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Effects of Chemical Materials Application and Storage Periods on Quality of Ambient Stored Lettuce

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Abstract: This study was conducted on the effects of chemical materials application (CMA) and storage periods (STP) on Iranian white lettuce during ambient storage at temperature of 25°C and 65% relative humidity. Four CMA (calcium chloride, citric acid, acetic acid and no chemical material application as control) and five STP (0, 2, 5, 7 and 10 days) were investigated for some quality characteristics including water content, total soluble solids (TSS) and vitamin C. The statistical results of the study indicated that CMA and STP significantly (P # 0.01) affected all quality characteristics. Interaction of CMA × STP for all studied quality characteristics was also significant (P # 0.01). Results of the study indicated that calcium chloride was the best CMA for preserving water content, TSS and vitamin C of lettuce during ambient storage. In addition, water content, TSS and vitamin C of lettuce decreased by increasing STP.

Key words: Lettuce % Ambient storage % Chemical materials % Storage periods % Quality % Iran

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 was first cultivated by the ancient Egyptians, who turned it from a weed whose seeds were used to make oil into a plant grown for its leaves. Lettuce is easily cultivated, although it requires relatively low temperatures to prevent it from quickly flowering. It can be plagued with numerous nutrient deficiencies, as well as insect and mammal pests and fungal and bacterial diseases [1]. Lettuce 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_9), 4 mg vitamin C, 102 µg vitamin K, 1.2 mg iron and 238 mg potassium [2, 3]. World production of lettuce for calendar year 2010 stood at 23,620,000 tons, over half of which came from China. The top ten lettuce producers for the mentioned calendar year 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^{th} 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

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[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 ($CaCl_2$) 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. Calcium chloride 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 wit 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, 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 $C_3H_4OH\ (COOH)_3$. 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 an organic compound with the chemical formula CH_3COOH. It is a colorless liquid that when undiluted is also called glacial acetic acid. Acetic acid is the main component of vinegar (apart from water; vinegar is roughly 8% acetic acid by volume) and has a distinctive sour taste and pungent smell. Although it 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. These burns or blisters may not appear until hours after exposure [1].

In this paper, the effects of chemical materials application (CMA) and storage periods (STP) on some quality characteristics of Iranian white lettuce including water content, total soluble solids (TSS) and vitamin C during ambient storage at temperature of 25°C and 65% 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, 2, 5, 7 and 10 days) at temperature of 25°C and 65% relative humidity.

Calcium Chloride Application: Only lettuces of this treatment were sprayed with aqueous solution of calcium chloride (10 g LG^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 25°C and 65% 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°C in 10 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 25°C and 65% relative humidity.

Acetic Acid Application: Again, 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°C in 10 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 25°C and 65% 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 25°C and 65% relative humidity.

Water Content Determination: The water content of lettuces was determined using the following formula:

$$\text{Water content (\%)} = 100 \times (M_1 - M_2)/M_1$$

where:

M_1 = Mass of sample before drying (g)

M_2 = Mass of sample after drying (g)

Total Soluble Solids (TSS) Measurement: The total soluble solids of lettuces (TSS) was measured using an ATC-1E hand-held refractometer (ATAGO, Japan) at temperature of 20°C.

Ascorbic Acid (Vitamin C) Determination: 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 ($I_2 + I^- \rightleftharpoons I_3^-$). 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

Water Content: CMA and STP significantly affected water content (Table 1). The highest water content of 94.32% was observed in the first CMA (calcium chloride application) and lowest (91.51%) in the no chemical material application and CMA affected water content in the order of calcium chloride > citric acid > acetic acid > no chemical material application (Table 2). Moreover, the highest water content of 96.15% was observed in 0 days STP and lowest (90.67%) in 10 days STP and water content decreased with increased STP (Table 2). Furthermore, interaction of CMA × STP showed significant effect ($P \leq 0.01$) on water content (Table 1). The study of CMA and STP combinations on water content showed that in each CMA, water content had the highest value in 0 days STP and lowest value in 10 days STP. The maximum mean value for water content (96.15%) was observed in 0 days STP of all CMA and minimum mean value for water content (86.17%) was observed in 10 days STP of no chemical material application. Also, in each STP, CMA affected water content in the same order as mentioned before, i.e. calcium chloride > citric acid > acetic acid > no chemical material application (Table 3). These results are in agreement with those of Izumi and Watada [18] who concluded that water loss of carrots substantially decreased when they were treated with calcium chloride solution and the differential between control and the treated carrots was greater at the extended

Table 1: Analysis of variance for quality characteristics of ambient stored lettuces

		Mean square		
Source of variation	Df	Water content	TSS	Vitamin C
Chemical materials application (CMA)	3	28.33 **	0.534 **	0.316 **
Storage period (STP)	4	57.10 **	1.231 **	12.47 **
CMA × STP	12	4.450 **	0.134 **	0.030 **
Error	32	0.028	0.000	0.001
C.V. (%)	---	0.18	0.56	0.26

** = Significant at 0.01 probability level

Table 2: Means comparison for quality characteristics of ambient stored lettuces under different treatments using DMRT at 1% probability level

	Treatment	Water content (%)	TSS (%)	Vitamin C (mg/100g)
CMA	Calcium chloride	94.32 a	4.037 a	9.025 a
	Citric acid	94.29 a	3.902 b	8.917 b
	Acetic acid	94.14 a	3.883 b	9.007 a
	No chemical material	91.51 b	3.589 c	8.709 c
STP	0 days	96.15 a	4.107 b	10.09 a
	2 days	95.09 b	4.173 a	9.605 b
	5 days	93.63 c	3.903 c	9.017 c
	7 days	92.28 d	3.688 d	8.336 d
	10 days	90.67 e	3.391 e	7.520 e

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

Table 3: Means comparison for quality characteristics of ambient stored lettuces under different combinations of chemical materials application (CMA) and storage period (STP) using DMRT at 1% probability level

CMA ×	STP	Water content (%)	TSS (%)	Vitamin C (mg/100g)
Calcium chloride	0 days	96.15 a	4.107 b	10.09 a
	2 days	95.33 ab	4.173 a	9.663 b
	5 days	94.33 bcd	4.063 b	9.143 d
	7 days	93.42 cde	3.977 c	8.530 g
	10 days	92.38 efg	3.863 e	7.693 j
Citric acid	0 days	96.15 a	4.107 b	10.09 a
	2 days	95.35 ab	4.163 a	9.620 b
	5 days	94.40 bc	3.943 cd	9.023 e
	7 days	93.36 cdef	3.773 f	8.333 h
	10 days	92.20 fg	3.523 h	7.513 k
Acetic acid	0 days	96.15 a	4.107 b	10.09 a
	2 days	95.26 ab	4.163 a	9.670 b
	5 days	94.15 bcd	3.927 d	9.133 d
	7 days	93.20 def	3.750 f	8.497 g
	10 days	91.93 g	3.467 i	7.643 j
No chemical material	0 days	96.15 a	4.107 b	10.09 a
	2 days	94.43 bc	4.193 a	9.467 c
	5 days	91.64 g	3.680 g	8.770 f
	7 days	89.15 h	3.253 j	7.983 i
	10 days	86.17 i	2.710 k	7.230 l

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

STP. These results are also in line with the results reported by El-Hammady *et al*. [19] that application of calcium chloride reduced water loss of citrus fruits. This might be due to the fact that the anhydrous hygroscopic $CaCl_2$ salt, in atmosphere of high relative humidity, has the ability of absorbing water. These results are also in agreement with those of Smith and Stow [5], Baldwin *et al*. [10], El Ghaouth *et al*. [20], Bahri and Rashidi [21], Rashidi and Bahri [22] and Rashidi *et al*. [23] who concluded that water loss significantly increased with increased STP.

Total Soluble Solids (TSS): The effect of CMA and STP on TSS was found significant (Table 1). The highest TSS of 4.037% was observed in the first CMA (calcium chloride application) and lowest (3.589%) in the no chemical material application and CMA affected TSS in the order of calcium chloride > citric acid > acetic acid > no chemical material application (Table 2). Moreover, the highest TSS of 4.173% was observed in 2 days STP and lowest (3.391%) in 10 days STP and TSS decreased with increased STP (Table 2). Furthermore, interaction of CMA × STP showed significant effect on TSS (Table 1). Mean comparison of CMA × STP combinations on TSS revealed that in each CMA, TSS had the highest value in 2 days STP and lowest value in 10 days STP. The maximum mean value for TSS (4.193%) was observed in 2 days STP and no chemical material application and the minimum mean value for TSS (2.710%) was observed in 10 days STP and no chemical material application. Also, in each STP, CMA affected TSS in the same order as mentioned before, i.e. calcium chloride > citric acid > acetic acid > no chemical material application (Table 3). These results are in agreement with those of Mahajan and Sharma [24] who concluded that application of $CaCl_2$ solutions on peach plants at pit hardening stage of the fruits significantly increased TSS of the fruits. However, these results are not in line with the results reported by Bahri and Rashidi [21], Rashidi and Bahri [22], Rashidi *et al*. [23], Park *et al*. [25, 26] and Hussain *et al*. [27] that TSS significantly increased by increasing STP for carrot, tomato and mango, respectively. This might be due to the fact that TSS of lettuce initially increased as a result of reducing water content of lettuce during 2 days STP and subsequently decreased because of starting sugar fermentation in lettuce after 2 days STP.

Vitamin C: The effect of CMA and STP on vitamin C was also found significant (Table 1). The highest vitamin C of 9.025 mg/100g was observed in the first CMA (calcium chloride application) and lowest (8.709 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 (7.520 mg/100g) in 10 days STP and vitamin C decreased with increased STP (Table 2). Furthermore, interaction of CMA × STP showed significant effect on vitamin C (Table 1). 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 10 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 (7.230 mg/100g) was observed in 10 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 [28] and Poovaiah) [29] 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*. [19] 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 and 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.

CONCLUSIONS

Chemical materials application (CMA) and storage periods (STP) significantly (P#0.01) affected water content, total soluble solids (TSS) and vitamin C of Iranian white lettuce during ambient storage at temperature of 25°C and 65% relative humidity. Results of the study indicated that calcium chloride was the best CMA for preserving water content, TSS and vitamin C of lettuce during ambient storage. In addition, water content, TSS and vitamin C of lettuce decreased by increasing STP.

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