 50. https://www.inchem.org/documents/jecfa/jecmono/v50je07.htm.

Jokinen, K., Salovaara, A. K., Wasonga, D. O., Edelmann, M., Simpura, I., & Mäkelä, P. S. A. (2022). Root-applied glycinebetaine decreases nitrate accumulation and improves quality in hydroponically grown lettuce. *Food Chemistry, 366*, Article 130558. https://doi.org/10.1016/j.foodchem.2021.130558

Kalisz, A., Kornaś, A., Gil, J., Rudolphi-Szydło, E., Gawrońska, K., Sieprawska, A., & Fotopoulos, V. (2024). Foliar spraying with amino acids and their chitosan nanocomposites as promising way to alleviate abiotic stress in iceberg lettuce grown at different temperatures. *Scientific Reports, 14*, 17208. https://doi.org/10.1038/s41598-024-68005-6

Konstantopoulou, E., Kapotis, G., Salachas, G., Petropoulos, S. A., Karapanos, I. C., & Passam, H. C. (2010). Nutritional quality of greenhouse lettuce at harvest and after storage in relation to N application and cultivation season. *Scientia Horticulturae, 125*, 93. https://doi.org/10.1016/j.scienta.2010.03.003

Liu, H., Fu, Y., Yu, J., & Liu, H. (2016). Accumulation and primary metabolism of nitrate in lettuce (*Lactuca sativa* L. var. Youmaicai) grown under three different light sources. *Communications in Soil Science and Plant Analysis, 47*, 1994–2002. https://doi.org/10.1080/00103624.2016.1225076

Ma, Q., Ma, J., Wang, J., Wang, Q., Feng, Y., & Wu, L. (2018). Glucose and sucrose supply regulates the uptake, transport, and metabolism of nitrate in Pak Choi. *Agronomy Journal, 110*, 535–544. https://doi.org/10.2134/agronj2017.09.0541

Mäkelä, P., Munns, R., Colmer, T. D., Condon, A. G., & Peltonen-Sainio, P. (1998). Effect of foliar applications of glycinebetaine on stomatal conductance, abscisic acid and solute concentrations in leaves of salt-or drought-stressed tomato. *Functional Plant Biology, 25*, 655–663. https://doi.org/10.1071/PP98024

Mensinga, T. T., Speijers, G. J., & Meulenbelt, J. (2003). Health implications of exposure to environmental nitrogenous compounds. *Toxicological Reviews, 22*, 41–51. https://doi.org/10.2165/00139709-200322010-00005

Min, Q., Marcelis, L. F., Nicole, C. C., & Woltering, E. J. (2021). High light intensity applied shortly before harvest improves lettuce nutritional quality and extends the shelf life. *Frontiers in Plant Science, 12*, Article 615355. https://doi.org/10.3389/fpls.2021.615355

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology, 59*, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Ozturk, M., Turkyilmaz Unal, B., García-Caparrós, P., Khursheed, A., Gul, A., & Hasanuzzaman, M. (2021). Osmoregulation and its actions during the drought stress in plants. *Physiologia Plantarum, 172*, 1321–1335. https://doi.org/10.1111/ppl.13297

Pannala, A. S., Mani, A. R., Spencer, J. P., Skinner, V., Bruckdorfer, K. R., Moore, K. P., & Rice-Evans, C. A. (2003). The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans. *Free Radical Biology and Medicine, 34*, 576–584. https://doi.org/10.1016/S0891-5849(02)01353-9

Ragaert, P., Verbeke, W., Devlieghere, F., & Debevere, J. (2004). Consumer perception and choice of minimally processed vegetables and packaged fruits. *Food Quality and Preference, 15*, 259–270. https://doi.org/10.1016/S0950-3293(03)00066-1

Rhodes, D., & Hanson, A. D. (1993). Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual Review of Plant Biology, 44*, 357–384. https://doi.org/10.1146/annurev.pp.44.060193.002041

Roitsch, T., & González, M. C. (2004). Function and regulation of plant invertases: Sweet sensations. *Trends in Plant Science, 9*, 606–613. https://doi.org/10.1016/j.tplants.2004.10.009

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M., & Prado, F. E. (2009). Soluble sugars: Metabolism, sensing and abiotic stress: A complex network in the life of plants. *Plant Signaling & Behavior, 4*, 388–393. https://doi.org/10.4161/psb.4.5.8294

Roughan, P. G., & Batt, R. D. (1969). The glycerolipid composition of leaves. *Phytochemistry, 8*, 363–369. https://doi.org/10.1016/S0031-9422(00)85432-1

Santamaria, P., Elia, A., Gonnella, M., Parente, A., & Serio, F. (2001). Ways of reducing rocket salad nitrate content. *Acta Horticulturae, 548*, 529–537. https://doi.org/10.17660/ActaHortic.2001.548.64

Santamaria, P., Elia, A., Parente, A., & Serio, F. (1998). Fertilization strategies for lowering nitrate content in leafy vegetables: Chicory and rocket salad cases. *Journal of Plant Nutrition, 21*, 1791–1803. https://doi.org/10.1080/01904169809365524

Shahbaz, M., Ashraf, M., Al-Qurainy, F., & Harris, P. J. (2012). Salt tolerance in selected vegetable crops. *Critical Reviews in Plant Sciences, 31*, 303–320. https://doi.org/10.1080/07352689.2012.656496

Shen, W., Zhang, W., Li, J., Huang, Z., Tao, Y., Hong, J., & Zhou, Y. (2024). Pre-harvest short-term continuous LED lighting improves the nutritional quality and flavor of hydroponic purple-leaf lettuce. *Scientia Horticulturae, 334*, Article 113304. https://doi.org/10.1016/j.scienta.2024.113304

Singh, M., Kumar, J., Singh, S., Singh, V. P., & Prasad, S. M. (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: A review. *Reviews in Environmental Science and Biotechnology, 14*, 407–426. https://doi.org/10.1007/s11157-015-9372-8

Stagnari, F., Galieni, A., & Pisante, M. (2015). Shading and nitrogen management affect quality, safety and yield of greenhouse-grown leaf lettuce. *Scientia Horticulturae, 192*, 70–79. https://doi.org/10.1016/j.scienta.2015.05.003

Trip, P., Krotkov, G., & Nelson, C. D. (1964). Metabolism of mannitol in higher plants. *American Journal of Botany, 51*, 828–835. https://www.jstor.org/stable/2439888.

Uusitupa, J., Edelmann, M., Simpura, I., Jokinen, K., & Mäkelä, P. S. (2023). Root-applied glycinebetaine controls nitrate content and edible quality of lettuce in continuous commercial hydroponic greenhouse production. *Frontiers in Sustainable Food Systems, 7*, Article 1125456. https://doi.org/10.3389/fsufs.2023.1125456

Werner, T., & Schmülling, T. (2009). Cytokinin action in plant development. *Current Opinion in Plant Biology, 12*, 527–538. https://doi.org/10.1016/j.pbi.2009.07.002

Wingler, A., & Roitsch, T. (2008). Metabolic regulation of leaf senescence: Interactions of sugar signalling with biotic and abiotic stress responses. *Plant Biology, 10*, 50–62. https://doi.org/10.1111/j.1438-8677.2008.00086.x

Yi, Z., Cui, J., Fu, Y., Yu, J., & Liu, H. (2021). Optimization of light intensity and nitrogen concentration in solutions regulating yield, vitamin C, and nitrate content of lettuce. *The Journal of Horticultural Science and Biotechnology, 96*, 62–72. https://doi.org/10.1080/14620316.2020.1804468

Yildirim, E., Ekinci, M., Turan, M., Dursun, A., Kul, R., & Parlakova, F. (2015). Roles of glycine betaine in mitigating deleterious effect of salt stress on lettuce (*Lactuca sativa* L.). *Archives of Agronomy and Soil Science, 61*, 1673–1689. https://doi.org/10.1080/03650340.2015.1030611

Zhang, T., Shi, Y., Piao, F., & Sun, Z. (2018). Effects of different LED sources on the growth and nitrogen metabolism of lettuce. *Plant Cell, Tissue and Organ Culture, 134*, 231–240. https://doi.org/10.1007/s11240-018-1415-8