

# Effect of Foliar Spraying with Different Sources of Calcium on Browning, Quality and Storability of Lettuce Crop

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## ABSTRACT

This study examined the influence of foliar spray containing various sources of calcium (calcium chloride, calcium nitrate, and calcium oxide) at two different concentrations (1% and 2%) of lettuce (cv. Iceberg). The study was carried out over two consecutive winter seasons (2021-2022 and 2022-2023), comparing treated plants with untreated control plants (sprayed only with water) on total yield, quality characteristics and storability of lettuce heads. Results showed that the plant treated with calcium nitrate at 1% or 2% was the most favorability treatment for enhancing the total yield/plot, head weight and number of leaves/head. However, plant treatment with calcium chloride at 2% treatment improved physical properties of head (head compactness, firmness, and dry weight) and chemical properties [ascorbic acid, total chlorophyll content, and total phenolic compounds (TPC)], also, gave the lowest values of browning% of the leaves at harvest and increased storability of heads, which provided the lowest weight loss value, browning (score), polyphenol oxidase (PPO) activity, maintained head firmness, total soluble solids, ascorbic acid, total chlorophyll content, and (TPC) during the storage period and gave heads with an excellent appearance without any decay after 20 days of storage at 0 °C.

**Key Words:** Lettuce, calcium chloride, calcium nitrate, calcium oxide, quality, storability.

## INTRODUCTION

The phenomenon of brown color on the leaf surface is one of the important reasons for the decline in the quantity and quality of head lettuce. This physiological disturbance also leads to huge financial losses (Khumjing *et al.*, 2011). There is a link between the occurrence of browning in leaves and a lack of calcium (Saure, 2001) involving lettuce (Martyn *et al.*, 2007). Lack of calcium also causes enzymatic browning of the heads and is associated with a loss in membrane integrity (Franck *et al.*, 2007). Since the phenomenon limits consumer acceptance of the product, it reduces the market value of the product, so damage is considered the major problem in production of lettuce (Saltveit, 2000).

Altunkaya and Gökmen (2008) pointed out that lettuce leaves are extremely vulnerable to browning caused by enzymes. Hence, browning of leaves is the main factor influencing product losses leading to

shortened shelf life (Zhang *et al.*, 2001). Polyphenol oxidase enzymes (PPO) contributes to enzymatic browning. The primary cause of fresh lettuce becoming brown in the presence of oxygen is the oxidation of phenolic compounds to quinones in the cell (Nicolas *et al.*, 1994). Visually we can observe the appearance of physiological disorders on the surfaces of leaves (Franck *et al.*, 2007) in the preharvest phase (Kays, 1999).

Furthermore, the breakdown of cell membranes is the direct cause of the enzymatic browning phenomena. Therefore, its causes must be sought in processes that affect the integrity of the cell membrane (Franck *et al.*, 2007). Advanced maturation stages of plants cause cellular compartments to inactivate, allowing substrate and enzymes in the chloroplast to come into contact and interact (Rocha and Morais, 2001). Therefore, we need to investigate how to regulate enzymatic browning during plant growth, in ways that maintain the integrity of the cell membrane, to improve the quality and economic value of the harvested lettuce.

Calcium (Ca) is a macronutrient essential for the life and growth of plants, and it carries out a number of tasks inside the cell based on its location and concentration. Calcium in the cell wall is mainly concentrated in the middle lamina, where it binds to pectin in the form of calcium pectate, the main component of cell walls, acts as a secondary messenger in the cytosol and acts in the vacuole as an antidote (Hepler, 2005 and Hepler & Winship, 2010). Additionally, calcium is involved in the process of photosynthesis, increasing cell size, assisting in cell division, cytoplasmic movements, maintaining cytoskeletal functions, and stabilizing the plasma membrane (Hepler, 2005). Not only that, but it is also considered an important intracellular transmitter that acts as a defense mechanism for plants by stimulating the synthesis of some genes and proteins that regulate plant stress (White & Broadley, 2003 and Hepler, 2005).

It's been demonstrated that calcium salt treatments before and after harvest can effectively control a number of plant physiological disorders, lower the prevalence of fungal infections, and maintaining firmness of lettuce heads (Bakshi *et al.*, 2005).

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Foliar spraying reduces many of the problems associated with calcium deficiency in species with short cycles. The foliar application offers direct nutrients throughout crucial phases of plant growth and causes a quick response when plant demand is strong or excessive, or when soil conditions or stress limit the supply of nutrients (Fageria *et al.*, 2009; Fernández *et al.*, 2013; Jusoh *et al.*, 2015 and Salama & Abou-Zaid, 2023).

Raising the cellular level of calcium ions  $\text{Ca}^{2+}$  in plants is one of the ways to delay tissue aging processes and is essential to the ripening of fruit and the process of vegetative aging. Additionally, it slows down the rate at which protein and chlorophyll break down, improving the fruit's quality (Perucka *et al.*, 2013) and reduces the browning of vegetable salads (Degl'Innocenti *et al.*, 2007).

A large portion of the literature regarding the effectiveness of calcium foliar application on lettuce is debatable because this efficiency depends on the source of calcium used and the dose applied (Almeida *et al.*, 2016).

In this regard, foliar application with calcium chloride ( $\text{CaCl}_2$ ) and calcium nitrate [ $\text{Ca}(\text{NO}_3)_2$ ] are common to enhance head lettuce quality and raise its calcium content (Matchima, 2013). As the level of calcium in plants increased, so did the calcium content (Yuan, 2018). Calcium chelate (200 or 300 ppm) improved quality and productivity characteristics of lettuce (iceberg) (Gomaa and Elsagan, 2020).

Calcium spraying reduces post-harvest losses, improves durability and increases time to market (Almeida *et al.*, 2016).

Khumjing *et al.* (2011) found that treatments with  $\text{CaCl}_2$  at 2% resulted in the lowest PPO activity and decrease browning of leaves compared to  $\text{Ca}(\text{NO}_3)_2$  or  $\text{CaO}$  on lettuce plants and increased chlorophyll contents in lettuce leaves during storage (Perucka *et al.*, 2013).

$\text{CaCl}_2$  can be utilized to obtain calcium and chloride (Rab and Haq, 2012). Additionally, Michałojć and Horodko (2006) reported that  $\text{CaCl}_2$  is the best absorbed among the calcium compounds used to spray plants. It is an agricultural chemical that enhances plant vegetative growth, crop productivity, and some physiological parameters for many vegetable crops, especially lettuce (Youssef *et al.*, 2017).

Youssef *et al.* (2017) showed positive effect of  $\text{CaCl}_2$  as foliar application on lettuce growth, productivity, and physiological parameters.

Tzortzakis (2009) mentioned that tip-burn symptoms and black heart of lettuce were reduced by  $\text{Ca}(\text{NO}_3)_2$  application.

Almeida *et al.* (2016) noticed that foliar application of three calcium sources ( $\text{CaO}$ ,  $\text{CaCl}_2$ , calcium chelate) in four doses increased the biomass and number of lettuce leaves. In addition, by raising the calcium concentrations in leaf tissue, calcium spraying improves the biofortification of plant foods. A product with higher calcium concentrations in the cells has better durability, a longer shelf life, and a lower rate of post-harvest losses and decomposition and thus higher profits.  $\text{CaCl}_2$  at the recommended dose showed optimal cost and efficiency benefits compared to  $\text{CaO}$  and  $\text{Ca}$  chelate. Therefore, the aim of this study was to determine the effect of foliar spraying with different calcium sources at various concentrations on the total yield, quality characteristics and storability of heads of lettuce (Iceberg).

## MATERIALS AND METHODS

### 1. Filed experiment

Field experiments were conducted on lettuce cv. Iceberg during the winter seasons of 2021-2022 and 2022-2023 at a private farm located in Alexandria Governorate. The plot area was 12.6 m<sup>2</sup>, and each plot consisted of six rows 3.5 m length and 60 cm width with around 210 plants per plot. Lettuce seeds were sown in nursery beds on the 3<sup>rd</sup> and 2<sup>nd</sup> of October in both seasons and then transplants of nearly 40 days were transplanted on the 15<sup>th</sup> and 13<sup>th</sup> of November in the permanent field on both sides of rows at a spacing of 20 cm between plants and 60 cm between rows. The treatments were arranged in a complete randomized block design with three replicates. Each replicate consisted of one plot.

The study investigated the effect of three different calcium sources {calcium chloride ( $\text{CaCl}_2$ ), calcium nitrate [ $\text{Ca}(\text{NO}_3)_2$ ], and calcium oxide ( $\text{CaO}$ )} at two concentrations (1% and 2%) were applied as foliar sprays beside untreated plants (control) which sprays with tap water. These treatments were done three times during the growth period of lettuce plants, after 20, 30, and 40 days of transplanting. All other agricultural practices including irrigation, fertilization and pest control were conducted uniformly across all plots according to the recommendations of the Egyptian Ministry of Agriculture for lettuce production. The physical and chemical properties of the two experimental sites under study are presented in Table (1), which were determined by the Soil and Water Research Institute, Agricultural Research Center.

**Table 1. Physical and chemical characteristics of the experimental soil as average of both seasons 2021/2022 and 2022/2023**

Physical properties								
Sand %		Silt %			Clay %		Texture	
32.7		21.2			44.8		Clay loam	
Chemical properties								
EC (ds/m)	pH	Cations (Meq.L <sup>-1</sup> )			Anions (Meq.L <sup>-1</sup> )			
		Ca <sup>++</sup>	Mg <sup>++</sup>	K <sup>+</sup>	Na <sup>+</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
3.4	7.9	12.6	9.8	0.68	15.9	26.8	9.2	3.4

**The following data were recorded:****Yield and its components:**

Yield parameters, viz., average head weight and total yield/plot (estimated as the weight of harvested plants over the growing season expressed as Kg/ plot).

**Head quality parameters:****i. Physical properties**

A random sample of thirty heads from each treatment was randomly chosen to determine the following properties:

- Head compactness: Score rating from 5 to 1, where 5= tight, 4= few basal bracts pointing a way from rather than toward tip of bud, 3= several whorls of bracts pointing a way from rather than toward tip of bud, 2= all or most outer bracts open, 1= all outer and more centrally located bracts open.
- Number of leaves/head, the standing leaves on each individual head were counted.
- Fruit firmness (kg/cm<sup>2</sup>) was determined as mentioned in the storage experiment.
- Head dry weight (g) was recorded. Dry leaf was obtained by drying in an electric oven at 70°C until it reached a consistent weight.
- Browning %: The browning levels on leaf surfaces were scored by determining visually and expressing as a percentage (Khumjing *et al.*, 2011).

**ii. Chemical properties**

The following properties were determined as mentioned in the storage experiment.

- Ascorbic acid (mg/100g F.W.).
- Total chlorophyll content (mg/100g F.W.) .
- Total phenolic compounds (TPC) (mg/100g F.W.).
- Polyphenol oxidase (PPO) activity (mg/100g F.W.).

**2. Storage experiment**

Lettuce heads (*Lactuca sativa* L.) cultivar Iceberg obtained from the previous experiment were harvested at suitable maturity stage for marketing on January 18<sup>th</sup> and 16<sup>th</sup> (70 days from transplanting) during the first and second season, respectively. Non-uniform and

uncompacted heads were discarded and choose healthy heads with dark green outer leaves, symmetrical shapes and good ones of heads were transported directly to the laboratory of Handling of Vegetable Crops Research Department, Horticulture Research Institute, Agriculture Research Center, Giza Governorate, Egypt. The dry and damaged outer leaves were removed and the stem of head lettuce were cut by a sharp knife (1cm in length). All treatments were arranged in a complete randomized design with three replicates. Every head was wrapped individually in a polypropylene bag (30µm thickness) served as one replicate. Fifteen replications were prepared for each treatment and packed in carton boxes (each box containing three bags) and stored at 0°C and 95% relative humidity. Three replicates from each treatment were selected at random and assessed immediately after harvest, as well as after 5, 10, 15 and 20 days from storage period to determine the following data:

- The percentage of weight loss was calculated according to the following equation, weight loss % = [(initial weight of heads - weight of heads at sampling date) / initial weight of heads] × 100.
- Decay was evaluated as score system of 1= none, 2= slight, 3= moderate, 4= moderately severe, 5= severe.
- General appearance: The appearance was evaluated using a scale from (1 to 9) where 9 = excellent, 7 = good, 5 = fair and 3= poor, heads rating (5) or below was considered unmarketable the general appearance assessment includes symptoms of deterioration (leaf dryness, leaf wilting and yellowing, browning in the cut stem surface and decay).
- Firmness: The firmness of lettuce heads as recorded by TA- 1000 texture analyzer instrument using a penetrating cylinder of 1mm diameter, to a constant distance (3 and 5mm) inside the pulp of heads and by a constant speed 2mm per sec.
- Browning (score): The browning appearance on leaf surfaces were scored for evaluating browning as described in González-Aguilar *et al.* (2004) by determining visually using a scale of 1-5, where 1 = none, 2 = slight, 3 = moderate, 4 = severe and 5 =

extreme browning. Degree of browning evaluations was carried out during storage.

6. Total soluble solids percentage (TSS): TSS was determined as a composite juice sample by digital refractometer, "Model Abbe Leica" according to AOAC (2000).
7. Ascorbic acid content (mg/100g F.W.) was determined by titration method using 2, 6-dichlorophenol indophenols as described in AOAC (2000).
8. Total chlorophyll content as determined according to AOAC (1990).
9. Total phenolic compounds (TPC): TPC content was determined by the colorimetric modified method of Velioglu *et al.* (1998) using Folin-Ciocalteu reagent.
10. Polyphenol oxidase (PPO) activity: The activity of polyphenol oxidase was determined by the colorimetric modified method of Dogan *et al.* (2002).

#### Statistical analysis

All data were statistically analyzed to calculate means, variances, and standard errors. Least significant difference test (LSD, 5 % of probability) was used to evaluate differences between the means by using MSTAT-C software program as stated by Snedecor and Cochran (1980).

## RESULTS AND DISCUSSIONS

### 1. Field experiment

#### Yield and its components:

Data in Table (2) demonstrate that lettuce plants treated with different concentrations of calcium sources significantly increased their total yield/plot, including head weight, compared to untreated plants (control). Nevertheless, plants that have been treated with  $\text{Ca}(\text{NO}_3)_2$  at 2% and 1% were the best treatments for increasing the total yield/ plot and head weight with significant difference between them.  $\text{CaCl}_2$  at 2% or 1% was less efficient treatment in this regard. The lowest values were observed in the untreated plants in this respect. These results are in agreement with Al-Hamzawi (2010) and Attallah & Abdalla (2021).

The increase in the growth and productivity of heads possibly because calcium raising the indicators of vegetative growth in lettuce and the leaf content of NK and photosynthetic pigments, which positively affects plant growth and increases its productivity (Almeida *et al.*, 2016).

Here, calcium plays a role in promoting plant growth by maintaining stability of the cell membrane and cell wall when sprayed externally (Song *et al.*, 2020). Furthermore, calcium accelerates the transfer of

carbohydrates produced from photosynthesis to the sinks (Navazio *et al.*, 2020).

#### Head quality parameters

##### Physical properties

Data in Table (2) showed that applying foliar sprays with varying calcium concentrations resulted in a significant increase in head quality expressed in number of leaves/head and head compactness compared to heads obtained from untreated plants. In this regard,  $\text{Ca}(\text{NO}_3)_2$  at 2% or 1% was the most successful treatment for raising number of leaves/head, followed by  $\text{CaO}$  at 2% or 1 % with significant differences between them in the first season. However,  $\text{CaCl}_2$  at 2% or 1% was the best treatment for enhancing head compactness, followed by  $\text{CaO}$  and  $\text{Ca}(\text{NO}_3)_2$  at 2% or 1 % with no significant differences between them. The least value of compactness resulted by untreated plants. These results were in agreement with Almeida *et al.* (2016); Youssef *et al.* (2017) and Gomaa & Elsagan (2020).

One of the essential elements for growth is calcium, because, it is necessary for the formation and stability of cell walls, proper root growth, and stimulates enzymes for various metabolic processes (Thor, 2019).

Youssef *et al.* (2017) indicated that the increase in the weight of the head of lettuce (yield) as a results to treatment lettuce plant with calcium is mainly due to the rise in the number of leaves, and not to increase in the leaf area. The stimulating effect of  $\text{CaCl}_2$  on growth highlights the reality that calcium ions ( $\text{Ca}^{2+}$ ) appear to take part in regulating the division process in cells, as calcium is an essential ion in formation of the mitotic spindle (Hepler, 1994).

Almeida *et al.* (2016) noticed that foliar application of three calcium sources ( $\text{CaO}$ ,  $\text{CaCl}_2$ , calcium chelate) was effective in promoting growth because it promoted high cell biomass accumulation, number of leaves per head and final concentration of calcium in tissues and increases the rate of cell division. The application of calcium paper goes along with the division processes by raising calcium concentrations inside the cell. It promotes microtubule depolymerization and controls the rate of chromosome movement during anaphase (Zhang *et al.*, 1992). The lettuce leaf has a thin cuticle that easily absorbs calcium due to the presence of stomata on each side, which facilitates the absorption and penetration of product into the cells of the applied solutions (Weryszko-Chmielewska and Michałojć, 2009).

Calcium accumulation in the cell wall increases biomass because applying calcium has little effect on leaf thickness, it does not expand the cell, and treatments with higher calcium concentrations showed

the greatest biomass accumulation (Almeida *et al.*, 2016).

In terms of head firmness, dry weight % and browning percentage, heads obtained from plants sprayed with CaCl<sub>2</sub> at 2% or 1% had the highest firmness and dry weight % values and the lowest browning % values, followed by CaO at 2% or 1%, with large differences between them. Ca(NO<sub>3</sub>)<sub>2</sub> treatment had lower effectiveness in this regard. However, control gave the least values of head firmness and dry weight % and the highest values of browning %. These findings were consistent with Khumjing *et al.* (2011); Attallah & Abdalla (2021) and EL-Sayed *et al.* (2023).

The gradual improvement of rigidity is due to calcium ions involved in the formation of the thickness of the cell wall's middle lamella resulting from increased formation of calcium pectate and the deposition and stabilization of the cell membrane (Singh *et al.*, 2007).

Additionally, Ca raises the levels of calcium in the cell walls and provides increased plant mechanical strength due to increased total pectin content and delayed pectin dissolution (Singh *et al.*, 2007 and Li *et al.*, 2012).

Calcium spray treatment raises the Ca content in mesocarp cells, resulting in a firmer tissue at harvest (Naradison *et al.*, 2006).

CaCl<sub>2</sub> stabilizes the plant membrane, delaying PPO activity and lowering the occurrence of browning. CaCl<sub>2</sub> had a significant impact in delaying the cell wall's modifications (Brummell *et al.*, 2004).

### Chemical properties

Data in Table (3) demonstrate that all treatments significantly maintained Total chlorophyll, ascorbic acid and total phenol content and reduced PPO activity compared with untreated control. However, CaCl<sub>2</sub> foliar spraying at 2% or 1% were observed to have the highest values of ascorbic acid, Total chlorophyll, and total phenol contents and the least values of PPO activity followed by CaO at 2% or 1% with significant differences between them in the two seasons. These findings were consistent with Keshavarzpour (2012); Youssef *et al.* (2017) and EL-Sayed *et al.* (2023).

CaCl<sub>2</sub> foliar spraying increases chlorophyll content due to increased absorption of nitrogen and magnesium (Youssef *et al.*, 2017). Also, Ritchey *et al.* (1995) found that CaCl<sub>2</sub> causes a rise in exchangeable Ca<sup>2+</sup> ions inside the cells, resulting in the activation of the chlorophyll molecule's metabolism in lettuce leaves (Heaton and Marangoni, 1996).

**Table 2. Effect of foliar spray treatments with different sources of calcium on physical properties of lettuce heads during 2021/2022 and 2022/2023 seasons**

Treatments	Total yield / plot (kg)	Head weight (gm)	Head compactness (Score)	Number of leaves/head	Firmness (kg/cm <sup>2</sup> )	Head dry weight (g)	Browning (%)
<b>2021/2022 season</b>							
Control	124.00 G	590.70 G	2.33 B	13.33 D	3.16 G	7.07 G	20.33 A
Oxide Calcium 1%	151.70 D	722.30 D	4.33 A	19.67 B	4.62 D	8.42 D	5.40 D
Oxide Calcium 2%	163.20 C	777.30 C	4.67 A	21.00 B	4.99 C	8.95 C	4.40 E
Chloride Calcium 1%	132.20 F	629.30 F	4.67 A	16.67 C	5.45 B	9.87 B	3.30 F
Chloride Calcium 2%	141.40 E	673.40 E	5.00 A	17.33 C	5.87 A	10.47 A	2.07 G
Nitrate Calcium 1%	173.50 B	826.30 B	4.33 A	23.33 A	3.73 F	7.95 F	7.43 B
Nitrate Calcium 2%	185.10 A	881.40 A	4.33 A	25.33 A	4.07 E	8.14 E	6.50 C
<b>2022/2023 season</b>							
Control	128.40 G	611.50 G	2.67 B	14.67 F	3.21 G	7.13 G	21.30 A
Oxide Calcium 1%	155.40 D	739.90 D	4.67 A	20.00 CD	4.68 D	8.90 D	5.60 D
Oxide Calcium 2%	166.70 C	793.70 C	4.67 A	22.00 BC	5.04 C	9.36 C	4.57 E
Chloride Calcium 1%	136.30 F	648.80 F	4.67 A	17.33 E	5.51 B	10.14 B	3.43 F
Chloride Calcium 2%	145.30 E	692.00 E	5.00 A	18.67 DE	5.95 A	10.93 A	2.17 G
Nitrate Calcium 1%	176.80 B	841.80 B	4.33 A	24.00 AB	3.79 F	8.14 F	7.53 B
Nitrate Calcium 2%	188.10 A	895.80 A	4.33 A	26.00 A	4.14 E	8.43 E	6.67 C

The values that contain the same capital letters in the same columns indicate that there are no significant variations between each other at level 0.05.

**Table 3. Effect of foliar spray treatments with different sources of calcium on chemical properties of lettuce heads during 2021/2022 and 2022/2023 seasons**

Treatments	Ascorbic acid (mg/100g F.W.)	Total chlorophyll content (mg/100g F.W.)	Total phenolic content (mg/100g F.W.)	Polyphenol oxidase activity (mg/100g F.W.)
<b>2021/2022 season</b>				
Control	21.57 F	35.66 G	27.90 F	41.03 A
Oxide Calcium 1%	24.96 D	43.24 D	35.54 C	30.35 D
Oxide Calcium 2%	25.51 C	44.23 C	36.25 C	29.57 D
Chloride Calcium 1%	27.80 B	45.77 B	38.28 B	25.43 E
Chloride Calcium 2%	29.95 A	48.89 A	40.43 A	22.33 F
Nitrate Calcium 1%	24.31 E	37.62 F	30.87 E	36.28 B
Nitrate Calcium 2%	24.85 D	38.48 E	32.96 D	34.44 C
<b>2022/2023 season</b>				
Control	21.66 G	35.72 G	28.22 F	40.10 A
Oxide Calcium 1%	25.65 D	43.70 D	36.97 C	30.11 D
Oxide Calcium 2%	27.02 C	44.80 C	37.67 C	29.12 E
Chloride Calcium 1%	27.93 B	48.76 B	39.64 B	25.20 F
Chloride Calcium 2%	30.24 A	51.46 A	41.83 A	22.14 G
Nitrate Calcium 1%	23.90 F	37.65 F	31.36 E	36.18 B
Nitrate Calcium 2%	24.56 E	38.84 E	33.25 D	34.12 C

The values that contain the same capital letters in the same columns indicate that there are no significant variations between each other at level 0.05.

Ilias *et al.* (2007) reported that Ca treatment stimulated the total chlorophyll content and enhanced K, B, Mg, Ca and Fe accumulation in plant leaves.

Ca<sup>2+</sup> directly contributes to the synthesis of phenols. Foliar dose of 10 µM CaCl<sub>2</sub> raised the phenols accumulation in lemon seedlings (Castaneda and Perez, 1996).

A decrease in activity of PPO appeared in treatment with 2% CaCl<sub>2</sub> due to her role in metabolic activities, such as stabilizing cell membranes, controlling enzymatic activity, and alleviating the harmful effects of activity of PPO enzyme (Arshi *et al.*, 2006).

## 2. Storage experiment:

### Weight loss percentage:

Data in Table (4) noticed that weight loss percentage of lettuce heads increased considerably and consistently as the storage period is extended. These findings are consistent with Abdullah *et al.* (2023). Loss of water from fresh produce causes many metabolic changes that are unfavorable for cells, which activates enzymes, which in turn accelerate aging and reduce nutritional values during storage, thus increasing weight loss (Breda *et al.*, 2017).

It was evident that all the treatments, used, resulting in significant reducing in the loss percentage in head weight in comparison to the control treatment. As for, CaCl<sub>2</sub> and CaO at 2% or 1%, are the best treatments for

lowering the percentage of weight loss, although they showed significant differences between them. Ca(NO<sub>3</sub>)<sub>2</sub> had lower effectiveness in this regard. In contrast, untreated heads gave the greatest values of weight loss percentage and these were consistent with those obtained by Al-Hamzawi (2010) and EL-Sayed *et al.* (2023).

The reduced weight loss caused by CaCl<sub>2</sub> could be because of Ca<sup>2+</sup> in the formation of calcium pectate hydrogel, which retains more water and delays the leaves' dehydration processes (Turmanidze *et al.*, 2017) and reduced respiration rate resulted in lower weight loss (Keshavarzpour, 2012).

Calcium also restricts weight loss by inhibiting respiratory rates, maintaining the integrity of cell structure, and thus decreasing electrolyte leakage and water evaporation (Erbaş and Koyuncu, 2023) and inhibiting physiological process, which thus decreased weight loss (Luna-Guzman and Barrett, 2000).

With respect to the interactions between all pre-harvest treatments and post-harvest storage periods, the weight loss percentage during storage at 0°C was significant in both seasons. At the end of storage period, CaCl<sub>2</sub> at 2% showed the lowest percentage of weight loss compared to all treatments and the untreated control treatment.

**Table 4. Effect of foliar spray treatments with different sources of calcium on weight loss (%) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	0.00 u	1.53 m	3.55 e	9.66 b	12.11 a	5.37 A
Oxide Calcium 1%	0.00 u	0.74 r	1.12 o	1.77 k	3.62 e	1.45 D
Oxide Calcium 2%	0.00 u	0.72 r	0.95 p	1.72 kl	3.47 f	1.37 E
Chloride Calcium 1%	0.00 u	0.64 s	0.85 q	1.65 l	2.86 g	1.20 F
Chloride Calcium 2%	0.00 u	0.35 t	0.68 rs	1.54 m	2.45 h	1.00 G
Nitrate Calcium 1%	0.00 u	0.86 q	1.36 n	1.97 i	6.35 c	2.11 B
Nitrate Calcium 2%	0.00 u	0.82 q	1.02 p	1.87 j	5.44 d	1.83 C
Mean	0.00 E	0.81 D	1.36 C	2.88 B	5.19 A	
<b>2022/2023 season</b>						
Control	0.00 t	1.46 l	3.45 e	9.53 b	11.06 a	5.10 A
Oxide Calcium 1%	0.00 t	0.64 pq	0.94 n	1.66 j	3.37 e	1.32 D
Oxide Calcium 2%	0.00 t	0.56 qr	0.93 n	1.56 k	2.96 f	1.20 E
Chloride Calcium 1%	0.00 t	0.54 r	0.73 op	1.48 kl	2.55 g	1.05 F
Chloride Calcium 2%	0.00 t	0.25 s	0.63 q	1.42 l	2.23 h	0.91 G
Nitrate Calcium 1%	0.00 t	0.76 o	1.23 m	1.86 i	6.23 c	2.01 B
Nitrate Calcium 2%	0.00 t	0.74 o	1.02 n	1.84 i	5.23 d	1.77 C
Mean	0.00 E	0.70 D	1.28 C	2.76 B	4.80 A	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

#### Decay (score):

Data in Table (5) indicate that decay (score) of lettuce heads increased with the extension of storage periods. These findings agree with Abdullah *et al.* (2023) on lettuce heads. Bacteria and fungi have been connected to some enzymatic alteration processes while fruit is ripening in long-term storage, leading to fruit damage and deterioration of the middle lamina, especially enzymes found in plants linked to aging process (Zapata *et al.*, 2008).

There were significant differences in decay between treatments and heads sprayed with 2% CaCl<sub>2</sub> which did not show any spoilage during all storage periods up to 20 days of storage at 0°C, followed by CaCl<sub>2</sub> at 1% and CaO 2% or 1%, with no significant difference between Ca(NO<sub>3</sub>)<sub>2</sub> at 2% or 1%, which was less effective in reducing this trait. The untreated heads gave the greatest decay incidence. These findings were consistent with Madani *et al.* (2015) and Mandour (2017).

Calcium may directly reduce the incidence of fungal diseases by inhibiting fungal germination and enzymes that degrade fungal cell walls, as well as indirectly through its impact on the integrity of the cell wall (Wisniewski *et al.*, 1995 and Bakshi *et al.*, 2005).

CaCl<sub>2</sub> is essential for thickening and strengthening the cell walls' middle lamina because it promotes the formation and deposition of calcium pectate. Thus, it

makes the cell wall more resistant to putrefactive organisms (Garcia and Herrera, 1996).

#### General appearance (GA):

Data in Table (6) demonstrate that GA score of lettuce heads decreased as storage periods were extended. Similar findings were reported by Abdullah *et al.* (2023) on lettuce heads. The decrease in GA could result from morphological defects like the cut surface's dryness, shriveling, discoloration, wilting, color changing, and deterioration or macroscopic decay (Shehata *et al.*, 2012).

The heads obtained from all preharvest treatments showed better GA than control, however, CaCl<sub>2</sub> at 2% was most successful in preserving GA, followed by CaCl<sub>2</sub> at 1% and CaO at 2% or 1% with no significant differences between them. The untreated control had the worst GA. These findings were consistent with Sun *et al.* (2015) and Sinha *et al.* (2019), and due to CaCl<sub>2</sub> treatment stimulated peroxidase activities, superoxide dismutase, decreased microbial growth, enhanced overall visual quality, and decreased tissue electrolyte leakage (Sun *et al.*, 2015).

The interaction between pre-harvest treatments and post-harvest storage periods on GA was significant in both seasons. However, 2% CaCl<sub>2</sub> gave an excellent appearance of the heads and no changes were seen until the end of storage (20 days at 0°C). CaCl<sub>2</sub> in the 1%

treatment gave a good appearance in the same period, while CaO in the 2% or 1% treatment gave a good appearance for up to 15 days. On the contrary, the

untreated heads showed a bad appearance at the end of the storage periods.

**Table 5. Effect of foliar spray treatments with different sources of calcium on decay (score) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	1.00 i	2.33 ef	3.33 d	4.33 b	5.00 a	3.20 A
Oxide Calcium 1%	1.00 i	1.00 i	1.00 i	1.66 gh	2.00 fg	1.33 C
Oxide Calcium 2%	1.00 i	1.00 i	1.00 i	1.33 hi	2.00 fg	1.27 C
Chloride Calcium 1%	1.00 i	1.00 i	1.00 i	1.00 i	1.66 gh	1.13 CD
Chloride Calcium 2%	1.00 i	1.00 i	1.00 i	1.00 i	1.00 i	1.00 E
Nitrate Calcium 1%	1.00 i	1.00 i	1.00 i	2.66 e	4.00 bc	1.93 B
Nitrate Calcium 2%	1.00 i	1.00 i	1.00 i	2.33 ef	3.66 cd	1.80 B
Mean	1.00 D	1.19 CD	1.33 C	2.04 B	2.76 A	
<b>2022/2023 season</b>						
Control	1.00 j	1.66 hi	3.00 de	4.00 b	4.66 a	2.86 A
Oxide Calcium 1%	1.00 j	1.00 j	1.00 j	1.66 hi	2.00 gh	1.33 C
Oxide Calcium 2%	1.00 j	1.00 j	1.00 j	1.33 ij	2.00 gh	1.27 C
Chloride Calcium 1%	1.00 j	1.00 j	1.00 j	1.00 j	1.66 hi	1.13 CD
Chloride Calcium 2%	1.00 j	1.00 j	1.00 j	1.00 j	1.00 j	1.00 E
Nitrate Calcium 1%	1.00 j	1.00 j	1.00 j	2.66 ef	3.66 bc	1.86 B
Nitrate Calcium 2%	1.00 j	1.00 j	1.00 j	2.33 fg	3.33 cd	1.73 B
Mean	1.00 D	1.09 CD	1.29 C	2.00 B	2.62 A	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

**Table 6. Effect of foliar spray treatments with different sources of calcium on general appearance (score) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	9.00 a	8.33 ab	7.00 cd	4.33 g	3.00 h	6.33 D
Oxide Calcium 1%	9.00 a	9.00 a	9.00 a	7.00 cd	6.33 de	8.07 B
Oxide Calcium 2%	9.00 a	9.00 a	9.00 a	7.67 bc	6.33 de	8.20 B
Chloride Calcium 1%	9.00 a	9.00 a	9.00 a	7.67 bc	7.00 cd	8.33 B
Chloride Calcium 2%	9.00 a	9.00 a	9.00 a	9.00 a	8.33 ab	8.87 A
Nitrate Calcium 1%	9.00 a	9.00 a	7.67 bc	6.33 de	5.67 e	7.53 C
Nitrate Calcium 2%	9.00 a	9.00 a	8.33 ab	6.33 de	5.67 e	7.67 C
Mean	9.00 A	8.90 A	8.43 B	6.90 C	6.05 D	
<b>2022/2023 season</b>						
Control	9.00 a	8.33 ab	6.33 de	5.67 e	3.67 f	6.60 D
Oxide Calcium 1%	9.00 a	9.00 a	9.00 a	7.67 bc	6.33 de	8.20 B
Oxide Calcium 2%	9.00 a	9.00 a	9.00 a	7.67 bc	6.33 de	8.20 B
Chloride Calcium 1%	9.00 a	9.00 a	9.00 a	7.67 bc	7.00 cd	8.33 B
Chloride Calcium 2%	9.00 a	9.00 a	9.00 a	9.00 a	8.33 a	8.87 A
Nitrate Calcium 1%	9.00 a	9.00 a	8.33 ab	6.33 de	5.67 e	7.67 C
Nitrate Calcium 2%	9.00 a	9.00 a	8.33 ab	6.33 de	5.67 e	7.67 C
Mean	9.00 A	8.90 A	8.43 B	7.19 C	6.14 D	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

**Head firmness:**

Data in Table (7) notice that firmness of lettuce head reduced continuously with extending the storage periods. One aspect that lowers the quality of harvested fruits is the loss of fruit firmness, which is caused by the polymerization and breakdown of cell wall components. Along with cell membrane deterioration, softening changes are caused by cell wall hydrolases like polygalactosidases, pectin methylesterases, b-galactosidase, and xylanase (Ghahremani *et al.*, 2021), this causes a significant rise in pectin solubilization (García *et al.*, 2014).

Pre-harvest treatments gave greater firmness compared to the untreated control. While CaCl<sub>2</sub> at 2% was the most efficient treatment in decreasing firmness loss, followed by CaCl<sub>2</sub> at 1%, with significant differences between them. Ca(NO<sub>3</sub>)<sub>2</sub> at 2% or 1% was less impact in this regard. The untreated control showed the greatest loss of head firmness. These findings are consistent with Madani *et al.* (2015); Sinha *et al.* (2019) and EL-Sayed *et al.* (2023).

Wójcik & Lewandowski (2003) and Nguyen *et al.* (2020) recorded that Calcium is an essential element for building the cell membrane and cell wall and regulates the stability of pectin and protein compounds and is essential for the formation of the middle lamella.

The positive effects of calcium treatments on firmness are demonstrated by the activation of the enzyme pectin methylesterase (PME) which cleaves the methoxyl groups of methylated pectic substances, leading to the generation of free pectic acids inside the cell that contain newly available carboxyl groups. Endogenous and added calcium can make plant tissues more rigid by binding to pectin carboxyl groups that are generated through the action of PME (Stanley *et al.*, 1995). Ca maintains the structure of the cell wall by interacting with pectic acid present in the cell walls to form solid calcium pectates, thus maintaining water activity (Suutarinen *et al.*, 1999).

Calcium binds to the cell wall to generate calcium pectate, which makes the middle lamella more rigid and stops the cell wall activity, which causes the enzymes to decompose, and thus the outer membrane of the cell wall becomes stronger and more solid (Pila *et al.*, 2010 and Khaliq *et al.*, 2015). However, the CaCl<sub>2</sub> also controls resistance to fruit-softening enzymes that break down fruit, such as polygalacturonase (Genanew, 2013).

CaCl<sub>2</sub> may delays the decrease in firmness because it can stabilize plasma membrane systems and form calcium pectate, which makes the middle lamella more rigid and raises resistance to polygalactouronase activity (Atrass *et al.*, 2010).

**Table 7. Effect of foliar spray treatments with different sources of calcium on firmness (kg/cm<sup>2</sup>) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	3.16 pq	2.63 st	2.23 u	1.89 v	1.21 w	2.22 G
Oxide Calcium 1%	4.62 g	4.54 gh	4.37 hi	4.11 jk	3.93 kl	4.32 D
Oxide Calcium 2%	4.99 e	4.93 ef	4.75 fg	4.53 g-i	4.31 ij	4.70 C
Chloride Calcium 1%	5.45 cd	5.40 cd	5.25 d	5.02 e	4.85 ef	5.19 B
Chloride Calcium 2%	5.87 a	5.84 a	5.79 ab	5.57 bc	5.36 cd	5.69 A
Nitrate Calcium 1%	3.73 lm	3.48 no	3.20 p	2.96 qr	2.52 t	3.18 F
Nitrate Calcium 2%	4.07 k	3.83 l	3.54 mn	3.26 op	2.81 rs	3.50 E
Mean	4.56 A	4.38 B	4.16 C	3.91 D	3.57 E	
<b>2022/2023 season</b>						
Control	3.21 pq	2.72 st	2.30 u	1.95 v	1.29 w	2.29 G
Oxide Calcium 1%	4.68 g-i	4.62 hi	4.44 ij	4.16 kl	3.99 lm	4.38 D
Oxide Calcium 2%	5.04 ef	4.99 ef	4.80 f-h	4.56 h-j	4.36 jk	4.75 C
Chloride Calcium 1%	5.51 cd	5.47 cd	5.31 d	5.06 e	4.91 e-g	5.25 B
Chloride Calcium 2%	5.95 a	5.93 a	5.87 ab	5.63 bc	5.44 cd	5.76 A
Nitrate Calcium 1%	3.79 mn	3.56 no	3.27 pq	3.03 qr	2.58 t	3.25 F
Nitrate Calcium 2%	4.14 kl	3.92 lm	3.62 n	3.34 op	2.88 rs	3.58 E
Mean	4.62 A	4.46 B	4.23 C	3.96 D	3.64 E	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

Kaya *et al.* (2002) discovered that adding  $\text{Ca}(\text{NO}_3)_2$  to melon and cucumber restored their membrane permeability. Also, calcium increases the cell wall's rigidity, which could make it more resistant to the cellulase enzyme's activity (Sinha *et al.*, 2019) and decreases the activity of the pectinase enzyme, which causes cell wall degradation, as well as delays tissue softening (Vicente *et al.*, 2009).

The interaction between pre-harvest treatments and storage periods had a significant impact on head hardness.  $\text{CaCl}_2$  at 2% was more efficient at preserving the hardness of the heads until the end of the storage periods, whereas the untreated heads had the lowest values.

#### Browning (score):

The results in Table (8) show that there had been increases in browning (score) for lettuce heads as the period of storage extended in both seasons. These findings coincide with those of Rageh and Abou-Elwafa (2018) on lettuce. The primary cause of the color change is the oxidation of phenolic compounds to o-quinones by polyphenol oxidase (PPO) in presence of oxygen. Quinones have the ability to polymerize dark brown, black or red polymers (Hussein *et al.*, 2020).

Leaf pigments of lettuce (chlorophyll and carotenoids) degrade or tissue discolours during storage (Ferrante and Maggiore, 2007).

All preharvest treatments decreased the incidence of browning (score) in comparison to untreated heads. After 20 days of storage, lettuce heads sprayed with different sources of calcium reducing browning and had lower score of browning. However,  $\text{CaCl}_2$  at 2% was the most effective followed by  $\text{CaCl}_2$  at 1% with significant difference between them, while  $\text{Ca}(\text{NO}_3)_2$  at 2% and 1% treatments were less efficient in this regard. Conversely, the untreated control resulted in the highest score in browning and resulted in extreme browning at the end of storage. These results are consistent with those of Khumjing *et al.* (2011).

Reduced leaf browning might be achieved with 2%  $\text{CaCl}_2$  treatment (Khumjing *et al.*, 2011), who, also, revealed that PPO activity was discouraged and made more prone to browning disorder as a result of browning incidence brought on by the breakdown of tissue cellular compartmentation. Lettuce is considered to be quite vulnerable to enzymatic browning (Altunkaya and Gökmen, 2008).  $\text{CaCl}_2$  application at 2% decreased PPO activity, which could be mostly brought on by  $\text{CaCl}_2$  propensity to lessen browning disorder. These matched the findings of Fujita *et al.* (2006) who reported a correlation between PPO activity and browning occurrence. In addition, browning disorder develops as a result of the leaf membrane disintegration (Felicetti and Schrader, 2009).

**Table 8. Effect of foliar spray treatments with different sources of calcium on browning (score) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	1.00 h	2.67 cd	3.33 b	4.67 a	5.00 a	3.33 A
Oxide Calcium 1%	1.00 h	1.00 h	1.00 h	2.00 ef	2.67 cd	1.53 CD
Oxide Calcium 2%	1.00 h	1.00 h	1.00 h	2.00 ef	2.33 de	1.47 CD
Chloride Calcium 1%	1.00 h	1.00 h	1.00 h	1.33 gh	2.33 de	1.33 D
Chloride Calcium 2%	1.00 h	1.00 h	1.00 h	1.00 h	1.33 gh	1.07 E
Nitrate Calcium 1%	1.00 h	1.00 h	1.00 h	2.33 de	3.33 b	1.73 B
Nitrate Calcium 2%	1.00 h	1.00 h	1.00 h	2.00 ef	3.00 bc	1.60 BC
Mean	1.00 E	1.24 D	1.33 C	2.19 B	2.86 A	
<b>2022/2023 season</b>						
Control	1.00 g	2.33 cd	3.00 b	4.33 a	4.67 a	3.07 A
Oxide Calcium 1%	1.00 g	1.00 g	1.00 g	2.00 de	2.67 cd	1.53 CD
Oxide Calcium 2%	1.00 g	1.00 g	1.00 g	2.00 de	2.67 bc	1.53 CD
Chloride Calcium 1%	1.00 g	1.00 g	1.00 g	1.67 ef	2.33 cd	1.40 D
Chloride Calcium 2%	1.00 g	1.00 g	1.00 g	1.00 g	1.33 fg	1.07 E
Nitrate Calcium 1%	1.00 g	1.00 g	1.00 g	2.33 cd	3.00 b	1.67 B
Nitrate Calcium 2%	1.00 g	1.00 g	1.00 g	2.00 de	2.67 bc	1.53 BC
Mean	1.00 D	1.19 D	1.29 C	2.19 B	2.76 A	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

There have already been reports on the potential impact of  $\text{CaCl}_2$  on the occurrence of browning in a number of plants (Carvajal *et al.*, 2000). The fact is that  $\text{CaCl}_2$  acts as a plant film stabilizer and retards the activity of PPO which reduces the occurrence of browning. It also has a significant impact on delaying modifications in the cell wall (Brummell *et al.*, 2004). Additionally, Renault (2005) said that calcium could prevent browning damage in plants by preserving the integrity of cell membranes and regulating ion transport (Picchioni *et al.*, 1995).

#### **Total soluble solids percentage:**

Data in Table (9) indicate that TSS of lettuce heads was significantly reduced with extension of storage periods. Similar findings were obtained by Abdullah *et al.* (2023) on lettuce head. The decline of TSS may result from the use of sugar in the respiratory process, which lowers TSS levels (Gol *et al.*, 2013).

$\text{CaCl}_2$  at 2% was the most successful treatment in maintaining TSS of the heads, followed by  $\text{CaCl}_2$  at 1% with significant differences between them during storage.  $\text{Ca}(\text{NO}_3)_2$  at 2% or 1% treatment was less efficient in this regard. The untreated control had the lowest TSS value. These results were true in both seasons and are consistent with Al-Hamzawi (2010) and Keshavarzpour (2012).

$\text{CaCl}_2$  treatment possibly conserve TSS by slowing down the respiration process and reducing senescence and fruit ripening (Turmanidze *et al.*, 2017) hence minimizing TSS loss during storage.

In general, the interaction between all pre-harvest treatments and storage periods was significant for the TSS in the two seasons.

#### **Ascorbic acid content:**

Data in Table (10) demonstrate that the ascorbic acid content (AsA) of lettuce heads reduced significantly as storage periods increased. Similar finding were found by Abdullah *et al.* (2023) on lettuce heads. This decrease in AsA might result from the use of AsA in the respiratory process (Hesami *et al.*, 2021). The decrease in ascorbic acid content may be attributed to ascorbate oxidase activity, which facilitates the oxidation of AsA to dehydroascorbic acid (de Siqueira Oliveira *et al.*, 2018).

All pre-harvest treatments had significantly greater ascorbic acid content in comparison to untreated heads. However,  $\text{CaCl}_2$  at 2% or 1% was the most effective treatment in maintaining ascorbic acid followed by  $\text{CaO}$  at 2% with significant differences between them. The untreated control had the lowest ascorbic acid content. These finding were true in the both seasons and consistent with Keshavarzpour (2012).

Mazumder *et al.* (2021) found that tomato fruits treated with  $\text{CaCl}_2$  had the highest levels of ascorbic acid, while control fruits had the lowest levels. Peroxidase activity in treated tomatoes reduced as ascorbate decreased the peroxidation of living cell wall polymers, which led to a more expansive cell wall and delayed metabolic activity of the fruits, extending their life (Madani *et al.*, 2016).

Keshavarzpour (2012) reported that  $\text{CaCl}_2$  treatment helps to maintain vitamin C content of lettuce. This could be explained by calcium regulation of the oxidative process in the cytosol (Haleema *et al.*, 2020). Also, calcium slowed down ascorbic acid's rapid oxidation (Turmanidze *et al.*, 2017). Applying calcium to horticultural products helps to preserve antioxidants like ascorbic acid (Luna-Guzman and Barrett, 2000), reducing the rate of respiration and moisture loss (Goodarzi, 2009) and retarding the overall ripening and senescence (Asrey and Jain, 2004), thus, it retains ascorbic acid content during storage. Also, application of calcium increased the activity of several of catalytic enzymes that were crucial to the biosynthesis of vitamins, including vitamin C (Kadir, 2005).

Concerning the impact of interaction between all pre-harvest treatments and storage periods was significant for ascorbic acid content of heads in two seasons.

#### **Total chlorophyll content:**

Data presented in Table (11) demonstrate that total chlorophyll content in lettuce heads declined dramatically as storage periods increased. Similar findings were obtained by Mohammed *et al.* (2019) on lettuce. The decline in chlorophyll content is the result of a steady rise in destruction by chlorophyll-degrading peroxidase activity, including the conversion of chloroplasts into chromoplasts by the enzyme chlorophyllase (Forney and Rij, 1991).

**Table 9. Effect of foliar spray treatments with different sources of calcium on total soluble solids (%) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	4.00 j-l	3.50 r	2.56 u	2.71 v	1.53 w	2.86 G
Oxide Calcium 1%	4.39 ef	4.14 i-k	3.85 n	3.57 p	3.16 s	3.82 D
Oxide Calcium 2%	4.54 cd	4.22 h-j	4.01 lm	3.72 o	3.33 r	3.96 C
Chloride Calcium 1%	4.64 c	4.32 f-h	4.05 kl	3.91 mn	3.56 p	4.10 B
Chloride Calcium 2%	4.95 a	4.78 b	4.46 de	4.25 g-i	3.88 n	4.46 A
Nitrate Calcium 1%	4.11 j-l	3.83 no	3.40 qr	3.05 s	2.92 t	3.46 F
Nitrate Calcium 2%	4.34 fg	4.02 k-m	3.81 no	3.31 r	3.09 s	3.71 E
Mean	4.42 A	4.12 B	3.73 C	3.50 D	3.07 E	
<b>2022/2023 season</b>						
Control	4.16 fg	3.70 no	2.86 r	2.74 t	1.90 u	3.07 G
Oxide Calcium 1%	4.54 d	4.23 g	3.86 jk	3.46 op	3.19 q	3.86 D
Oxide Calcium 2%	4.74 c	4.32 ef	3.92 j	3.69 l	3.38 p	4.01 C
Chloride Calcium 1%	4.93 b	4.37 e	4.05 i	3.92 j	3.58 mn	4.17 B
Chloride Calcium 2%	5.20 a	4.96 b	4.78 c	4.19 gh	3.92 j	4.61 A
Nitrate Calcium 1%	4.26 fg	3.94 j	3.48 o	3.03 r	2.74 s	3.49 F
Nitrate Calcium 2%	4.47 d	4.14 h	3.78 k	3.25 q	2.96 r	3.72 E
Mean	4.61 A	4.24 B	3.82 C	3.47 D	3.10 E	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

**Table 10. Effect of foliar spray treatments with different sources of calcium on ascorbic acid (mg/100g F. W.) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	21.57 s	21.11 t	19.51 u	17.73 v	14.34 w	18.85 G
Oxide Calcium 1%	24.96 l	24.47 m	23.88 o	23.32 p	22.94 q	23.91 D
Oxide Calcium 2%	25.51 j	25.17 k	24.86 l	24.24 n	23.92 o	24.74 C
Chloride Calcium 1%	27.80 e	27.57 f	27.27 g	26.96 h	26.33 i	27.19 B
Chloride Calcium 2%	29.95 a	29.44 b	29.05 c	28.75 d	27.66 ef	28.97 A
Nitrate Calcium 1%	24.31 mn	23.74 o	22.81 q	22.13 r	21.25 t	22.85 F
Nitrate Calcium 2%	24.85 l	23.79 o	22.93 q	22.14 r	21.96 r	23.14 E
Mean	25.56 A	25.04 B	24.33 C	23.61 D	22.63 E	
<b>2022/2023 season</b>						
Control	21.66 u	21.17 w	20.61 x	18.12 y	15.07 z	19.32 G
Oxide Calcium 1%	25.65 i	25.18 jk	24.32 n	23.45 p	22.65 s	24.25 D
Oxide Calcium 2%	27.02 f	26.30 h	25.32 j	24.43 mn	22.95 qr	25.20 C
Chloride Calcium 1%	27.93 d	27.44 e	26.53 g	25.09 k	24.86 l	26.37 B
Chloride Calcium 2%	30.24 a	29.81 b	28.74 c	28.07 d	27.17 f	28.81 A
Nitrate Calcium 1%	23.90 o	23.30 p	22.94 r	22.17 t	21.43 v	22.75 F
Nitrate Calcium 2%	24.56 m	23.95 o	23.12 q	22.74 s	21.64 u	23.20 E
Mean	25.85 A	25.31 B	24.51 C	23.44 D	22.25 E	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

**Table 11. Effect of foliar spray treatments with different sources of calcium on total chlorophyll content (mg/100g F.W.) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	35.69 r	33.59 s	30.51 v	29.06 w	27.37 z	31.24 G
Oxide Calcium 1%	43.24 hi	42.96 ij	42.20 k	41.79 k	39.89 m	42.02 D
Oxide Calcium 2%	44.23 ef	43.79 fg	42.68 j	41.88 k	40.83 l	42.68 C
Chloride Calcium 1%	45.77 cd	45.42 d	44.62 e	43.41 gh	41.92 k	44.23 B
Chloride Calcium 2%	48.89 a	48.57 a	47.50 b	45.94 c	44.66 e	47.11 A
Nitrate Calcium 1%	37.62 o	36.86 p	35.78 q	34.53 r	33.06 t	35.57 F
Nitrate Calcium 2%	38.48 n	37.87 o	36.88 p	35.75 q	34.44 r	36.68 E
Mean	41.99 A	41.29 B	40.02 C	38.91 D	37.45 E	
<b>2022/2023 season</b>						
Control	35.72 rs	33.91 tu	32.58 uv	30.48 w	28.30 x	32.20 G
Oxide Calcium 1%	43.70 gh	43.22 hi	42.65 ij	41.93 jk	40.77 l	42.45 D
Oxide Calcium 2%	44.80 f	44.34 fg	43.70 gh	42.46 ij	41.39 kl	43.34 C
Chloride Calcium 1%	48.76 bc	48.37 cd	47.63 d	46.70 e	44.98 f	47.29 B
Chloride Calcium 2%	51.46 a	50.98 a	50.55 a	49.44 b	48.17 cd	50.12 A
Nitrate Calcium 1%	37.65 no	37.18 op	36.18 qr	35.29 r-t	33.35 v	35.93 F
Nitrate Calcium 2%	38.84 m	38.31 mn	37.67 no	36.70 pq	34.70 st	37.24 E
Mean	42.99 A	42.33 B	41.57 C	40.43 D	38.81 E	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

The pre-harvest treatments gave the greatest significant value for total chlorophyll content in heads compared to the untreated. CaCl<sub>2</sub> at 2% had the greatest significant value for total chlorophyll content, followed by CaCl<sub>2</sub> at 1% with a significant difference between them, whereas the untreated control had the lowest ones. These results were consistent with those found by Perucka *et al.* (2013) and Youssef *et al.* (2017).

The pre-harvest application of the 0.1 M CaCl<sub>2</sub> solution to lettuce prevented the leaf blades' chlorophyll "a", "b" and total chlorophyll from degrading (Perucka *et al.*, 2013) by decrease the chlorophyllase enzyme (Ritchey *et al.*, 1995). In addition, calcium regulates vital hormone processes in aged tissues (Perucka *et al.*, 2013).

Concerning the effect of interaction between all pre-harvest treatments and storage periods was significant for total chlorophyll of lettuce heads in both seasons.

#### **Total Phenolic Content:**

Data in Table (12) indicates that total phenolic content of lettuce heads declined significantly as storage

time increased. These findings agree with those found by Abdullah *et al.* (2023) on lettuce and probably due to the PPO enzyme oxidizing to produce the colored quinones and quercetin was directly oxidized by PPO (Queiroz *et al.*, 2008). Lichanporn *et al.* (2009) discovered that phenolic compounds function as both substrates for browning reactions and antioxidants, but enzymatic oxidation continues and the quinones that are produced undergo non-enzymatic polymerization to produce a darker pigment, which explains why phenol consumption and the development of darkening during storage occur simultaneously.

The pre-harvest treatments gave the greatest significant value for total phenolic content in comparison to the untreated heads. CaCl<sub>2</sub> treatments of 2% and 1% were the most efficient with significant differences between them, followed by CaO treatments of 2% and 1%, with significant differences between them in both seasons. The least values were obtained from untreated heads after 20 days in both seasons. These findings agreed with EL-Sayed *et al.* (2023).

**Table 12. Effect of foliar spray treatments with different sources of calcium on total phenolic (mg/100g F.W.) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	27.90 r	26.12 st	24.70 u	23.11 v	21.14 w	24.59 G
Oxide Calcium 1%	35.54 g-i	34.57 i	33.45 j	32.18 k-m	31.45 m-o	33.44 D
Oxide Calcium 2%	36.25 fg	35.64 gh	34.69 hi	33.59 j	32.62 j-l	34.56 C
Chloride Calcium 1%	38.28 bc	37.93 b-d	37.26 de	36.66 ef	35.83 fg	37.19 B
Chloride Calcium 2%	40.43 a	40.37 a	39.78 a	38.75 b	37.72 cd	39.41 A
Nitrate Calcium 1%	30.87 no	29.37 p	28.00 qr	26.53 s	25.17 tu	27.99 F
Nitrate Calcium 2%	32.96 jk	31.76 l-n	30.48 o	28.90 pq	27.67 r	30.35 E
Mean	34.60 A	33.68 B	32.62 C	31.39 D	30.23 E	
<b>2022/2023 season</b>						
Control	28.22 rs	26.67 tu	25.34 v	23.82 w	21.97 x	25.20 G
Oxide Calcium 1%	36.97 hi	36.09 ij	35.06 jk	33.83 l	33.15 lm	35.02 D
Oxide Calcium 2%	37.67 f-h	37.12 g-i	36.22 ij	35.16 jk	34.24 kl	36.08 C
Chloride Calcium 1%	39.64 cd	39.33 c-e	38.73 d-f	38.23 e-g	37.44 gh	38.67 B
Chloride Calcium 2%	41.83 a	41.55 a	41.24 ab	40.24 bc	39.23 c-e	40.82 A
Nitrate Calcium 1%	31.36 no	29.92 pq	28.61 r	27.20 st	25.87 uv	28.59 F
Nitrate Calcium 2%	33.25 lm	32.16 mn	30.96 op	29.41 qr	28.25 rs	30.81 E
Mean	35.56 A	34.69 B	33.74 C	32.56 D	31.45 E	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

CaCl<sub>2</sub> increases cell wall strength as well as plasma membrane permeability, this also slows down the aging process and reduces polyphenol oxidase activity (Torres *et al.*, 2010) resulted in retention of phenolic contents (Abbasi *et al.*, 2020).

#### Polyphenol Oxidase Activity (PPO):

Data in Table (13) indicate that PPO activity of lettuce heads increased significantly with the extension of storage period. These findings agree with those found by Abdullah *et al.* (2023) on lettuce. The increase of PPO activity throughout storage is mainly due to transformation of a latent form into a fully active one (Abdullah and Zakaria, 2024). Actually, as Cantos *et al.* (2001) discovered, demineralization entails the removal of minerals from crucial cellular components followed by the release of proteases, which sets off a series of reactions that activate the latent PPO.

Pre-harvest treatments decreased PPO activity throughout storage in comparison to the untreated heads and CaCl<sub>2</sub> at 2% and 1% were the most efficient treatments in delaying activity with significant

differences between them, followed by 2% CaO treatment. However, the 2% or 1% Ca(NO<sub>3</sub>)<sub>2</sub> was less efficient treatment in this case, whereas PPO enzyme activity increased more in the untreated control treatment over both seasons. These findings are agree with those reported by EL-Sayed *et al.* (2023).

Little PPO activity appeared in treatment with 2% CaCl<sub>2</sub> due to the role of CaCl<sub>2</sub> in metabolic activities, such as stabilizing cell membranes and controlling the activity of enzyme, like mitigating the harmful effects of PPO activity (Arshi *et al.*, 2006). Thus, it was proposed that 2% CaCl<sub>2</sub> would protect cell membrane disintegration from the browning reaction's impacts. These are consistent with the findings of Raese and Drake (2002) who found that the best formulations to increase the calcium concentration were those that contained CaCl<sub>2</sub>. Additionally, it is thought that CaCl<sub>2</sub> stimulates ionic homeostasis in plant cells and caused adverse conditions for activity of chromogenic enzyme (Beirao-da-Costa *et al.*, 2008).

**Table 13. Effect of foliar spray treatments with different sources of calcium on polyphenol oxidase activity (mg/100g F.W.) of lettuce heads during storage at 0°C in 2021/2022 and 2022/2023 seasons**

Treatments	Storage Period (days)					Mean
	0	5	10	15	20	
<b>2021/2022 season</b>						
Control	41.03 f	44.57 e	49.30 c	53.93 b	58.77 a	49.52 A
Oxide Calcium 1%	30.35 pq	31.86 no	33.67 lm	35.47 jk	37.43 h	33.76 D
Oxide Calcium 2%	29.57 qr	30.91 op	32.66 mn	34.36 kl	36.05 ij	32.71 E
Chloride Calcium 1%	25.43 u	25.93 tu	27.55 s	29.10 r	30.51 pq	27.70 F
Chloride Calcium 2%	22.33 w	22.74 w	24.15 v	25.40 u	26.76 st	24.28 G
Nitrate Calcium 1%	36.28 h-j	38.93 g	41.42 f	44.11 e	46.62 d	41.47 B
Nitrate Calcium 2%	34.44 kl	36.93 hi	38.93 g	41.32 f	43.64 e	39.05 C
Mean	31.35 E	33.12 D	35.38 C	37.67 B	39.97 A	
<b>2022/2023 season</b>						
Control	40.10 f	43.63 e	48.06 c	52.60 b	57.30 a	48.34 A
Oxide Calcium 1%	30.11 mn	31.52 l	33.24 k	34.94 j	36.86 h	33.33 D
Oxide Calcium 2%	29.12 no	30.34 m	32.03 l	33.63 k	35.14 ij	32.05 E
Chloride Calcium 1%	25.20 qr	25.60 qr	27.11 p	28.64 o	30.02 mn	27.31 F
Chloride Calcium 2%	22.14 t	22.47 t	23.72 s	24.84 r	26.13 pq	23.86 G
Nitrate Calcium 1%	36.18 hi	38.67 g	41.04 f	43.56 e	46.02 d	41.09 B
Nitrate Calcium 2%	34.12 jk	36.51 h	38.45 g	40.81 f	43.08 e	38.59 C
Mean	31.00 E	32.68 D	34.81 C	37.00 B	39.22 A	

The values that contain the same capital or small letters in the same columns and rows indicate that there are no significant variations between each other at level 0.05.

## CONCLUSION

From the previous results, it could be concluded that lettuce heads cv. Iceberg obtained from plants sprayed with CaCl<sub>2</sub> at 2% increase the physical properties (head compactness, head firmness, and head dry weight) and chemical properties (ascorbic acid, total chlorophyll content, and total phenolic compounds) and reduce activity of PPO enzyme and browning% at harvest, and enhanced storability, which preserved head quality attributes, and gave excellent appearance of heads after 20 days of storage at 0 °C without any decay.

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## الملخص العربي

### تأثير الرش الورقي بمصادر مختلفة من الكالسيوم على التلون البني والجودة والقدرة التخزينية لمحصول الخس

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الكيميائية (المحتوى من حمض الأسكوربيك، ومحتوى الكلوروفيل الكلي، ومحتوى الفينولات الكلية) وأيضاً أعطت أقل القيم من نسبة التلون البني للأوراق عند الحصاد كما أدت الى زيادة القدرة التخزينية للرؤوس حيث أعطت أقل قيمة للفق في الوزن، والتلون البني، ونشاط انزيم البولي فينول أكسيداز PPO، والمحافظة على صلابة الرؤوس، ونسبة المواد الصلبة الذائبة الكلية، والمحتوى من حمض الأسكوربيك، ومحتوى الكلوروفيل الكلي، ومحتوى الفينولات الكلية خلال فترات التخزين، وأعطت مظهرًا ممتازًا للرؤوس بدون أي تالف بعد ٢٠ يوم من التخزين على صفر درجة مئوية.

**الكلمات الدالة:** الخس، كلوريد الكالسيوم، نترات الكالسيوم، أكسيد الكالسيوم، الجودة، القدرة التخزينية.

أجريت هذه الدراسة لتقييم تأثير الرش الورقي المحتوي على مصادر مختلفة من الكالسيوم (كلوريد الكالسيوم، نترات الكالسيوم، وأكسيد الكالسيوم) بتركيزين مختلفين (١% و ٢%) على الخس (صنف آيسبرج). أجريت الدراسة خلال الموسم الشتوي (٢٠٢١-٢٠٢٢ و ٢٠٢٢-٢٠٢٣)، وتم المقارنة بين النباتات المعاملة والنباتات غير المعاملة الكنترول (الرش بالماء فقط) على المحصول وخصائص الجودة والقدرة التخزينية لرؤوس الخس. أشارت النتائج إلى أن النباتات المعاملة بنترات الكالسيوم بتركيز ١% أو ٢% كانت المعاملة الأكثر فاعلية لتحسين المحصول الكلي ومتوسط وزن الرأس، وعدد الأوراق/الرأس. ومع ذلك، فإن معاملة النباتات بكلوريد الكالسيوم بتركيز ٢% أدت الى تحسين الصفات الطبيعية للرأس (اندماج الرأس، والصلابة، والوزن الجاف) والصفات