

## Effect of Chemical Materials Application and Storage Periods on Vitamin C of Lettuce During Cold Storage

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**Abstract:** This study was conducted on the effect of chemical materials application (CMA) and storage periods (STP) on vitamin C of Iranian white lettuce during cold storage at temperature of 2°C and 90% relative humidity. Four CMA (calcium chloride, citric acid, acetic acid and no chemical material application as control) and five STP (0, 7, 14 21 and 28 days) were investigated. The statistical results of the study indicated that CMA and STP significantly ( $P \leq 0.01$ ) affected vitamin C. Interaction of CMA  $\times$  STP for vitamin C was also significant ( $P \leq 0.01$ ). Results of the study indicated that calcium chloride was the best CMA for preserving vitamin C of lettuce during cold storage. In addition, vitamin C of lettuce decreased by increasing STP.

**Key words:** Lettuce • Vitamin C • Cold storage • Calcium chloride • Citric acid • Acetic acid • Storage period

### INTRODUCTION

The lettuce (*Lactuca sativa*) is an annual plant of the aster or sunflower family Asteraceae. It is most often grown as a leaf vegetable, but also sometimes for its stem and seeds. Lettuce is easily cultivated, although it requires relatively low temperatures to prevent it from quickly flowering [1]. It is most often used for salads, although it is also seen in other kinds of food, such as soups, sandwiches and wraps. One type is grown for its stems, which are eaten either raw or cooked. Lettuce is a good source of vitamin A and potassium, as well as a minor source for several other vitamins and nutrients and 100 g of lettuce contains 13 cal energy, 2.2 g carbohydrates, 1.1 g dietary fiber, 0.2 g fat, 1.4 g protein, 96 g water, 166 µg vitamin A, 73 µg folate (vitamin B<sub>9</sub>), 4 mg vitamin C, 102 µg vitamin K, 1.2 mg iron and 238 mg potassium [2, 3]. The top ten lettuce producers are China (12,574,500 tons), United States (3,954,800 tons), India (998,600 tons), Italy (843,344 tons), Spain (809,200 tons), Japan (537,800 tons), Iran (402,800 tons), France (398,215 tons), Turkey (358,096 tons), Mexico (340,976 tons). Although Iran produces 402,800 tons

lettuce and is ranked 7<sup>th</sup> in the world, Iranian lettuce are not exported due to the product's short shelf life and quality decline [4].

The best methods that are being used to preserve fruits and vegetables during storage and marketing are generally based on refrigeration with or without control of composition of the atmosphere [5, 6]. However, temperature, atmosphere, relative humidity and sanitation must be regulated to maintain quality of them [7, 8]. In this direction, several methods that have been used are refrigeration, controlled atmosphere packaging, modified atmosphere packaging and chemical preservatives [9-11]. The most prevalent method is rapid cooling at a low temperature with high relative humidity [12]. However, low temperature storage is not economically feasible in most developing countries [6, 13]. To ensure high quality of freshly cut vegetables, preserving the correct coloring and preventing the browning of tissues, a variety of processes are employed. The plants are subjected to various treatments during their growth or after harvest. Such processes comprise mainly biochemical transformations related with the occurrence of plant browning [14, 15]. One of the causes for leaf browning is

a deficit of calcium ions. The most effective method is foliar supplementation of this element. Among the calcium compounds that are used for plant spraying, calcium chloride ( $\text{CaCl}_2$ ) is absorbed the best [16].

Calcium chloride is a salt of calcium and chloride. It behaves as a typical ionic halide and is solid at room temperature. It can serve as a source of calcium ions in a solution, as it is soluble. Calcium chloride can be produced directly from limestone, but large amount are also produced as a byproduct of the Solvay process. As an ingredient, it is listed as a permitted food additive in the European Union for use as a sequestrant and firming agent with the E number E509 and considered as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration. As a firming agent, calcium chloride is used in canned vegetables, in firming soybean curds into tofu and in producing a caviar substitute from vegetable or fruit juices. It is commonly used as an electrolyte in sport drinks and other beverages, including bottle water. The extremely salty taste of calcium chloride is used to flavor pickles while not increasing the food's sodium content. Also, it is frequently added to sliced apples to maintain texture. Calcium chloride can act as an irritant by desiccating moist skin [1]. It has been found that a high content of calcium in fruits reduces the rate of respiration and delays ageing [17]. Earlier studies showed that foliar application of calcium chloride solution on plants of sweet and hot peppers at the seedling stage caused accelerated ripening of the fruits but did not have any significant effect on the level of vitamins [16].

Citric acid is a weak organic acid with the chemical formula  $\text{C}_3\text{H}_4\text{OH}(\text{COOH})_3$ . It is a natural preservative/conservative and is also used to add an acidic, or sour, taste to foods and soft drinks. Citric acid is a commodity chemical and more than a million tons are produced every year by fermentation. It is used mainly as an acidifier, as a flavoring and as a chelating agent. The dominant use of citric acid is as a flavoring and preservative in food and beverages, especially soft drinks. Within the European Union it denoted by E number E330. Citric acid can be added to ice cream as an emulsifying agent to keep fats from separating, to caramel to prevent sucrose crystallization, or to recipes in place of fresh lemon juice. Citric acid is also often used in cleaning products and sodas or fizzy drinks [1]. Acetic acid is another organic compound with the chemical formula  $\text{CH}_3\text{COOH}$ . It is the main component of vinegar and has a distinctive sour taste and pungent smell. Although acetic acid is classified as a weak acid, concentrated acetic acid

is corrosive to skin and must, therefore, be handled with appropriate care, since it can cause burns, permanent eye damage and irritation to the mucous membranes [1].

In this paper, the effects of chemical materials application (CMA) and storage periods (STP) on vitamin C of Iranian white lettuce during cold storage at temperature of  $2^\circ\text{C}$  and 90% relative humidity are reported.

## MATERIALS AND METHODS

**Experimental Material:** The experimental material was Iranian white lettuce. Lettuces were purchased from a green house in Varamin, Iran.

**Experimental Method:** A split plot experiment was laid out in a randomized complete block design (RCBD) with three replications to randomize the chemical materials application (CMA) and storage periods (STP) in the main and sub-plots, respectively. The experiment comprised of four CMA (calcium chloride, citric acid, acetic acid and no chemical material as control) and five STP (0, 7, 14, 21 and 28 days) at temperature of  $2^\circ\text{C}$  and 90% relative humidity.

**Calcium Chloride Application:** Lettuces were sprayed with aqueous solution of calcium chloride ( $10 \text{ g L}^{-1}$ ) after three and five weeks from planting in the green houses. After harvesting, they were visually inspected for freedom of defects and blemishes. Lettuces were then washed with tap water and then air dried for approximately 15 minutes. After that, they were individually wrapped with cellophane film and stored at temperature of  $2^\circ\text{C}$  and 90% relative humidity.

**Citric Acid Application:** Lettuces were visually inspected for freedom of defects and blemishes after harvesting. They were then washed with tap water, placed in a 50-liter plastic bucket and soaked for 5 minutes at  $20^\circ\text{C}$  in  $10 \text{ g L}^{-1}$  aqueous solution of citric acid. After that, lettuces were air dried for approximately 15 minutes, individually wrapped with cellophane film and stored at temperature of  $2^\circ\text{C}$  and 90% relative humidity.

**Acetic Acid Application:** Lettuces were visually inspected for freedom of defects and blemishes after harvesting. After that, they were washed with tap water, placed in a 50-liter plastic bucket and soaked for 5 minutes at  $20^\circ\text{C}$  in  $10 \text{ g L}^{-1}$  aqueous solution of acetic acid. Then, lettuces were air dried for approximately 15 minutes, separately wrapped with cellophane film and stored at temperature of  $2^\circ\text{C}$  and 90% relative humidity.

**No Chemical Material Application:** Lettuces were visually inspected for freedom of defects and blemishes after harvesting. They were only washed with tap water and then air dried for approximately 15 minutes. After that, lettuces were placed in the polyethylene boxes and stored at temperature of 2°C and 90% relative humidity.

**Vitamin C (Ascorbic Acid):** The vitamin C of lettuces was determined with a redox titration. Redox titration (also called oxidation-reduction titration) is a type of titration based on a redox reaction between the analyte and titrant. The redox reaction is better than an acid-base titration since there are additional acids in a juice, but few of them interfere with the oxidation of ascorbic acid by iodine. Iodine is relatively insoluble, but this can be improved by complexing the iodine with iodide to form triiodide ( $I_2 + I^- \rightarrow I_3^-$ ). Triiodide oxidizes vitamin C to form dehydroascorbic acid ( $C_6H_8O_6 + I_3^- + H_2O \rightarrow C_6H_6O_6 + 3I^- + 2H^+$ ). As long as vitamin C is present in the solution, the triiodide is converted to the iodide ion very quickly. However, when the all the vitamin C is oxidized, iodine and triiodide will be present, which react with starch to form a blue-black complex. The blue-black color is the endpoint of the titration. This titration procedure is appropriate for testing the amount of vitamin C in vitamin C tablets, juices and fresh, frozen, or packaged fruits and vegetables. The titration can be performed using just iodine solution and not iodate, but the iodate solution is more stable and gives a more accurate result [1].

**Data Analysis:** The data were subjected to analysis of variance (ANOVA) using MSTAT-C statistical software. Moreover, the means of different treatments were separated by Duncan's Multiple Range Test (DMRT) at 1% probability level.

## RESULTS AND DISCUSSION

The effect of CMA and STP on vitamin C was found significant (Table 1). The highest vitamin C of 8.259 mg/100g was observed in the first CMA (calcium chloride application) and lowest (7.369 mg/100g) in the no chemical material application and CMA affected vitamin C in the order of calcium chloride > acetic acid > citric acid > no chemical material application (Table 2). Moreover, the highest vitamin C of 10.09 mg/100g was observed in 0 days STP and lowest (6.944 mg/100g) in 28 days STP and vitamin C decreased by increasing STP (Table 2). Furthermore, interaction of CMA × STP showed significant effect on vitamin C (Table 1).

Table 1: Analysis of variance for vitamin C of cold stored lettuce

Source of variation	Df	Mean square Vitamin C
Chemical Materials Application (CMA)	3	2.277 **
Storage Period (STP)	4	22.45 **
CMA × STP	12	0.198 **
Error	32	0.001
C.V. (%)	---	0.32

\*\* = Significant at 0.01 probability level

Table 2: Means comparison for vitamin C of cold stored lettuce under different treatments using DMRT at 1% probability level

	Treatment	Vitamin C (mg/100g)
CMA	Calcium chloride	8.259 a
	Citric acid	7.983 c
	Acetic acid	8.103 b
	No chemical materials	7.369 d
STP	0 days	10.09 a
	7 days	8.487 b
	14 days	7.007 d
	21 days	7.112 c
	28 days	6.944 e

Means in the same column with different letters differ significantly at 0.01 probability level according to DMRT

Table 3: Means comparison for vitamin C of cold stored lettuce under different combinations of Chemical Materials Application (CMA) and Storage Period (STP) using DMRT at 1% probability level

	CMA × STP	Vitamin C (mg/100g)
Calcium chloride	0 days	10.09 a
	7 days	8.667 b
	14 days	7.500 ef
	21 days	7.593 e
	28 days	7.443 fg
Citric acid	0 days	10.09 a
	7 days	8.510 c
	14 days	7.097 ij
	21 days	7.207 h
	28 days	7.007 j
Acetic acid	0 days	10.09 a
	7 days	8.653 b
	14 days	7.227 h
	21 days	7.357 g
	28 days	7.183 hi
No chemical material	0 days	10.09 a
	7 days	8.117 d
	14 days	6.203 kl
	21 days	6.290 k
	28 days	6.143 l

Means in the same column with different letters differ significantly at 0.01 probability level according to DMRT

The study of CMA and STP combinations on vitamin C showed that in each CMA, vitamin C had the highest value in 0 days STP and lowest value in 28 days STP. The maximum mean value for vitamin C (10.09 mg/100g) was observed in 0 days STP of all CMA and minimum mean value for vitamin C (6.143 mg/100g) was observed in 28 days STP of no chemical material application. Also, in each STP, CMA affected vitamin C in the same order as mentioned before, i.e. calcium chloride > acetic acid > citric acid > no chemical material application (Table 3). These results are in line with the results reported by Conway and Sams [18] and Poovaiah [19] that calcium chloride treatment helps to increase vitamin C content of apple. These results are also in agreement with those of El-Hammady *et al.* [20] who confirmed the positive effects of calcium chloride on vitamin C content of citrus fruits. However, these results are not in line with the results reported by Perucka & Olszowka [16] that foliar application of calcium chloride solution on plants of sweet and hot peppers at the seedling stage did not have any significant effect on the level of vitamin C.

### CONCLUSION

Chemical materials application (CMA) and storage periods (STP) significantly ( $P \leq 0.01$ ) affected vitamin C of Iranian white lettuce during cold storage at temperature of 2°C and 90% relative humidity. Results of the study indicated that calcium chloride was the best CMA for preserving vitamin C of lettuce during cold storage. In addition, vitamin C decreased by increasing STP for cold stored lettuce.

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