DISSERTATION

EFFECTS OF ASCORBIC ACID APPLIED BY TWO HYDROCOOLING METHODS ON PHYSICAL, CHEMICAL AND SENSORY ATTRIBUTES OF GREEN LEAF LETTUCE STORED AT 5 °C

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JUAN RAMON ESPARZA RIVERA ENTITLED EFFECTS OF ASCORBIC ACID APPLIED BY TWO HYDROCOOLING METHODS ON PHYSICAL, CHEMICAL AND SENSORY ATTRIBUTES OF GREEN LEAF LETTUCE STORED AT 5 °C BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

EFFECTS OF ASCORBIC ACID APPLIED BY TWO HYDROCOOLING METHODS ON PHYSICAL, CHEMICAL AND SENSORY ATTRIBUTES OF GREEN LEAF LETTUCE STORED AT 5 °C

One of the most popular vegetables around the world is lettuce (Lactuca sativa L.). In recent years, the consumption of this vegetable has increased notably, making it one of the most preferred vegetable products in the U.S. However, the quality and shelf life of lettuce are limited by detrimental textural changes (flaccid tissue and reduced turgidity) and browning. Textural changes are caused mostly by dehydration, resulting in a decrease in turgor pressure within cells as well as cellular wall degradation. Browning in lettuce is caused by the production of melanins (dark pigments) from the oxidation of phenolic compounds. These negative changes mainly occur during post harvesting stages (processing and storage). Hence, the implementation of preventive measures to maintain produce quality and nutritional value is necessary. Two treatments used to retard lettuce deterioration include hydrocooling, a procedure used to remove heat from freshly harvested commodities, and the use of antioxidants or reducing agents such as ascorbic acid, which is an alternative to the use of sulfites as a browning inhibitory agent in raw produce.

The effects of ascorbic acid applied by two hydrocooling methods on physical, chemical and sensory attributes of fresh cut leaf lettuce during storage at 5 °C were evaluated. Waldmann's dark green lettuce heads were grown in Fort Collins, CO, and harvested manually. Four harvest lots of 55 lettuce heads each were obtained and randomly distributed across 5 treatments, with 11 lettuce heads per treatment. Lettuce heads were treated within 4 hours of harvesting. Treatments included 1) immersion in, or 2) spraying with 1% ascorbic acid solution, 3) immersion in, or 4) spraying with tap water, and 5) control (untreated). Treatment solutions for immersion or spraying were applied at 5 °C for 2 min. After treatment application, lettuce heads were packaged in moisture impermeable polyethylene bags and stored at 5 °C for up to 21 d. Analytical assays included total ascorbate content, Trolox equivalent antioxidant capacity ($ABTS^+$ radical cation assay), total phenolic content (Folin-Ciocalteu method), instrumental color (L^*, a^* and b^* values), texture measurement using TA-XT 2 Texture analyzer, relative water content and a consumer sensory evaluation. All analytical assays were completed at 5 time intervals: before treatment application, and after 1, 7, 14 and 21 days of storage at 5 °C. Sensory evaluation panels were conducted on the fourth harvest at 3 storage time intervals: on days 1, 7 and 14 of storage at 5 °C, with a minimum of 30 panelists per session. Lettuce sensory attributes evaluated included appearance, color intensity, flavor, texture, bitterness, tartness and overall acceptability. Sensory attributes were rated using a 15-cm semi-structured scale with 0 = lowest score for the attribute and 15 = highest score for the attribute. Data analyses for all variables were performed using 2-way Analysis of Variance. Differences between means were determined using Least Significant Differences ($P < 0.05$).

Hydrocooling of leaf lettuce by immersion or spraying using 1% ascorbic acid solution increased total ascorbate content for up to 7 days, with an increase of more than 300% in total ascorbate content on day 1 of storage time compared to its initial value before treatments. Still, for all treatments the total ascorbate content of leaf lettuce decreased over time. Hydrocooling by immersion or spraying using cold water or 1% ascorbic acid solution did not prevent post harvest decreases in total ascorbate content in leaf lettuce. Antioxidant capacity of leaf lettuce did not vary among treatments throughout the study. It is possible that the antioxidant capacity of this lettuce variety is based mostly in compounds other than vitamin C, such as phenolics. These results imply that the increase in total ascorbate content in treatments using ascorbic acid solution was not sufficient to affect the antioxidant capacity of leaf lettuce. Hydrocooling with ascorbic acid by immersion increased the total phenolic content of leaf lettuce for up to 7 days, but this could be due to the known interference that ascorbic acid has on the Folin-Ciocalteu assay. Ascorbic acid immersed lettuce was the only treatment that maintained its relative water content throughout the 21 days of storage. Ascorbic acid immersion treatment held the percentage of relative water content in leaf lettuce during the entire experiment, thereby contributing to a firmer texture in leaf lettuce for a longer storage time.

Lettuce hydrocooled by spraying with 1% ascorbic acid increased in sensory firmness and became less bitter over the storage time, while hydrocooling by immersion using 1% ascorbic acid negatively affected lettuce appearance and resulted in a darker color over storage time. Water spraying was the treatment for lettuce with the least acceptable appearance and overall acceptability on day 14 of storage time. Further

experimentation with the application of ascorbic acid during hydrocooling is needed to fully assess its usefulness in maintaining the quality of leaf lettuce during storage.

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CHAPTER I

INTRODUCTION

One of the most popular vegetables around the world is lettuce *(Lactuca sativa L.)*, which is frequently used in green salads. In recent years, the consumption of this vegetable has increased notably, making it one of the most preferred vegetable products in the United States. However, quality and shelf life of minimally processed lettuce are usually limited by the development of detrimental texture changes (flaccid tissue and reduced turgidity) as well as browning. Browning in lettuce is a quality problem that has been linked to the enzymatic activity of phenylalanine ammonia lyase (Peiser and others 1998) and polyphenol oxidase (Tomas-Barberan and others 1997a). Wounding caused during lettuce harvesting and processing (handling and cutting) triggers an increase in the enzymatic activity of phenylalanine ammonia lyase. The increase in the enzymatic activity of phenylalanine ammonia lyase results in the production of new phenolic compounds that are substrates for oxidative enzymes such as polyphenol oxidase (Lamikanra and Watson 2001; Cantos and others 2002). Brown color development in lettuce is related to the oxidation of phenolic compounds naturally present in the plant. This oxidative reaction is catalyzed by polyphenol oxidase in presence of oxygen, producing o-quinones which eventually polymerize to form melanins, which are dark brown, black or red pigments (Castañer and others 1996). Textural changes in vegetable products have been attributed mainly to dehydration, because of the decrease in turgor

pressure within cells as well as cellular wall degradation. These undesirable changes primarily occur during post harvesting stages of processing and storage; therefore it is necessary to implement preventive measures to maintain produce quality. The most important factor that affects lettuce storage life is temperature, which affects enzymatic and other chemical reaction rates (Bolin and Huxsoll 1991). Thus, it is essential that minimally processed fruits and vegetables be kept at temperatures just above freezing in order to ensure maintenance of original sensory attributes, such as flavor, color, texture and appearance (Bolin and Huxsoll 1991).

Some of the treatments used to retard lettuce deterioration include the use of antioxidants and/or reducing agents such as sulfites and ascorbic acid (Li and others 2001), and hydrocooling. Hydrocooling is a procedure for removal of heat from freshly harvested commodities by using pure water at lower temperature than the produce. According to Ferreira (1994) cooling retards respiration, ripening, senescence, dehydration and decay, thus helping to maintain quality attributes such as appearance, texture and color, as well as to extend shelf life. Sulfites are highly effective in controlling browning, but are currently subject to regulatory restrictions because of adverse effects on health, such as asthmatic crises, or even in rare cases anaphylactic-like reactions may occur. In 1986 the FDA revoked the Generally recognized as safe (GRAS) status of sulfites, prohibiting their use in fruits and vegetables intended to be served or sold raw to consumers (Sapers 1993). Hence, the development of sulfite alternatives as browning inhibitors in raw vegetables is imperative. One of the most promising choices is the use of L-ascorbic acid. This reducing agent, also known as vitamin C, has been used as a browning inhibitor in some fruits and vegetables, as well as in several processed

products, i.e. fruit juices (Bauernfeind 1985). Dong and others (2000) reported that dipping pear slices in a 1% ascorbic acid solution for 2 minutes delayed browning development in that product. The browning inhibitory effect of ascorbic acid consists of the reduction of the o-quinones generated from the oxidation of phenolic substrates of polyphenol oxidase, thus stopping the formation of dark pigments (Sapers and Hicks 1989). One additional advantage of using this compound as a food additive is that it is naturally present in many vegetable products (Bauernfied 1985). Actually ascorbic acid is one of the most abundant antioxidant compounds in produce (Howard and others 1999). Vitamin C is a water soluble compound which is easily oxidized to form dehydroascorbic acid, a relatively stable free radical whose antioxidant activity is due to the ease of losing electrons. Hence, it protects compounds in the water soluble portion of cells and tissues by donating electrons to reactive oxidant species. Ascorbic acid also reactivates *in vivo* the oxidized tocopherol radicals at the cellular membranes (Kaur and Kapoor 2001). Ascorbic acid has also proven to be an effective bactericidal agent (Burnham and others 2001). Moreover, this compound is GRAS for its use as a food additive, without having any adverse reactions.

The purpose of the current study is to evaluate the effectiveness of ascorbic acid and water applied by two hydrocooling methods (immersion and spraying) on controlling browning and textural changes in green leaf type lettuce (Waldmann's dark green variety) stored at 5 ^{o}C, thereby increasing its shelf life, antioxidant capacity and general acceptability. Thus, the overall objectives of this study are as follows:

1. To evaluate the effect of ascorbic acid and water applied by two hydrocooling methods on the physical and chemical properties of green leaf lettuce stored at 5 ^{o}C over storage time.
2. To evaluate the effect of ascorbic acid and water applied by two hydrocooling methods on the sensory attributes of green leaf lettuce stored at 5 ^{o}C over storage time.

CHAPTER II

LITERATURE REVIEW

INTRODUCTION

Lettuce *(Lactuca sativa L.)* is one of the most popular green salad vegetables, and is used around the world. In recent years, the consumption of this vegetable has increased notably, making it one of the most preferred vegetable products in the United States. Glaser and others (2001) reported that in 2000 the average American consumed 33 pounds of all types of lettuce, the highest per capita rate on record. The most consumed lettuce variety was a crisphead type, Iceberg lettuce, with 24.9 pounds consumed per capita in the U.S. in 2000, making this product the second most popular fresh vegetable in this nation. Overall, U.S. lettuce production has increased by 16% since 1992 (Glaser and others 2001), and 25-30% of the total lettuce production corresponds to varieties other than crisphead, such as leaf and butterhead (Ryder 1999).

This vegetable provides relatively little nutrient value per unit weight. However, because its per capita consumption is high, lettuce is an important contributor of dietary fiber and other nutrients, such as vitamin A, vitamin C, calcium, potassium and magnesium (Table 2.1) (Ryder 1999).

Lettuce is a leafy vegetable which is mostly used as a fresh raw product, and it is never canned, dried, or frozen. This vegetable is processed in three main ways: as a commodity, value added lettuce, or as a fresh-cut product (Glaser and others 2001).

Produce sold as a commodity is sold in bulk and does not undergo any processing. Value-added lettuce is lettuce subjected to some processing steps such as washing and bagging.

Table 2.1 Nutritional value for leafy vegetables[a]

MINERALS (mg)	CRISP LETTUCE	BUTTERHEAD LETTUCE	LEAF LETTUCE
Calcium	22	35	68
Phosphorus	26	26	25
Iron	1.5	1.8	1.4
Sodium	7	7	9
Potassium	166	260	264
VITAMINS			
A (IU)	470	1065	1900
C (mg)	7	8	18
Water (%)	95.5	95.1	94.0
Fiber (g)	0.5	0.5	0.7

[a]Values are for 100 g of edible product

(Ryder 1999)

Fresh-cut or fresh-processed lettuce is produce sold as bagged lettuce, chopped alone or as salad blends (shredded lettuce combined with other fresh vegetables and ingredients) (Glaser and others 2001).

Lettuce is a sensitive product whose postharvest quality is affected by several unfavorable changes, e.g. browning and texture deterioration (softness, wilting, and loss of turgidity) (Mateos and others 1993; Peiser and others 1998). Browning and textural changes also have an important impact on lettuce shelf life, since sensory attributes such as appearance, color and texture are negatively affected, reducing the marketability of this vegetable (Sapers and Hicks 1989; Iyengar and McEvily 1992; Tomas-Barberan and others 1997a; Abbot and Harker 2004).

TYPES OF LETTUCE

Lettuce cultivars are generally grouped into four principal types: crisphead, butterhead, cos and leafy type.

Crisphead is the most important commercial type since it occupies most of the U.S. commercial acreage destined for lettuce production (70-75%) (Ryder 1999). Main physical characteristics of crisphead are a large sized firm head formed of overlapped leaves whose texture is crisp and tough (Figure 2.1). Crisphead lettuce outer leaves are large and green, meanwhile inner leaves are white-creamy color. Crisphead lettuce withstands adverse conditions of temperature and packaging better than other lettuce varieties. Thus, crisphead lettuce shelf life is longer than any other lettuce variety, with a shelf life of 3-4 weeks when stored at 0 °C and 98-100% of relative humidity (Lipton and Ryder 1989; Saltveit 2004b).

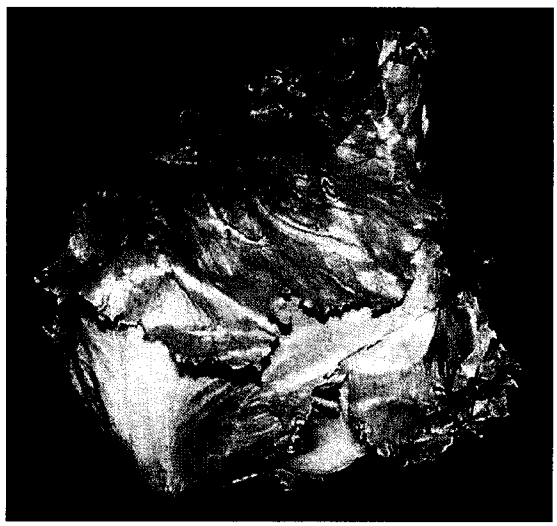


Figure 2.1 Crisphead lettuce

Quality grade of crisphead lettuce depends on size and texture (firmness). Heads of crisphead lettuce should be firm with the outer leaves turgid, dark green color, free of damage and firmly attached to the stem (USDA 1997b). If the outer leaves are not firmly

attached to the stem then it is indicative of a lettuce harvested when overmature. Flavor of overmature lettuce is bitter and is not appreciated in the market (Simonne and others 2002). Midrib color is an indicator of freshness of crisphead lettuce since the midrib is white when crisphead lettuce is just harvested, with some liquid leakage from the cut surface area (Lipton and Ryder 1989). Atmospheric oxygen oxidizes released liquid resulting in midrib (butt) darkening or discoloration 12-24 hours after harvesting (Lipton and Ryder 1989). Quality criteria for crisphead lettuce apply for the other lettuce varieties with some exceptions (USDA 1997a). Midrib discoloration is not an important problem in butterhead and leaf lettuce. Moreover, butterhead and leaf lettuce need to be protected from dehydration following harvesting, since these lettuce varieties tend to wilt faster than crisphead due to the larger surface-to-volume ratio (Lipton and Ryder 1989; Saltveit 2004b).

Butterhead lettuce is the principal type of lettuce produced and consumed in northern Europe. This lettuce variety forms a head less compact than crisphead lettuce which weighs 300-500 g at maturity (Figure 2.2). Moreover, leaves of this lettuce variety are tender, light green color outside, creamy yellow inside, and oily to the touch. This lettuce variety must be handled carefully since butterhead lettuce texture is more fragile than crisphead lettuce (Lipton and Ryder 1989).

Romaine or cos lettuce is an elongated lettuce type which does not form a head, and is one of the most preferred lettuce varieties in Europe, mainly in the Mediterranean

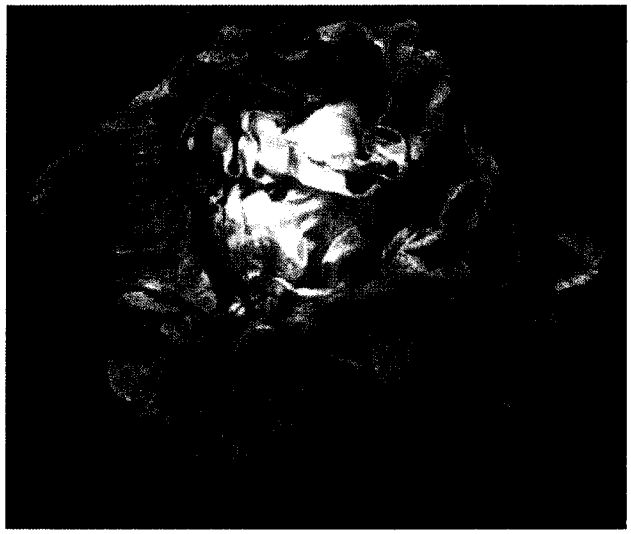


Figure 2.2 Butterhead lettuce

area. Color of outer leaves of this lettuce variety is green (Figure 2.3); meanwhile inner leaves are cream colored. Moreover, texture of Romaine lettuce is crisp and tender. Shelf life of Romaine lettuce is around 21 days when stored at 0 °C with a relative humidity

Figure 2.3 Romaine or cos lettuce

above 95%, and at 5 °C is reduced to about 14 days when the lettuce is not in contact with ethylene during storage (Cantwell and Suslow 2002b).

Leaf lettuce has experienced the highest increase in production, as well as in consumption in this country (Ryder 1999). Glaser and others (2001) reported an increase of 16% in leaf lettuce production since 1992. Furthermore, per capita consumption of leaf and romaine lettuce has doubled since early 1990's, resulting in a per capita consumption of 8.3 pounds in 2000 (Glaser and others 2001). There are many colored types of leaf lettuce, going from dark green to red-colored lettuce (due to anthocyanins). Leaves of this lettuce variety are ruffled and smooth, and their texture is crisp and soft (Figure 2.4) (Lipton and Ryder 1989). This lettuce type does not form heads but rosettes, and its shelf life is shorter than other varieties with only 1-2 weeks when stored at optimal conditions

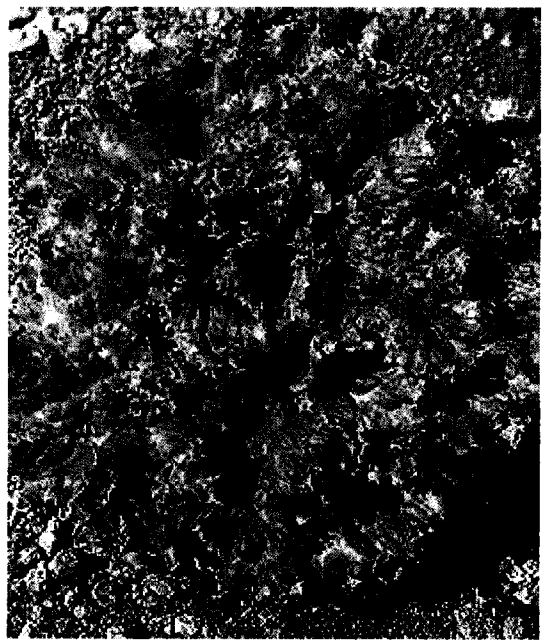


Figure 2.4 Waldmann's dark green leaf lettuce

(0 °C with 98-100% relative humidity) (Saltveit 2004b).

LETTUCE QUALITY

As in any other food product, quality is quite important. Regardless of lettuce type, quality of lettuce is based mainly on its appearance and visual quality (Peiser and others 1998). In general a high quality lettuce should be clean (free of soil, insects and dirt). Lettuce should also be free of browning spots, crisp, turgid, and firm without damaged parts (leaves or midrib), and its color should be bright green or red, depending on the variety (Saltveit 2004b). Some federal and state agencies have established quality grades or standards for many vegetable products including lettuce to regulate and standardize quality criteria of those commodities (USDA 1997a; 1997b). Those standards, such as the United States Standards for Grades of Lettuce (USDA 1997a), define quality grades for vegetable products by specifying criteria such as: tolerances for defects and diseases, maturity, presence of insects, degree and timeliness of precooling (USDA 1997b). According to those standards it is stated that the higher the specified grade of the product the higher its quality, since the allowed tolerance for defects on the product is lower (Lipton and Ryder 1989).

Impact of processing on lettuce quality is important. Harvest and postharvest handling have a determinant effect on produce quality and shelf life, since steps such as cutting or peeling involve certain degree of tissue injury or wounding (Saltveit 2000). Wounding triggers physiological and biochemical changes in the product which result in enzymatic browning (Bolin and Huxsoll 1991; Saltveit 1997) and/or texture deterioration due to induced-ethylene senescence (Saltveit 1997, Saltveit 2004a).

ENZYMATIC BROWNING

Browning discoloration in foods, or just browning, is a problem defined as the appearance of either dark spots or changes in produce surface coloration, and is one of the most concerning problems for food industry (Sapers 1993). Quality of many raw fruits, vegetables and beverages is reduced due to browning changes which could occur during processing and storage stages (Sapers 1993). Hence, browning can cause important economical losses for food producers since sensory attributes such as appearance, color and flavor are negatively affected (Sapers and Hicks 1989; Iyengar and McEvily 1992). These discolorations can be caused by oxidative reactions (nonenzymatic browning) or by enzymes (enzymatic browning). Browning negatively affects the appearance of a wide variety of food products, from seafood such as lobster and shrimp, to fruits and vegetables, including lettuce. Browning is one of the main causes of reduction of lettuce quality and shelf life, either as a raw product or as minimally processed lettuce (Sapers 1993). Increase in restrictions on the use of chemical browning inhibitors such as sulfites has caused increased interest in the study of these browning reactions (Sapers 1993, Castañer and others 1996). Thus, it is important to elucidate browning causes and how these color changes can be controlled or avoided (Peiser and others 1998).

The most frequent discoloration problem in lettuce is enzymatic browning, which is linked to increases in enzymatic activity of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) (Heimdal and others 1995; Tomas-Barberan and others 1997a). According to Castañer and others (1996), increases in enzymatic activity of phenylalanine ammonia lyase, polyphenol oxidase and peroxidase

causes the oxidation of phenolic compounds present in the produce, as well as the production and accumulation of new phenolic compounds which eventually may be oxidized (Tomas-Barberan and others 1997a).

Polyphenol oxidase (catechol oxidase or diphenol oxidase 1.10.3.2) is an enzyme involved in browning reactions, and is located in plastid thylakoids in vegetable cells (Mayer 1987). Copper is an integral part of polyphenol oxidase, thus this metal is important for its enzymatic activity. Plants grown under copper-deficient conditions did not have polyphenol oxidase enzymatic activity even if Cu^{2+} was provided to the plant at a later stage (Mayer 1987). It seems that the holoenzyme (active enzyme) is formed only during growing developmental stages. It is speculated that the apoenzyme (inactive enzyme) can be formed at a mature plant stage, and it does not incorporate copper. Moreover, it is thought that the active polyphenol oxidase is not formed during copper deficiency at any time (Mayer 1987). Some acids naturally present in plants, such as citric, malic, phosphoric, ascorbic and tartaric acids are used to inhibit enzymatic browning by lowering the pH, thus reducing the polyphenol oxidase enzymatic activity. Besides, citric acid negatively affects the enzymatic activity of the polyphenol oxidase by chelating the copper of the prosthetic group of the polyphenol oxidase (Almeida and Nogueira 1995).

Phenolic compounds are secondary plant metabolites that mostly act like pigments as a defensive mechanism to protect the plant against excessive light (Hopkins and Huner 2004a). Thus, phenolic compounds are present in fruits and vegetables at low concentrations when the produce has not been exposed to stressful conditions such as wounding (Tomas-Barberan and others 1997b).

Enzymatic browning is initiated when a monophenolic compound is hydroxylated by PPO in the presence of atmospheric oxygen to produce an *ortho*-dihydroxiphenol compound (Sapers 1993) (Figure 2.5). The *ortho*-dihydroxiphenol compound formed is eventually oxidized by PPO in the presence of atmospheric oxygen, and forms an ortho-quinone. *Ortho*-quinones are highly reactive colorless compounds which

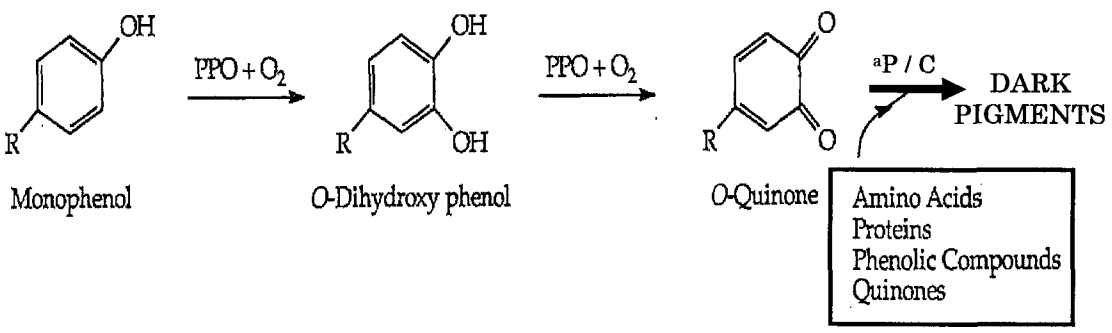


Figure 2.5 Enzymatic browning reactions
[a] P/C = Polymerization/Conjugation

(Sapers 1993)

polymerize and react spontaneously with other cellular components such as amino acids, proteins, other phenolic compounds and quinones, forming dark pigments called melanins (Iyengar and McEvily 1992; Sapers 1993). Polymerization reactions do not require enzymes or catalytic agents (Sapers and Hicks 1989; Sapers 1993). Thus, the initial concentration of monophenolic compounds (oxidizable substrates) is important as it is the postharvest production of new phenolic compounds. Monophenolic compounds may be naturally present in produce or synthesized postharvest (Tomas-Barberan and others 1997b). Synthesis of monophenolic compounds is triggered by physical injuries or wounding during postharvest handling, since wounding increases the activity of PAL,

which is the main rate controlling enzyme in the phenylpropanoid pathway (Kang and Saltveit 2002).

One of the biochemical responses induced by wounding includes the activation of the phenylpropanoid pathway (Figure 2.6) (Ke and Saltveit 1989; Saltveit 1997;

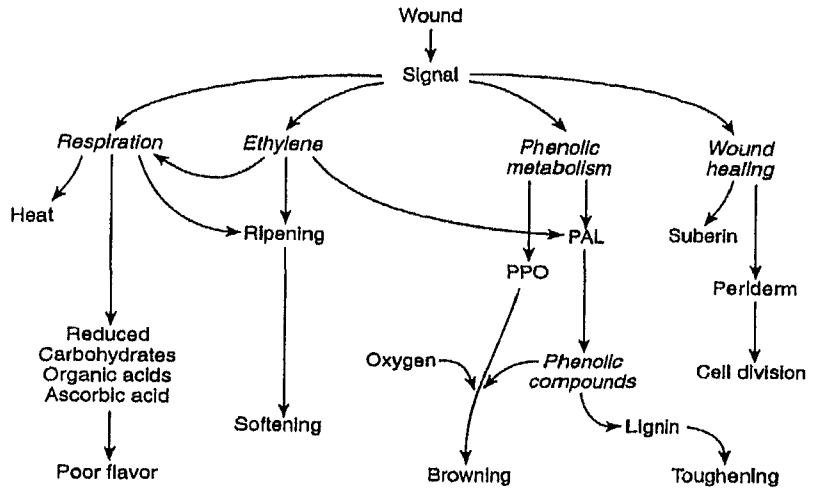


Figure 2.6 The interrelationships among the many effects of wounding on physiological processes in minimally processed fruits and vegetables

(Saltveit 1997)

Tomas-Barberan and others 1997a). A wound signal is formed at the site of injury and disseminates into adjacent tissue. Cells near the wound site respond first and with the greatest intensity to the injury (Saltveit 1997). Moreover, wounding stimulates an increase in the respiratory rate of the produce as a first response, as well as an increase in the production of phenolic compounds. Thus, wounding promotes an increase in respiratory rate and ethylene production (Barth and others 2004). After wounding a signal is propagated not only to the injured cells but also to distant cells away from the injury localization (Saltveit 1997).

A wound signal may be physical, e.g., hydraulic or pressure wave, or physiological, e.g., a hormone such as traumatin produced in response to wounding, or a combination of both processes (e.g., a bioelectrical wave of potential or current) (Saltveit 1997). It has also been proposed that the wound signal is a response to the presence of pectic fragments from degraded cell walls (Saltveit 1997). Calcium concentration within cells is important in wound signal transmission, because calcium affects the cellular membrane stability as well as many signal transduction pathways. Wounding affects calcium distribution within tissues, thus application of calcium during lettuce processing may interfere with wound signaling, retarding wound-induced processes such as elevation of ethylene production (Ke and Saltveit 1986; Saltveit 1997). A wound signal also induces an increase in leaf thickness as a plant response to the perceived stimuli. It was suggested that wound (hydraulic) signals form part of a plant mechanism to regulate the responses to localized stress (Saltveit 1997).

One of the first effects provoked by wounding is an increase in ethylene production (Figure 2.6). Ethylene is a plant hormone produced at low rates by lettuce (Saltveit 2004b), however wounding increases ethylene production considerably (Saltveit 2004a). This plant hormone causes senescence and increases respiratory rate in the plant tissue (Saltveit 2004a), as well as changes in cellular structure of wounded tissue (Ke and Saltveit 1988). Ke and Saltveit (1988) found higher lignin content in lettuce exposed to 3 ppm of ethylene compared to control tissue exposed to air (no ethylene). Moreover, ethylene caused the accumulation of (+) catechin and (-) epi-catechin, 3-caffeoyl-quinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid. These soluble phenolic compounds were oxidized by PPO. Ethylene also increased the concentration of bound

indole-3 acetic acid (IAA) oxidase activity (Ke and Saltveit 1988). IAA oxidase is an enzyme that oxidizes IAA, which is one of the most important auxins, a group of vegetable hormones (Hopkins and Huner 2004c). In addition, IAA application provoked an opposite effect to the ethylene: a reduction in ethylene-induced phenylalanine ammonia lyase, peroxidase, and indole-3 acetic acid oxidase activities, soluble phenolic content, and russet spotting development (Ke and Saltveit 1988).

There are other structural changes in vegetable cells as a result of exposure of plant tissue to ethylene. Cell wall thickening and cell discoloration are two cellular changes associated with russet spotting (Ke and Saltveit 1988). Cell wall thickening (lignification) is a defensive mechanism in response to wounding. Lignins are insoluble polymers which accumulate on and around the wounded area to prevent or retard penetration of microorganisms (Ke and Saltveit 1989). Ke and Saltveit (1988) reported that there was an increase in lignin content and POD activities in wounded lettuce tissue. POD activity was measured because this enzyme participates in the lignin pathway. Bound POD is localized in the cell wall where lignin production occurs (Ke and Saltveit 1988).

Ethylene also induces an increase in phenolic metabolism and RS development whereas IAA and the auxin-type synthetic growth regulator (2,4-D) inhibits the ethylene-induced effects (RS development) (Ke and Saltveit 1988). IAA exerts important functions in plants, since it stimulates cell elongation as well as regulates the development of flowers and fruits (Hopkins and Huner 2004c). Hence, it seems that there is a mutual inhibitory action between ethylene and auxin (Figure 2.7), since application of auxin (1.0 mM) reduced PAL activity, total soluble phenolic content, and flavonoid content in

iceberg lettuce (Ke and Saltveit 1988). Therefore, IAA also reduced POD activity, as well as inhibited ethylene-induced IAA oxidase activity in this lettuce variety (Ke and Saltveit 1988).

Wounding in iceberg lettuce increased the enzymatic activity of PAL not only in cells near the wounded area but also in tissue cells 2.5 cm. away from the wound site (Ke and Saltveit 1989). This indicates that a wound signal for PAL induction was transmitted from wounded to non-wounded cells. The average speed reported for the transmission of the wound-induced PAL activity was about 0.5 cm/h from wounded to non-wounded cells (Ke and Saltveit 1989)

Other physiological effects caused by wounding include increases in produce respiratory rate, production of carbon dioxide, oxygen consumption and heat production (Saltveit 1997; Barth and others 2004). An increase in respiration rate in a product enclosed in a place without gas exchange may cause carbon dioxide concentration to increase and oxygen to deplete to levels that induce anaerobic respiration. Iceberg lettuce is very sensitive to CO_2 damage, since it presents brown stain at low CO_2 concentrations such as 1% (Siriphanich and Kader 1985). Plant tissues are tolerant to products of anaerobic respiration such as ethanol and acetaldehyde, but the problem is that those compounds cause off-flavor in the product (Saltveit 1997).

There are some different types of browning discoloration problems that affect lettuce quality, such as russet spotting, senescent browning, brown stain and butt discoloration (Saltveit 1997; Tomas-Barberan and others 1997a). Russet spot is a physiological disorder that serves as an excellent model system for analyzing the regulation of phenolic metabolism at both the molecular and cellular levels (Figure 2.7)

(Ke and Saltveit 1988). Russet spotting is a type of discoloration induced by ethylene which may affect the epidermis, as well as the mesophyl and vascular tissues (Ilker and others 1977). This physiological disorder is characterized by colored spot-like lesions (yellow-brown, tan or olive-green) that may appear initially either in the mesophyl or in the epidermal cells (Ke and Saltveit 1988).

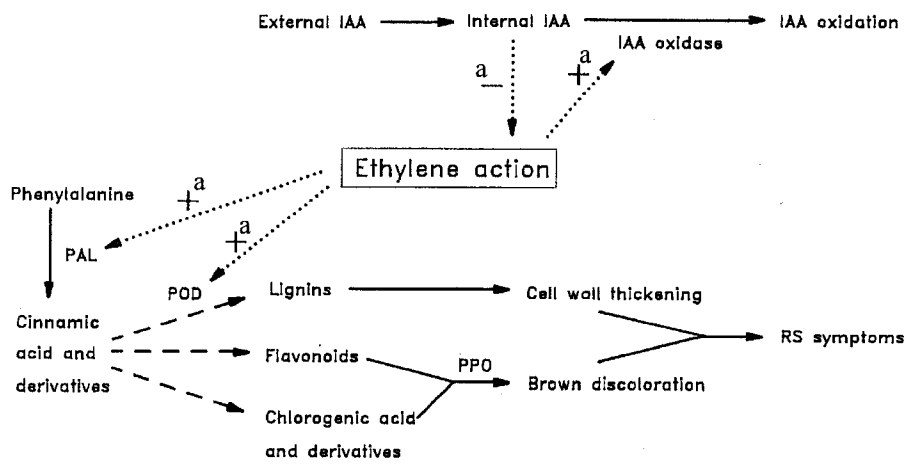


Figure 2.7 The pathways related to the regulation of russet spot development.
[a] Symbols: + = activation, - = inhibition.

(Ke and Saltveit 1988)

Ilker and others (1977) showed that once the russet spotting lesions affect subepidermal mesophyll cells (advanced stages of this disorder) the mesophyll cell walls collapse forming a pit-like depression. Russet spotting (RS) is a physiological disorder characterized by the appearance of numerous small brown spots in the midrib, and those spots may spread over the leaf blade. Exposure of iceberg lettuce to ethylene at concentrations as low as 3 ppm at storage temperatures of 5 ^{o}C causes RS development (Ke and Saltveit 1988). Brown stain is a discoloration problem provoked by exposure of lettuce to carbon dioxide (Siriphanich and Kader 1985). Butt discoloration is one of the

most important browning changes occurring in this vegetable during postharvest stages, and is characterized by first the appearance of yellow spots, then reddish-brown, and finally an intense brown color in the stem butt end, usually caused by the inherent wounding from cutting the produce during harvesting (Castañer and others 1996; Tomas-Barberan and others 1997a).

Treatments intended to prevent the oxidation of accumulated wound induced phenolic compounds seem to be a better approach to avoid the browning in vegetable products. Such treatments may produce a healthier fresh-cut product as well, since phenolic compounds with antioxidant properties produced during postharvest stages would be enriching the produce (Kang and Saltveit 2002).

TEXTURAL CHANGES

Textural attributes of vegetables and fruits are the result of the chemical and physical interactions of the plant tissue structural components. To understand the mechanical properties of vegetable products it is necessary to identify how the different tissue structural levels (molecular, cellular and organ levels) contribute to the product's texture (Alzamora and others 2000).

Vegetables such as lettuce and spinach are mostly leaves, and like other vegetable products are composed mostly of parenchyma cells (Alzamora and others 2000). The parenchyma cells are 50-500 μm long and are not lignified. Parenchyma cell walls are separated by the middle lamella (Figure 2.8), which is rich in pectic substances and binds

Figure 2.8 Diagramatic representation of a parenchyma cell. cl = chloroplast; cw = cell wall; g = golgi; is = intercellular space; nu = nucleolus; n = nucleus; ml = middle lamella; pl = plasmodesmata; pm = plasmalemma; rer = rough endoplasmic reticulum; v = vacuole.

(Alzamora and others 2000)

adjacent cells. Cell walls are composed of pectic substances, hemicellulose and cellulose, and the combination of these polysaccharides will determine cell wall mechanical properties, such as elasticity or rigidity (Alzamora and others 2000; Abbot and Harker 2004). A cell wall has to be not only elastic to allow the cell expansion during plant growth, but also rigid to maintain cell shape and provide structural support for the plant tissue (Alzamora and others 2000; Abbot and Harker 2004).

Another important cell structure is the plasma membrane, which is a semipermeable phospholipids bilayer enclosing the cytoplasm. All the cell organelles, i.e. nucleus, chloroplasts, mitochondria, plastids, endoplasmic reticulum and vacuoles are also enclosed by membranes. Other cell components that also contribute to the texture

properties of the plant cells are the vacuoles, which contain a mixture of water, organic acids, salts and pigments (Alzamora and others 2000).

The three most important cellular structural factors contributing to the textural properties of vegetable produce are cell turgor pressure, cell wall rigidity and cell-to-cell adhesion (Alzamora and others 2000). Other factors that also determine plant tissue mechanical properties are size, shape, arrangement and hydration grade of cells, presence of interstitial spaces filled with gas, as well as concentration of starch and other solutes within the cells (Alzamora and others 2000, Abbot and Harker 2004).

Turgor pressure is the pressure exerted by the cellular content over the cell wall, and is dependent on the concentration of sugars, salts and other hydrophilic solutes contained in its vacuoles, as well as the cellular water status (Alzamora and others 2000). These two factors combined determine the cell water potential (ψ), which is the capacity of a cell to accept water (from a hypotonic media) (Hopkins and Huner 2004b). Cell turgor increases when hydrophilic solutes such as salts and sugars provoke an osmotic pressure gradient on the semi-permeable plasma membrane (higher osmotic pressure inside the cell than outside), thus water moves toward inside the cell through the plasma membrane. Water will continue flowing towards inside the cell theoretically until reaching an osmotic equilibrium between both sides of the plasma membrane. In non-growing plant cells water flows towards inside the cell increasing the pressure exerted over the cell wall until a maximum turgor pressure is reached (Alzamora and others 2000; Hopkins and Huner 2004b). Afterwards, the elastic energy stored in the polymer chains (or strains) forming the cell wall is released as a compression force that stresses the protoplasm. Pressure exerted over the protoplasm membrane stops water flow towards

inside the cell, even though the osmotic equilibrium has not been reached between the inside and outside of the cell. At this point the cell is fully turgid, and has an inner pressure of 3-4 atmospheres (Alzamora and others 2000). Thus, in mature plant cells changes in turgor pressure will be directly related to changes in their water potential. In growing stages water continues flowing into the cell even when the maximum turgor pressure is reached inside the cell, since an irreversible cell wall expansion occurs. Such increase in the cell wall volume results in a permanent cell growth (plant growth) (Alzamora and others 2000, Abbot and Harker 2004).

Cell wall properties are also important for vegetable texture, since the cell wall provides support and stability to the cell not only during plant growth but also when the produce is going mature and overripe. The cell wall undergoes functional changes during tissue maturation that impact produce texture. Some physiological changes induced during ripening are increases in transpiration, respiratory rate, and ethylene production (Saltveit 2004c). One of the most important chemical and functional changes during vegetable tissue maturation is lignification of the cell wall, which results in toughening of the product. As a product ripens, texture is softening due to degradation of cell wall components such as pectin, which becomes water soluble (Alzamora and others 2000; Scheiner and others 2000). There is an inverse relationship between water status in the cell and degradation of cell wall, which leads to textural changes. Scheiner and others (2000) reported that there was no hydrolysis of cell wall pectic substances in lettuce when it contains high water content. Another cell wall component hydrolyzed during ripening is hemicellulose, which is degraded into lower molecular polysaccharides. Some enzymes involved in the hydrolysis of cell wall components are polygalacturonases and some

glycosidases, such as xyloglucanase, endotransglycosylases and cellulases (Abbot and Harker 2004).

Some treatments intended to preserve vegetable product firmness using calcium have proven to be effective. It has been suggested that calcium helps to maintain the stability of the cell wall structure by interacting with pectin. Calcium may bind to the pectin in cell walls stabilizing the cell wall structure during produce ripening (Abbot and Harker 2002).

Leaf lettuce is one of the most susceptible varieties to wilting, since its surface-to-volume ratio is higher than in other lettuce varieties, such as Romaine or iceberg (Saltveit 2004b). Hence, it may be expected to have a higher degree of product dehydration during postharvest stages, which may result in a less acceptable lettuce appearance and texture (wilted or flaccid tissue) (Lipton and Ryder 1989; Saltveit 2004b).

LETTUCE PROCESSING/PRESERVATION

Lettuce quality only declines after harvest, thus quality can never be better than at harvest (Lipton and Ryder 1989). This leafy vegetable is delicate and susceptible to damage during processing steps such as cutting and handling (Bachman and Earles 2000). After harvesting lettuce continues metabolic processes such as respiration and ethylene production at higher rates than in preharvesting stages (Saltveit 2004a). Lettuce processing consists mostly of these steps: harvesting, washing, cooling, draining, packaging and storage (Figure 2.9).

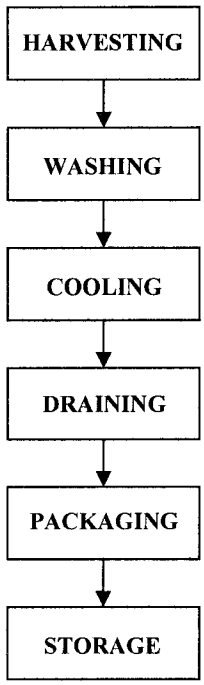


Figure 2.9 Flowchart of lettuce processing

(Hentges 1997)

Harvesting

Harvesting is performed manually by cutting the lettuce head in the midrib at ground level with a sharp knife (Hentges 1997). It is recommended to implement a training program for workers who will harvest the lettuce to minimize damage caused by handling (Bachman and Earles 2000). After the produce is obtained it can be bagged in plastic films to minimize moisture loss. Harvesting lettuce is recommended in the morning, so that the produce will be at the coolest temperature of the day (Suslow 2000). Produce temperature is an important factor to consider during the entire lettuce processing, since this leafy vegetable wilts easily due to its large surface-to-volume ratio. Thus, lettuce should be protected from direct sunlight exposure to avoid heat gain (Suslow 2000). It is critical to reduce the produce temperature as quickly as possible after

harvesting, since vegetable respiration and transpiration rates are high at field temperature (Suslow 2000; Saltveit 2004a).

Washing

Lettuce is washed following harvest to remove initial microbial load and dirt. Lettuce grows at ground level with direct contact with soil, thus it is expected to find a large microbial load in this vegetable when just harvested. Some pathogens such as *Escherichia coli* O157:H7, *Salmonella*, *Shigella* and *Listeria monocytogenes* have been found on lettuce (Suslow 2000). It is recommended to perform a prewashing step with chlorinated water (recommended 4-10 ppm) to remove soil, dirt and insects from the produce, as well as plant exudates released from harvest cuts or wounds (Suslow 2000). The effectiveness of the chlorinated water is affected by the pH, since if the pH is above 8.0 the chlorine activity is slow, while if it is below 6.5 chlorine is very reactive and corrosive to the equipment (Sargent and others 2003). In addition, it is important to adjust the pH of the chlorinated water between 6.5 and 7.5, because at this pH range most of the chlorine dissolved in the chlorinated water is in the form of hypochlorous acid (HClO), which is very effective as antimicrobial agent (Suslow 2000). It is necessary to monitor constantly the chlorine concentration of the washing solution to maintain effective chlorine levels. Water used to wash produce has to be changed constantly due to the accumulation of dirt and pathogens (Sargent and others 2003).

Other sanitizers used for washing vegetable products are ozone (O_3), hydrogen peroxide (H_2O_2) and peroxyacetic acid (Suslow 2000). These compounds are strong oxidizing compounds that have been proven to be effective sanitizer agents. Ozone acts faster than chlorine, and it has proven as an effective antibacterial agent against food

borne microbes (Suslow 2000). Regarding hydrogen peroxide and peroxyacetic acid, these two compounds are also good antimicrobial agents, but their high cost limits their use (Suslow 2000).

Cooling

Lettuce cooling is used to retard its quality deterioration, since this vegetable continues to be metabolically active after harvesting (Kang and Saltveit 2002). Lettuce is exposed after harvesting to 2 stress factors that promote an increase in respiratory rate and in transpiration: wounding (cutting) and dehydration. Hence, it is necessary to reduce the produce metabolic rate by cooling it down as soon as possible after harvesting (Suslow 2000). Cooling reduces lettuce respiratory rate, dehydration (Table 2.2), and decreases the risk of microbial growth (Erdman and Klein 1982; Sargent and others 2003; Saltveit 2004b).

Table 2.2 Post harvest respiration rates (in mg CO_2 kg^{-1} h^{-1}) of head and leaf lettuce

Temperature	Head lettuce	Leaf lettuce
0 °C	6 to 17	19 to 27
5 °C	13 to 20	24 to 35
10 °C	21 to 40	32 to 46
15 °C	32 to 45	51 to 74
20 °C	51 to 60	82 to 120
25 °C	73 to 91	120 to 173

(Saltveit 2004b)

Post harvest respiratory rates vary considerably between lettuce varieties as well as within the same variety depending mostly on the lettuce type (Table 2.3). Respiratory

Table 2.3 Respiration rates of specialty leafy greens[b] at 0 °C and 10 °C

Common name	Respiration rate at 0°C	Respiration rate at 10°C
Green Oak	3.4	19 to 27
Red Oak	4.8	24 to 35
Sierra[a]	4.1	32 to 46
Tango[a]	3.5	51 to 74
Green Romaine	3.6	82 to 120
Red Romaine	4.3	120 to 173

[a] Red-green leafy lettuce cultivars
[b] Data are averages of duplicate samples measured 2 to 5 days after harvest

(Cantwell and others 1998)

rate of vegetables during postharvest stages is very important since commodities with shorter storage life tend to have higher respiratory rates than vegetable products with longer storage life (Saltveit 2004c). Some produce sensory attributes are directly affected by the respiratory rate, since post harvest respiration produces compounds that determine quality attributes such as aroma, flavor, color, texture and appearance (Saltveit 2004c).

Postharvest respiratory rate of vegetable produce including lettuce is mostly affected by temperature, as well as by light and storage conditions (Saltveit 2004b; 2004c). There are several methods used to cool lettuce, but vacuum cooling and hydrocooling are the preferred methods (Thompson 2004). In vacuum cooling the lettuce is placed into an enclosed chamber at low pressure (Figure 2.10). The principle behind vacuum cooling method is that water boiling point decreases as the atmospheric pressure goes down. Thus, some of the water contained in the produce gets evaporated when the commodities are exposed to a lower pressure, and the produce temperature lowers when water evaporates (Thompson 2004).

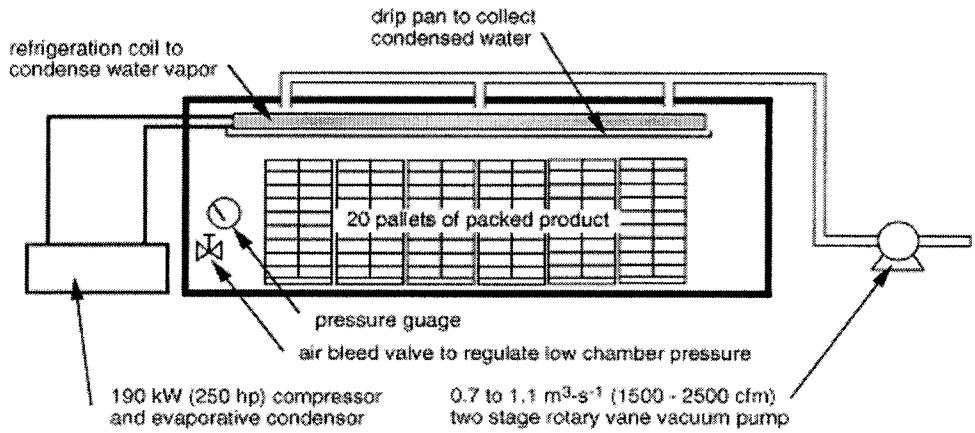


Figure 2.10 Key components of a 20-pallet capacity vacuum cooler

(Thompson 2004)

Vacuum cooling usually takes a shorter time to cool down the produce compared to other cooling methods (Table 2.4), especially when the surface area is as large as in

Table 2.4 Comparison of typical product effects and cost for six common cooling methods

	Forced-air	Hydro	Vacuum	Water spray	Ice	Room
Typical cooling time (h)	1 to 10	0.1 to 1	0.3 to 2	0.3 to 2	0.1 to 0.3[a]	20 to 100
Product moisture loss (%)	0.1 to 2.0	0 to 0.5	2.0 to 4.0	no data	no data	0.1 to 2.0
Water contact with product	no	yes	no	yes	yes, unless bagged	no
Potential for decay contamination	low	high[b]	none	high**	low	low
Capital cost	low	low	medium	N.A.[c]	high	low[d]
Energy efficiency	low	high	high	medium	low	low
Water-resistant packaging needed	no	yes	no	yes	yes	no
Portable	sometimes	rarely done	common	common	common	no
Feasibility of in-line cooling	rarely done	yes	no	no	rarely done	no

[a]Top icing can take much longer.
[b] Recirculated water must be constantly sanitized to minimize accumulation of decay pathogens.
[c] Not available.
[d] Low if product is also stored in cooler; otherwise long cooling times make it an expensive system.

(Thompson 2004)

leaf lettuce (Thompson 2004). Lettuce cooled by vacuum cooling loses 2-4% of its total weight due to dehydration during cooling step, thus it is recommended to spray the produce with water previous to cooling to minimize moisture losses (Saltveit 2004a; Thompson 2004). Hydrocooling is another method frequently used for cooling leafy vegetables such as leaf lettuce.

Efficiency of cooling depends on factors such as time, temperature and contact (Thompson 2004). Moreover, the cooling medium (air, water, crushed ice) must be maintained at constant low temperature during the cooling period. The cooling medium also must have continuous contact with most of the lettuce surface (Lipton and Ryder 1989; Sargent and others 2003).

Packaging

Packaging is intended to protect the product from contamination as well as to reduce lettuce dehydration and mechanical damage due to handling by customers. Lettuce sometimes is wrapped in polyethylene plastic at the retail market when processed as a value-added product. Polyethylene wraps are recommended to be perforated or be permeable to avoid CO_2 and ethylene accumulation (Saltveit 2004a; 2004c). Lettuce produces ethylene normally at very low rates (less than 0.2 μL/kg·hr at 0-5 °C). However, this vegetable is extremely sensitive to damage caused by ethylene (Kim and Wills 1995; Saltveit 2004a; 2004b). Ethylene is a vegetable hormone naturally produced by plants which affects many natural functions in vegetables, including ripening. Ethylene-induced damage differs among lettuce varieties, since ethylene damage appears as russet spotting in crisphead Iceberg lettuce, while in Romaine and leaf lettuce ethylene damage may produce midrib discoloration, as well as senescence. Therefore, some varieties are more

susceptible to ethylene-induced damage than others (Cantwell and Suslow 2002a; 2002b).

Storage

Adequate storage temperature is an important factor during postharvest handling of lettuce, since temperature is the most important single factor that affects postharvest lettuce quality (Suslow 2000, Saltveit 2004a). Temperature in storage cold rooms must be maintained close to 0 °C but never below –0.2 °C, since lettuce is very sensitive to chilling injury (Saltveit 2004a). The storage room should be maintained at a relative humidity of 98-100% to avoid produce dehydration and extend lettuce shelf life as much as possible. Crisphead lettuce lasts longer than other lettuce varieties, with a shelf life of 3-4 weeks when stored at a relative humidity of 98% and temperatures between 0 and 1 °C. Leaf lettuce lasts 1-2 weeks when stored at optimal conditions (0-0.5 °C and a relative humidity of 98-100% (Lipton and Ryder 1989). However, storage conditions rarely are ideal, thus it is necessary that lettuce be maintained in storage cold rooms as briefly as possible, since lettuce shelf life regardless of variety is reduced two-fold when storage temperature is 5 °C (Lipton and Ryder 1989; Jackson and others 1996; Saltveit 2004b).

HYDROCOOLING

Hydrocooling is a cooling method used to remove heat from freshly harvested commodities using pure water at a lower temperature than the produce (Thompson 2004). According to Ferreira and others (1994), cooling commodities after harvesting retards vegetable respiration, ripening, senescence, dehydration and decay. Thus, cooling helps to maintain produce quality attributes such as appearance, texture and color, as well as to

extend shelf life (Ferreira and others 1994). Hydrocooling is recommended for nonhead lettuce types such as romaine and leafy varieties, but not for head-forming types (crisphead and butterhead varieties) since the shape of head-forming varieties facilitates water retention, promoting decay (Saltveit 2004b). Hydrocooling is a rapid and effective cooling method, since water is a better heat-transfer medium than air, thus produce is cooled faster in hydrocoolers than in forced-air coolers (Table 2.4) (Thompson 2004). In this cooling method produce is cooled by circulating water at low temperature over the warmer commodities. The water absorbs heat from the produce, decreasing the temperature of the commodities. Water used for cooling the produce is circulated through a refrigeration system to cool it down again. There are several hydrocooling methods, but the most commonly used for cooling fresh commodities are hydrocooling by immersion (Figure 2.11) and the shower type or hydrocooling by spraying (Figure 2.12) (Salunhke and others 1984; Vigneault 2000; Thompson 2004). Hydrocooling by spraying spreads cold water over the warmer produce. Cold water after contacting the produce is recovered

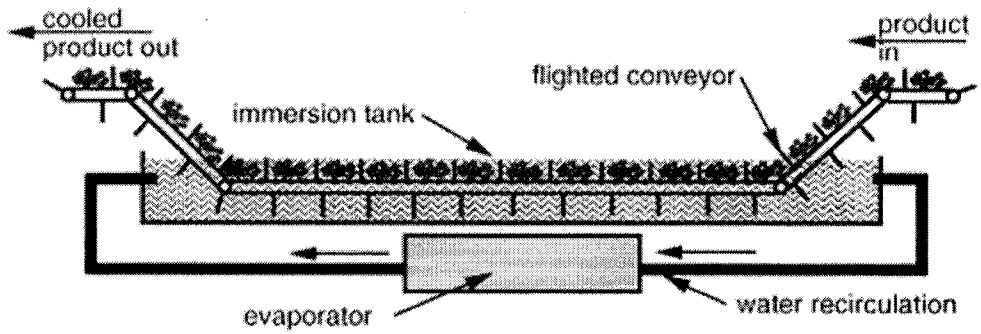


Fig 2.11 Cut-away side view of a continuous-flow immersion-type hydrocooler

(Thompson 2004)

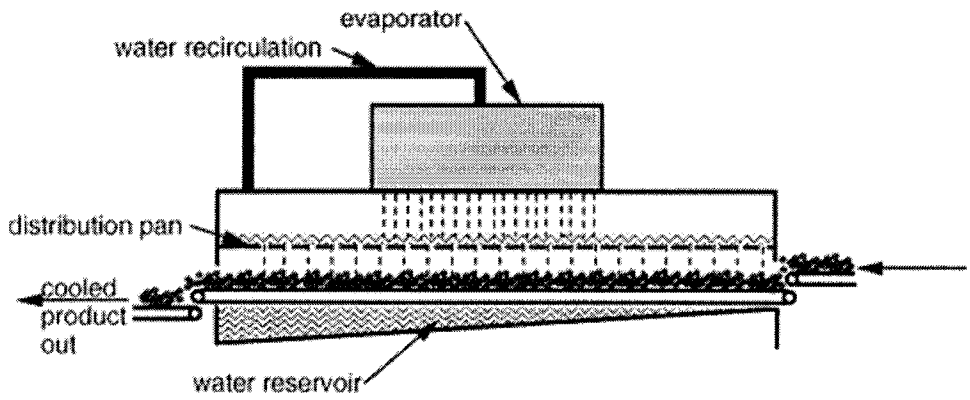


Fig 2.12 Cut-away side view of a continuous-flow spraying (shower)-type hydrocooler (Thompson 2004)

and circulated through a refrigeration system to cool it down, since water temperature increases due to the heat gain from the produce (Thompson 2004). Hydrocooler shower type can be implemented for continuous flow operation, or for operation in a batch mode. Hydrocooling by immersion consists in water containers where produce is soaked. Immersion hydrocoolers usually cool produce slower than shower coolers because water flows at slower rates past the product (Thompson 2004).

Hydrocooling does not cause moisture loss during cooling. In fact, the hydrocooling step hydrates commodities, since produce absorbs water during the cooling treatment. According to Vigneault (2000) during hydrocooling the cooling solution flows to inside the produce due to a pressure gradient created in the product surface. Such pressure gradient is caused by a volume reduction of gas contained in produce surface cells due to the reduction in the produce temperature. Temperature gradient between the cooling media and the warmer produce facilitates the diffusion of cooling solution into produce tissue during hydrocooling (Vigneault 2000). One of the safety issues related

with hydrocooling method is that dirt and microbial contaminants accumulate in water used for cooling, since it is collected and circulated (Sargent and others 2003; Thompson 2004). Thus, sanitation of water for cooling is important to avoid produce contamination (Sargent and others 2003).

ANTIOXIDANTS

The use of reducing agents or antioxidants is another preservation method intended to extend lettuce shelf life by avoiding its browning. The mechanism of action of the antioxidant agent consists of the reduction of intermediate products of enzymatic browning reactions, such as o-dihidroxyphenolic compounds, thus avoiding the formation of dark pigments (melanins) (Sapers 1993; Saltveit 1997; Li and others 2001).

Oxidative reactions in food products are one of the most concerning issues for the food industry, since these reactions unfavorably affect product sensory attributes such as color, flavor, odor, appearance and palatability (Sapers 1993). The problem consists in the oxidative action of atmospheric oxygen upon the unsaturated double bond linkages of many food components such as fats, proteins and phospholipids. Therefore, oxygen also reacts with other compounds also present in food products such as pigments (i.e. phenolics), flavor and odor compounds (Sapers 1993). Oxidative reactions are affected by light, temperature, presence of trace minerals such as copper or iron, and enzymatic activity (Bauernfeind 1985).

Some of the preventive practices to avoid oxidation of food products include the inactivation of oxidative enzymes, the addition of chelating agents to remove trace minerals, the modification of the atmosphere surrounding the product to reduce or

eliminate the contact of atmospheric oxygen with the oxidizable food components, and the use of antioxidants and synergists (Bauernfeind 1985; Sapers 1993).

Antioxidants are compounds that at low concentrations retard or avoid oxidative reactions (Sapers 1993; Halliwell 1997). Some antioxidant compounds have aromatic structure with one or more hydroxyl groups, such as vitamins C and E (Niki 1987; Sapers 1993). The mechanism of action of antioxidant compounds consists of the inactivation of free radicals to break oxidative chain reactions by donating hydrogen atoms (Bauernfeind 1985, Sapers 1993). Synergist compounds are additives used in combination with antioxidants. Synergist compounds exert a double action: they regenerate the active phenolic state of the primary antioxidant compounds by donating hydrogen atoms to the oxidized form of the antioxidants, and they deactivate trace minerals required by oxidative enzymes (Bauernfeind 1985).

There are several antioxidant compounds which have proven to be effective browning inhibitors, including sulfites, some sulfhydryl compounds, and ascorbic acid and its analogues (Sapers 1993). Sulfites are potent non-specific reducing agents (antioxidants) highly effective in controlling browning in a wide variety of food products (Sapers 1993). However, these reducing agents are currently subject to regulatory restrictions because of adverse effects on health, such as asthmatic crises, or even in rare cases anaphylactic-like reactions may occur (Iyengar and McEvily 1992; Castañer and others 1996). In 1986, FDA revoked the GRAS status to sulfites, prohibiting their use in fruits and vegetables intended to be served or sold raw to consumers (Sapers 1993; Castañer and others 1996). Thus, the development of sulfite alternatives as browning inhibitors in raw vegetables is imperative.

Regarding the sulfhydryl compounds, their mechanism of action consists in the reduction of the o-quinones formed from the oxidation of ortho-dihydroxiphenol by the enzyme polyphenoloxidase (Iyengar and McEvily 1992). These antioxidant agents, e.g. reduced glutathione, effectively inhibit browning although they are too expensive to be used commercially. Besides, other sulfhydryl agents like sulfur-containing aminoacids (L-cysteine, L-cystine or D,L-methionine) impart off-flavor to food products (Iyengar and McEvily 1992; Sapers 1993). Hence the use of sulfhydryl compounds as antibrowning agents has been limited.

ASCORBIC ACID

This reducing agent, also known as vitamin C, is used as a browning inhibitor in some fruits and vegetables, as well as in processed products, i.e. fruit juices (Bauernfeind 1985). Dong and others (2000) reported that dipping pear slices in a 1% ascorbic acid solution for 2 min considerably delayed browning development in that product. The browning inhibitory effect of ascorbic acid consists of the reduction of o-quinones generated from the oxidation of phenolic substrates by polyphenol oxidase (Figure 2.13), thus stopping the formation of dark pigments (Sapers and Hicks 1989; Sapers 1993). One additional advantage of using this compound as a food additive is that is its natural presence in many vegetable products (Bauernfeind 1985, Champion and others 2004); actually ascorbic acid is one of the most abundant antioxidant compounds in vegetables, along with β-carotene (Howard and others 1999).

Vitamin C is a water soluble compound whose antioxidant activity is due to the ease of losing electrons, thus producing dehydroascorbic acid, which is a relatively stable

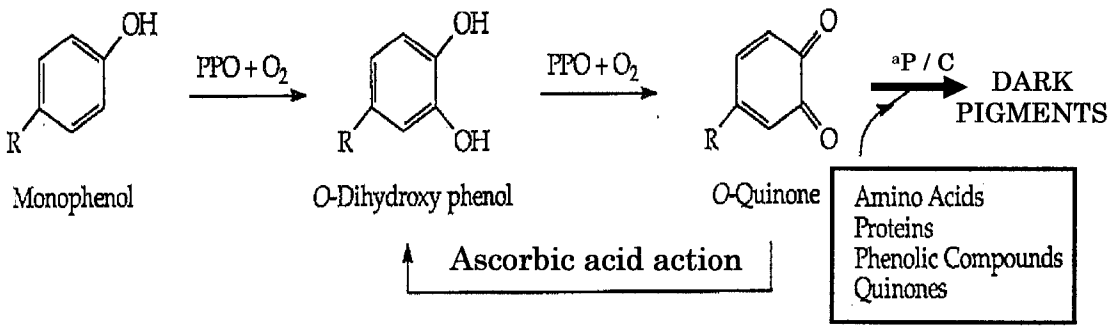


Figure 2.13 Enzymatic browning reactions showing the mechanism of action of the ascorbic acid as a browning inhibitor
[a]P/C stands for Polymerization/Conjugation

(Sapers 1993)

free radical (Bauernfeind 1985; Iyengar and McEvily 1992; Sapers 1993). Moreover, vitamin C protects compounds in the water soluble portion of cells and tissues from being oxidized by donating electrons to reactive oxidant species (Champion and others 2004). Ascorbic acid also reduces tocopherol radicals back to their active form at the cellular membranes (Figure 2.14) (Bauernfeind 1985; Niki 1987; Kaur and Kapoor 2001). Besides, ascorbic acid has also proven as an effective bactericidal

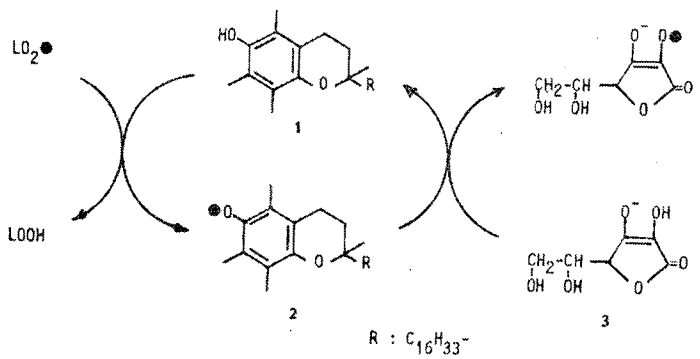


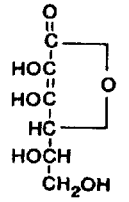
Figure 2.14 Regeneration of vitamin E from vitamin E radical by vitamin C

(Niki 1987)

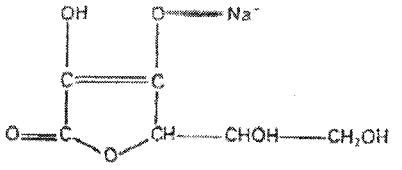
agent. Burnham and others (2001) reported that immersing apple slices inoculated with *Escherichia coli* O157:H7 in a 3.4% ascorbic acid solution for 15 min previous to home-drying at 57.2 or 62.8 °C, resulted in a decrease of 5.0-5.1 log colony forming units/g (CFU/g).

Ascorbic acid is recognized as safe (GRAS) for its use as a food additive, without reporting any adverse reactions like other additives do, i.e. sulfites. One disadvantage of the use of ascorbic acid as anti-browning agent is that its antioxidant activity is only temporary since it is rapidly oxidized (Iyengar and McEvily 1992). According to Roura and others (2003) ascorbic content of lettuce decreases over storage time. These researchers reported a decrease of 50 % of ascorbic acid content in Romaine lettuce after 4 days.

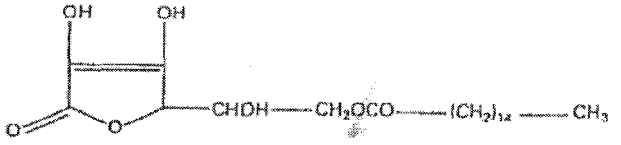
L-ascorbic acid and its derivatives sodium ascorbate and ascorbyl palmitate are considered as multifunctional antioxidants (Figure 2.15), since these antioxidants act as chelators by sequestering trace metals such as iron or copper inactivating oxidative



L-Ascorbic acid



Sodium ascorbate



Ascorbyl palmitate

Figure 2.15 Chemical structure of L-Ascorbic acid, sodium ascorbate and ascorbyl palmitate

(Bauernfeind 1985)

enzymes, as well these compounds donate hydrogen atoms to break oxidative reactions in food product and *in vivo* (Kaur and Kapoor 2001).

Ascorbic acid can be found in all plant subcellular compartments. In fact, ascorbic acid may be found in meristemic and reproductive tissues, as well as in chloroplasts, where it is found at higher concentrations (20-50 mM) (Smirnoff and others 2001). Ascorbic acid concentration varies in different plant tissue, since some leaves normally may contain 2-5 μmol/g fresh weight although few species can obtain up to 10 times more (Smirnoff and others 2001).

The biosynthesis pathway of ascorbic acid is still not thoroughly understood, as the enzymes which participate in its synthesis or the type of regulation which determines the concentration of ascorbic acid in plants have not been elucidated. It is known that in some plants exposure to sunlight and ascorbic acid content are positively correlated (Smirnoff and others 2001). Therefore, several compounds have been suggested as ascorbic acid transporters in plant cells, but little is known about their participation in the intracellular distribution of ascorbic acid in plant cells (Smirnoff and others 2001).

Ascorbic acid is a compound which degrades over time in harvested commodities (Roura and others 2003). The first reaction in the ascorbic acid degradation pathway is the oxidation of ascorbic acid by several factors: enzymes, oxygen, light and high temperatures (Erdman and Klein 1982). From the oxidation of ascorbic acid is produced dehydroxyascorbic acid. Dehydroxyascorbic acid can be reduced back to ascorbic acid by the enzyme dehydroxyascorbic acid reductase, or by chemical reagents, i.e. hydrogen sulfide. If dehydroxyascorbic acid is not reduced back into ascorbic acid then it may be converted into diketogulonic acid. This reaction is irreversible, and by then the biological

activity of ascorbic acid is lost. Diketogulonic acid is decarboxylated eventually, forming xylosome, and afterwards furfural (Figure 2.16) (Erdman and Klein 1982; Smirnoff and others 2001).

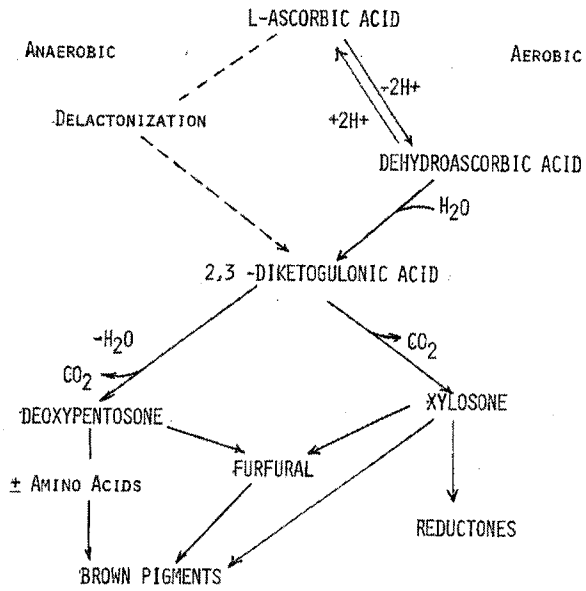


Figure 2.16 Degradation of *L*-Ascorbic acid

(Erdman and Klein 1982)

Quality and shelf life of minimally processed lettuce are usually limited by the development of detrimental texture changes (flaccid tissue and reduced turgidity) as well as browning. Browning in lettuce is related to the oxidation of phenolic compounds naturally present in the plant. This oxidative reaction is catalyzed by polyphenol oxidase in presence of oxygen, producing *ortho*-quinones which eventually polymerize to form melanins, which are dark brown, black or red pigments (Castañer and others 1996). Textural changes in vegetal products have been attributed mainly to dehydration, because of the decrease in turgor pressure within cells as well as cellular wall degradation. These

undesirable changes primarily occur during post harvesting stages of processing and storage. Therefore, it is necessary to implement preventive measures to maintain lettuce quality. Some of the treatments used to retard lettuce deterioration include the use of antioxidants and/or reducing agents such as ascorbic acid (Li and others 2001), and hydrocooling.

The purpose of the current study was to evaluate the effectiveness of ascorbic acid and water applied by two hydrocooling methods (immersion and spraying) on controlling browning and textural changes in green leaf type lettuce (Waldmann's dark green variety), thereby increasing its shelf life, antioxidant capacity and general acceptability. Thus, the overall objectives of this study are as follows:

1. To evaluate the effect of ascorbic acid and water applied by two hydrocooling methods on the physical and chemical properties of green leaf lettuce stored at 5 ^{o}C over storage time.
2. To evaluate the effect of ascorbic acid and water applied by two hydrocooling methods on the sensory attributes of green leaf lettuce stored at 5 ^{o}C over storage time.

CHAPTER III

EFFECTS OF ASCORBIC ACID APPLIED BY TWO HYDROCOOLING METHODS ON PHYSICAL AND CHEMICAL PROPERTIES OF GREEN LEAF LETTUCE DURING STORAGE AT 5 °C

ABSTRACT

The effectiveness of ascorbic acid applied by two hydrocooling methods to avoid textural changes and browning in Waldmann's dark green leaf lettuce (*Lactuca sativa*) was evaluated. Lettuce was immersed (in 1% ascorbic acid or tap water) or sprayed (in 1% ascorbic acid or tap water) or left untreated (control). Treatment solutions for immersion or spraying were applied at 5 °C for 2 min. Afterward lettuce was packaged and stored at 5 °C for up to 21 d. Analytical assays conducted included total ascorbate content, Trolox equivalent antioxidant capacity, total phenolic content, relative water content, instrumental color and texture. Lettuce was analyzed at 5 time intervals: before treatment application, and at d 1, 7, 14 and 21 of storage time. Hydrocooling of leaf lettuce by immersion or spraying using 1% ascorbic acid solution increased total ascorbate content for up to 7 days, with an increase of more than 300% in total ascorbate content on day 1 compared to its initial value before treatments. Application of ascorbic acid during hydrocooling of leaf lettuce by immersion maintained the relative water content of lettuce for 21 d, thereby resulting in a firmer texture compared to the other hydrocooling treatments and the untreated lettuce. Antioxidant capacity of leaf lettuce did

not increase in ascorbic acid treated lettuce that had increases in total ascorbate content. Hydrocooling with ascorbic acid by immersion increased the total phenolic content of leaf lettuce for up to 7 d, but this could be due to known interference that ascorbic acid has on the Folin-Ciocalteu assay. Further experimentation with the application of ascorbic acid during hydrocooling may result in its implementation as a preservation practice to extend shelf life of nutritionally fortified leaf lettuce.

Keywords: browning, textural changes, leaf lettuce, hydrocooling, ascorbic acid.

INTRODUCTION

Lettuce (*Lactuca sativa L.*) is one of the most popular green salad vegetables, and is used around the world. In recent years, the consumption of this vegetable has increased notably, making it one of the most preferred vegetable products in United States. Glaser and others (2001) reported that in 2000 the average American consumed 33 pounds of lettuce, the highest per capita amount on record. Overall, U.S. lettuce production has increased by 16% since 1992 (Glaser and others 2001). However, lettuce is a very perishable vegetable whose quality and shelf life are limited by the development of enzymatic browning and detrimental texture changes during postharvest stages.

Enzymatic browning in lettuce is a quality problem that has been linked to the enzymatic activity of phenylalanine ammonia lyase (Peiser and others 1998) and polyphenol oxidase (Tomas-Barberan and others 1997a). Wounding caused during lettuce harvesting and processing (handling and cutting) triggers an increase in the enzymatic activity of phenylalanine ammonia lyase and the production of new phenolic compounds that are substrates for oxidative enzymes such as polyphenol oxidase (Lamikanra and Watson 2001; Cantos and others 2002). Brown color development in lettuce is related to

the oxidation of phenolic compounds naturally present in the plant. This oxidative reaction is catalyzed by polyphenol oxidase in presence of oxygen, producing o-quinones which eventually polymerize to form melanins, which are dark brown, black or red pigments (Castañer and others 1996). Other physiological effects caused by wounding in lettuce include increases in respiratory rate and oxygen consumption, and the production of carbon dioxide and ethylene (Saltveit 1997; Barth and others 2004). Ethylene is a plant hormone produced at low rates by lettuce (Saltveit 2004b). However, wounding increases ethylene production considerably (Saltveit 2004a). Ethylene causes senescence in plant tissues (Saltveit 2004a), and also induces phenylalanine ammonia lyase activity (Ke and Saltveit 1988; Saltveit 1997). Detrimental textural changes in vegetable products such as leaf lettuce are caused mainly by dehydration, which provokes a decrease in turgor pressure within cells as well as cellular wall degradation (Alzamora and others 2000; Abbot and Harker 2004). These undesirable quality changes primarily occur during the post harvesting stages of processing and storage; therefore, it is necessary to implement preventive measures to maintain produce quality.

The most important factor that affects vegetables and fruit quality during postharvest stages is temperature, which influences enzymatic and other chemical reaction rates, as well as respiratory rate (Saltveit 2004c). Thus, lettuce should be cooled down promptly after harvest to maintain an adequate quality grade. According to Ferreira and others (1994), cooling retards respiration, ripening, senescence, dehydration and decay of vegetables and fruits, thus helping to maintain quality attributes such as appearance, texture and color, as well as to extend shelf life. Lettuce cooling may be performed by several methods such as vacuum-cooling, forced air-cooling, or by

hydrocooling. Hydrocooling is a procedure for removal of heat from freshly harvested commodities like leafy vegetables by using pure water at a lower temperature than the produce (Thompson 2004). Maintaining minimally processed fruits and vegetables at temperatures just above freezing throughout storage is also essential to the maintenance of their original sensory attributes (Bolin and Huxsoll 1991). Another treatment that has been used to retard lettuce deterioration during its storage is the use of antioxidants and/or reducing agents, such as sulfites (Li and others 2001). Sulfites are highly effective in controlling browning in vegetable products, but are currently subject to regulatory restrictions because of adverse effects on health such as asthmatic crises (Almeida and Nogueira 1995), or even in rare cases anaphylactic-like reactions may occur (Sapers 1993). Since 1986, the Food and Drug Administration has prohibited the use of sulfites on fruits and vegetables intended to be served or sold raw to consumers (Sapers 1993). Thus, the development of sulfite alternatives as browning inhibitors in raw vegetables is imperative.

One of the most promising choices as a browning inhibitor is ascorbic acid (Sapers 1993). This reducing agent, also known as vitamin C, has been used as a browning inhibitor in some fruits and vegetables, as well as in processed products, i.e. fruit juices (Bauernfeind 1985). Dong and others (2000) reported that dipping pear slices in a 1% ascorbic acid solution for 2 min delayed browning development in that product. The browning inhibitory effect of ascorbic acid consists of the reduction of the o-quinones generated from the oxidation of phenolic substrates of polyphenol oxidase, thus inhibiting the formation of dark pigments (Sapers and Hicks 1989). One additional advantage of using this compound as a food additive is that it is naturally present in many

vegetable products (Bauernfeind 1985). Vitamin C is one of the most abundant antioxidant compounds in produce, along with beta-carotene (Howard and others 1999).

Ascorbic acid is a water soluble compound whose antioxidant activity is due to its ease of losing electrons, thereby forming dehydroascorbic acid, which is a relatively stable dimer (Champion and others 2004). Hence, it protects compounds in the water soluble portion of cells and tissues by donating electrons to reactive oxidant species. Ascorbic acid also reduces tocopherol radicals back to their active form at the cellular membranes (Kaur and Kapoor 2001). This antioxidant has also proven to be an effective bactericidal agent (Burnham and others 2001). Moreover, ascorbic acid is Generally Recognized as Safe (GRAS) for its use as a food additive, without having any adverse reactions.

The purpose of the current study was to evaluate the effectiveness of ascorbic acid applied by two methods of hydrocooling (immersion and spraying) on controlling browning and textural changes in green leaf lettuce, thereby increasing its total ascorbate content and antioxidant capacity. Hydrocooling (immersion or spraying) with tap water and no treatment were used as comparative controls. Overall objectives of this study were to evaluate the effect of ascorbic acid and hydrocooling method on total ascorbate content, Trolox equivalent antioxidant capacity, total phenolic content, texture (firmness), relative water content and color of green leaf lettuce over 21 d of storage at 5 °C.

MATERIALS AND METHODS

Waldmann's dark green leaf lettuce heads were grown and harvested in Fort Collins, Co. Six lots of 60 lettuce seeds (Johnny's Selected Seeds, Albion, Me.) were

planted at different times in Colorado State University greenhouses from April to June 2004. Lettuce plants were transplanted to the field four weeks after the planting date. Four replicates of 55 lettuce heads were harvested at different dates from July to September of 2004. Harvesting was performed manually using a sharp knife to cut lettuce plants at the soil surface. Lettuce heads were transported to the laboratory in coolers containing ice within 30 min after harvesting, and prepared for treatments within one hr after harvesting by washing the heads under running tap water at 20 °C ± 1 °C for 5 min (washing step), and removing damaged outer leaves and dirt. Entire washed lettuce heads were placed into 68 L plastic containers (Sterilite, Townsend, Ma.) and allowed to drain for 10 min at room temperature (22-25 °C), 10 to 12 heads per container. After washing and draining the lettuce heads were randomly distributed for treatment application, with eleven heads per treatment.

Treatments

Five treatments were applied within 4 h after harvest time. These treatments included: 1) hydrocooling by immersion with 1% ascorbic acid w/v solution (ascorbic acid immersion), 2) hydrocooling by immersion with tap water (water immersion), 3) hydrocooling by spraying with 1% ascorbic acid w/v solution (ascorbic acid spraying), 4) hydrocooling by spraying with tap water (water spraying), 5) no treatment (control). Hydrocooling with tap water (immersion and spraying) and no treatment served as comparative controls. Treatment solutions for immersion or spraying (tap water or 1% ascorbic acid solution) were applied at 5 °C ± 1 °C for 2 min. The 1 % ascorbic acid solution was prepared 5-10 min before use diluting the ascorbic acid (Sigma, St. Louis, Mo.) in tap water at 5 °C. The pH of the 1% ascorbic acid solution was 3.0. Hydrocooling

by immersion was performed by soaking the washed lettuce heads for 2 min in a 68 L plastic container (Sterilite, Townsend, Ma.) containing 45 L of cooling solution (tap water or 1% ascorbic acid). Three or four lettuce heads were treated at a time, then removed from the cooling solution and placed in plastic containers to drain excess solution for 5 min. Hydrocooling by spraying was performed by placing the lettuce heads (one head at a time) into a 68 L plastic container (Sterilite, Townsend, Ma.), and spraying constantly (50 sprays/min for 2 min) using a 24 oz plastic spray bottle (The Bottle Crew®, West Bloomfield, Mi.) with the respective solution (tap water or 1% ascorbic acid) at a distance of 35-40 cm from the lettuce head. The heads were turned upside down at least twice during treatment, and then placed in a plastic container to drain excess solution for 5 min.

In all treatments and control the drained lettuce heads were packaged in 49 L closed twist tie 20 μm thick polyethylene moisture impermeable bags (Great Value, Wal-Mart®, Bentonville, Ar.) with one lettuce head per bag. Packaged lettuce heads were stored at 5 ^{o}C ± 1 ^{o}C for up to 21 d. The 11 lettuce heads within the same treatment where randomly assigned for analytical measurements as follows: three heads per treatment for texture analysis, two heads per treatment for relative water content, two lettuce heads per treatment for color analysis, and two lettuce heads per treatment for freeze-drying as a sample preparation step for total ascorbate content, total phenolic content and Trolox equivalent antioxidant capacity assays.

Samples were analyzed or freeze-dried at five different storage time intervals: before treatment application, and at d 1, 7, 14 and 21 of storage time. Four replicates were obtained for total ascorbate content, total phenolic content and Trolox equivalent

antioxidant capacity. Three replicates were run for texture analysis, relative water content and color analysis.

Sample preparation for total ascorbate content, Trolox equivalent antioxidant capacity and total phenolic content

At each analytical time, two outer entire leaves per lettuce head were removed and rinsed under running tap water at room temperature (~20 °C) for 2 min. This step was performed to remove the ascorbic acid adhered to the outside of the leaves, since in preliminary tests it was found that some ascorbic acid from the treatments remained on the outside of the leaves. Then the leaves were blotted dry with paper towels. Colorless lower leaf parts were removed (midrib), and the remaining colored leaf tissue (photosynthetic tissue) was frozen with liquid nitrogen. Afterward frozen tissue was freeze-dried and ground finely (sieve #20), and ground dried tissue was stored in 16 mL screw cap plastic tubes at -20 °C until either phenolic extraction or analysis. After taking the leaves to be freeze-dried, the lettuce heads were re-packaged and stored as previously described until the next sampling time.

Phenolic extraction for measurement of Trolox equivalent antioxidant capacity and total phenolic content

Four hundred mg of ground dried sample were dissolved in 10 mL of 80% acetone (Sigma Co., St. Louis, Mo.) in a 16 mL screw cap plastic tube. Each tube was rotated for 2 h, and then centrifuged at 4000 rpm for 20 min. One mL of supernatant was poured in a 1.7 mL Eppendorf tube and vacufuged at 45 °C to dryness (aprox 2.5-3 h). Tubes with extracts were stored at –20 °C for further analysis. Each tube with extract was reconstituted previous to analysis by adding 1 mL of 80% acetone.

Total ascorbate content

Total ascorbate content (TAC) was determined by using a modification of the HPLC method published by Dale and others (2003). Twenty five mg of ground dried sample were mixed with 300 μL of 3% w/v dithiothreitol (EMD Chemicals, Inc., Darmstadt, Germany) in HPLC grade methanol (EMD Chemicals, Inc., Darmstadt, Germany) in a 1.7 mL Eppendorf tube, and vortexed 15 sec. Then 700 μL of 36 m*M* phosphate buffer solution (pH 7.26) were added, and the mixture was vortexed for 15 sec. Tubes were rotated for 15 min at 4 °C, and centrifuged for 5 min at 4000 rpm, and the supernatant was collected and kept cool. A second extraction was performed, and all collected supernatant was mixed and filtered through a syringe filter 0.2 μm into an amber HPLC vial and analyzed by HPLC chromatography (Hewlett Packard Model 1050 Series, Palo Alto, Ca.) using Chem Station for LC Rev A. 09.01 (Agilent Technologies, Palo Alto, Ca.) software. Ascorbic acid was eluted in an Inertsil C4 column at a flow of 1 mL/min using a mobile phase of 50 m*M* phosphoric acid (EM Science, Gibbstown, N.J.) as eluent A, and 100% methanol (EMD Chemicals, Inc., Darmstadt, Germany) as eluent B, both solvents HPLC grade. Effluent was monitored at 254 nm. Results were obtained by using a standard curve for ascorbic acid (Sigma, St. Louis, Mo.) prepared the same day of determination, and reported as mg of total ascorbate content per g of sample dry weight basis (mg of TAC/g dw). Analyses were run in duplicate.

Trolox equivalent antioxidant capacity

Trolox equivalent antioxidant capacity (TEAC) was measured using a microplate ABTS (2,2'- azinobis-(3-ethylbenz-thiazoline-6-sulphonic acid) radical cation assay, which is a modification of the method developed by Wilson (2004) based on the

analytical assay published by Miller and Rice-Evans (1997). Twenty five μL of sample were mixed with 250 μL of ABTS solution in a microplate well, and after one min of reaction the absorbance was read at 734 nm in a Spectra Max Plus (Molecular Devices, Sunnyvale, Ca.) spectrophotometer using software Softmax Pro version (Molecular Devices, Sunnyvale, Ca). ABTS solution was prepared by mixing 40 mg of ABTS (Calbiochem, Darmstadt, Germany) with 15 mL of nanopure water. Then 2-3 g of manganese oxide (Sigma, St. Louis, Mo.) were added to the ABTS solution, and after 20 min the solution was filtered through 0.2 μm syringe-end filter. The absorbance of the ABTS solution was then read at 734 nm in a spectrophotometer, adjusting solution absorbance to 0.700 by diluting with 5 m*M* phosphate buffer solution pH 7.4. Once the ABTS solution absorbance was adjusted it was kept at 30 °C. Antioxidant capacity was reported as Trolox equivalent antioxidant capacity per g of sample dry weight basis (TEAC/g dw), and was calculated by comparing to a standard curve based upon Trolox (Aldrich Chemicals, Milwaukee, Wi.). Analyses were run in triplicate.

Total phenolic content

Total phenolic content was measured by using a modified method published by Spanos and Wrolstad (1990). Fifty μL of reconstituted extract were mixed with 450 μL of nanopure water in a 16 ml glass tube, then 2.5 mL of 1/10 dilution of Folin-Ciocalteu reagent (Sigma-Aldrich, Inc., St. Louis, Mo.) were added. The mixture was vortexed for 10 sec, and after 5 min of reaction 2 mL of 7.5% w/v sodium carbonate (Fisher Chemicals, Fair Lawn, N.J.) were added, and the mixture was vortexed for 10 sec. Tubes were placed in a hot water bath at 45 °C for 15 min. Then, samples were cooled at room temperature, and absorbance solution was read at 765 nm in a Spectra Max Plus

(Molecular Devices, Sunnyvale, Ca.) spectrophotometer using software Softmax Pro version (Molecular Devices, Sunnyvale, Ca.). Total phenolic content was calculated by derivation from a standard curve based upon gallic acid (Sigma, St. Louis, Mo.), and reported as mg of gallic acid equivalent per g of sample dry weight basis (mg of GAE/g dw). Analyses were run in triplicate.

Texture

Texture (firmness) was measured using a modification of a method used by Prakash and others (2000). Maximum force of compression was used as an indicator of firmness in this produce. Three entire leaves per lettuce head were taken at each analytical time, using only the outer leaves at the time. The lettuce heads were packaged and stored as previously described until needed for analysis. The leaves were cut into square analytical samples of aprox 5 x 5 cm taken exclusively from upper parts of leaves (excluding ribs), to obtain 3 analytical samples per leaf (n = 3). Analytical samples were measured individually by placing one sample at a time into a Kramer-Shear attachment (TA-91) of the TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y.) using software Texture Expert for Windows version 1.0 (Stable Micro Systems, Surrey, England). The Kramer-Shear blade (TA-91) was forced through the sample moving downward at 2.5 mm/s, and reported as maximum force in compression (g). Analytical samples were kept at 5 ^{o}C until measurement time.

Relative water content

Relative water content was calculated using the method described by Kramer (1983). Two outer entire leaves per lettuce head were taken at each respective analytical time; then the lettuce heads were packaged and stored as previously described until the

next analytical time. Entire leaves were weighed individually, and this measurement was recorded as fresh weight. The leaves then were placed into a humidified chamber made with a 1000 mL plastic beaker containing 200-250 mL of distilled water covered with a 16 x 22 cm impermeable plastic zip bag (Ziploc®, Racine, Wi.), and sealed to avoid moisture exchange. The leaves remained for 24 h in the humidified chamber at 4 °C in darkness. After this step the leaves were weighed and reported as turgid weight. The leaves were then dried for 48 h at 80 °C in a forced air oven, weighed and reported as dry weight. Relative water content was reported as percentage of relative water content (% RWC), and it was computed using the following formula:

$$\% \text{ RWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \times 100$$

Color

Color was determined using a method cited by Prakash and others (2000). Two entire leaves per lettuce head were taken at each respective analytical time, using only the outer leaves at the time. The remaining portion of the lettuce heads were packaged and stored as previously described until the next analytical time. The leaves were cut into pieces (approx 10 g) to cover the bottom of the spectrocolorimeter cell. Each analytical sample was placed into the chamber of a HunterLab ColorFlex spectrocolorimeter (Hunter Associates Laboratory, Inc., Reston, Va.) using HunterLab Software A60-1010-694 Manual version 5.0 (Hunter Associates Laboratory, Inc., Reston, Va.) and read. L^*, a^* and b^* values were obtained by direct measurement of the samples, and three measurements were obtained for each determination.

Statistical analysis

Data were analyzed using a 2-way ANOVA (5 x 5 factorial design) with 5 treatments (including control) x 5 time intervals when analytical measurements were run, that were: 0 (before application of treatments), 1, 7, 14 and 21 d of storage time. Statistical analysis of the data was conducted using the SAS System Mixed Procedure (SAS Inc. version 9.1, Cary, N.C.). When differences between means were found, comparisons were determined using Least Significant Differences test ($P < 0.05$).

RESULTS AND DISCUSSION

Total ascorbate content

Total ascorbate content (TAC) was not different ($P > 0.05$) among treatments before treatment application (Figure 3.1, Appendix I). Hydrocooling of leaf lettuce by immersion or spraying using 1% ascorbic acid solution increased total ascorbate content for up to 7 days, with an increase on day 1 of 318% and 308% in total ascorbate content for immersed and sprayed lettuce respectively, compared to their initial values before treatments. Ascorbic acid immersed lettuce had the highest total ascorbate content of all treatments ($P < 0.05$) on d 1, 7 and 14 (5.00, 4.50 and 1.80 mg of TAC/g dw respectively). Lettuce hydrocooled by ascorbic acid spraying treatment had the second highest total ascorbate content (4.01 mg of TAC/g dw, $P < 0.05$) on d 1, but dropped on d 7 and 14 (1.69 and 0.28 mg of TAC/g dw respectively), to levels similar to water treated and control lettuce samples. Differences in the increase of total ascorbate content of ascorbic acid treated lettuce were attributed to the hydrocooling method. Roura and others (2003) also reported an increase in ascorbic acid content of Romaine lettuce

dipped for 4 min in a 0.5% ascorbic acid solution at 20 °C. According to Vigneault (2000) during hydrocooling treatment the cooling solution flows to inside the produce due to a pressure gradient created in the product surface. Such pressure gradient is caused by a volume reduction of gas contained in produce surface cells due to the reduction in the produce temperature. Temperature gradient between the cooling media and the warmer produce facilitates the diffusion of cooling solution into produce tissue during hydrocooling (Vigneault 2000). Cooling solution uptake could have been larger in hydrocooling by immersion than in spraying because of the additional pressure exerted for the cooling solution over the produce when immersed (Vigneault 2000). Regardless of the analytical time, there were no significant differences ($P > 0.05$) in total ascorbate content for water treated and control samples. Therefore, hydrocooling by immersion or spraying using cold water did not prevent postharvest decreases in total ascorbate content in leaf lettuce.

Ascorbic acid is a labile compound that is depleted gradually in vegetable products during postharvest stages (Lipton and Ryder 1989; Heimdal and others 1995; Ihl and others 2003). In this study, total ascorbate content was measured using dithiothreitol as an antioxidant (Dale and others 2003) to reduce the dehydroascorbic acid of the sample to ascorbic acid. According to Roura and others (2003) ascorbic content of lettuce decreases over storage time. These researchers reported a decrease of 50% of ascorbic acid content in untreated Romaine lettuce after 4 d, but the decrease in total ascorbate content in the current study was slower. In the current study the total ascorbate content of untreated leaf lettuce decreased 39% by d 7, and 66% by d 14 (compared to its total ascorbate content before the application of treatments). Moreover, the total ascorbate

content of water treated lettuce by d 14 lowered 83% for immersed lettuce, and 88% for sprayed lettuce. The ascorbic acid immersed lettuce had a total ascorbate content on d 14 similar ($P > 0.05$, 1.80 mg TAC/gm dw) to its total ascorbate content before the application of treatments (1.57 mg TAC/gm dw), meanwhile there were no significant differences ($P > 0.05$) in the total ascorbate content of the ascorbic acid sprayed lettuce on d 7 (1.69 mg TAC/gm dw) and before treatment application (1.30 mg TAC/gm dw). Thus, hydrocooling of leaf lettuce using 1% ascorbic acid solution increased the total ascorbate content for this vegetable for up to 7 d, despite the fact that the total ascorbate content of leaf lettuce decreased over storage time.

Trolox equivalent antioxidant capacity

Mean results of Trolox equivalent antioxidant capacity of Waldsmann's leaf lettuce ranged from 250.2 to 371.1 μM TEAC/g dw (Table 3.2, Appendix II). There were no significant differences ($P > 0.05$) in Trolox equivalent antioxidant capacity among any treatments at any storage time interval. Also, although there were some differences within water sprayed and control products over storage time, there were no differences in Trolox equivalent antioxidant capacity for any treatment on d 21 compared to d 1. Trolox equivalent antioxidant capacity of water immersion and ascorbic acid treatments were not different ($P > 0.05$) throughout the entire study. These results imply that the increase in total ascorbate content in treatments using ascorbic acid solution was not sufficient to affect the antioxidant capacity of leaf lettuce. It is possible that the antioxidant capacity of this lettuce variety is based mostly in compounds other than vitamin C, such as phenolics. Some phenolic compounds have higher antioxidant capacity than β-carotene and vitamin C (Cao and others 1996). According to Chu and others (2002), the

contribution of vitamin C (total ascorbate) content to the total antioxidant activity in lettuce is low, since they reported vitamin C contributes 8.1% to the total antioxidant activity of lettuce.

There is a possible issue to discuss about sample preparation procedure for this assay. Extracts of phenolic compounds were subjected to 45 ^{o}C for 2 h during sample preparation. It is possible that the application of this mild heat treatment could have caused loss of vitamin C as well as other heat sensitive compounds. Thus, it is recommended to minimize heat application during sample preparation of phenolic extracts to avoid losses of ascorbic acid.

Total phenolic content

There were significant differences in total phenolic content among Waldmann's leaf lettuce ($P < 0.05$) before application of treatments, since water and control samples had a higher total phenolic content (treatment means ranged from 39 to 42.5 mg GAE/mg dw) than the other treatments (treatment means ranged from 34.2 to 38.3 mg GAE/mg dw) (Table 3.3, Appendix III). Lettuce treated by ascorbic acid immersion had higher total phenolic content ($P < 0.05$) on d 1 and 7 but not on d 14 and 21 compared with levels before treatment. Meanwhile ascorbic acid sprayed lettuce had higher total phenolic content on d 1, but not significantly. Total phenolic content of ascorbic acid immersed lettuce was higher ($P < 0.05$) than the total phenolic content of ascorbic acid sprayed samples on d 1 and 7, but not ($P > 0.05$) on d 14 or 21. Water treatments did not differ in total phenolic content ($P > 0.05$) at any storage time. Control and water immersion treatments maintained their total phenolic content throughout the experiment ($P > 0.05$), but water sprayed lettuce had a lower total phenolic content ($P < 0.05$) on d

21 compared with its total phenolic content before treatment application. There were significant differences in total phenolic content among treatments at all time intervals, except on d 14. Total phenolic content of all treatments on d 14 were not significantly different than their total phenolic content before the application of treatments ($P > 0.05$). However, all treatments presented browning on d 14. Total phenolic content in food products is important since phenolic compounds are the substrates for oxidative reactions to produce dark pigments (Sapers 1993), though only a few phenolics serve as a substrate for oxidative enzymes such as polyphenol oxidase (Lamikanra and Watson 2001). Therefore, browning in leaf lettuce depends mostly on the type of phenolic compound present in the lettuce that could serve as a substrate for the enzyme polyphenol oxidase, and not on the total phenolic content of the leaf lettuce. It is possible that the changes observed in total phenolic content of ascorbic acid treated lettuce may be attributable to the hydrocooling method, as well as to the analytical method used in the current study. It is known that reducing agents such as ascorbic acid and sulfites can contribute to the total phenolic content estimates, and thus interfere with the determination of total phenolic content by the Folin-Ciocalteu method (Singleton and Rossi 1966). Thus, the ascorbic acid solution absorbed by the lettuce samples during hydrocooling probably was sufficient to cause increases in total phenolic content measurable by the Folin-Ciocalteu method.

Relative water content

It has been observed that leaf lettuce is less tolerant to dehydration than other lettuce varieties such as Iceberg (Salveit 2004a). Relative water content (% RWC) of Waldmann's leaf lettuce was measured, and means ranged from 94.07 % to 99.79 %

RWC throughout the experiment, indicating that all samples were well hydrated during the experiment (Table 3.4, Appendix IV). There were no differences in relative water content among treatments ($P > 0.05$) before treatment application, or at d 1 or 7 of storage. The relative water content of untreated lettuce ($P < 0.05$) declined at d 14 and 21. Ascorbic acid immersed lettuce was the only treatment that maintained its relative water content ($P > 0.05$) for 21 d. Maintenance of water content in ascorbic acid immersion treatment could result from the production of cellular osmolyte compounds caused by the exposure of leaf lettuce to a stress factor, like the acidic pH of the ascorbic acid solution (3.0). Concentration of cellular osmolytes such as sugars, salts and other hydrophilic solutes contained in vacuoles is determinant for the cell water potential, which is the capacity of a cell to accept (and retain) water (Alzamora and others 2000). Water loss in vegetables and fruits produces wilting due to a decrease in turgor pressure within tissue cells (Hopkins and Huner 2004). It is important for the leaf lettuce to remain well hydrated to maintain sensory attributes such as texture or appearance for a longer storage time.

Texture

Texture (firmness) of Waldmann's leaf lettuce was measured, and means before treatments (2688 to 2746 g) did not differ ($P > 0.05$) (Table 3.5, Appendix V). All treatments showed a significant increase in texture (firmness) over the 21 d storage time ($P < 0.05$). Across treatments, firmness did not differ ($P > 0.05$) from that of the control treatment at any storage time, with the exception of the ascorbic acid immersion treatment, which had a firmer texture ($P < 0.05$) than all other treatments at days 1, 14 and 21. Thus, hydrocooling by spraying with any solution did not affect lettuce firmness.

Ascorbic acid immersed lettuce was firmer than untreated lettuce at d 1 ($P < 0.05$), and firmer than all other treatments at d 14 and 21. Textural changes in vegetable produce are associated with dehydration, which causes a loss in turgor pressure in tissue cells (Alzamora and others 2000, Abbot and Harker 2004). Ascorbic acid immersion treatment held its percentage of relative water content during the entire experiment, thereby contributing to a firmer texture in leaf lettuce for a longer storage time. Differences in the results for firmness of the ascorbic acid treated leaf lettuce could be attributed to the hydrocooling method. Perhaps ascorbic acid immersed lettuce absorbed a larger amount of ascorbic acid solution than ascorbic acid sprayed lettuce, based on the larger increase of total ascorbate content in immersed lettuce compared to the sprayed samples. The application of 1% ascorbic acid during hydrocooling of leaf lettuce by immersion maintained the relative water content of the leaf lettuce for 21 days, thereby resulting in a firmer texture compared to the other hydrocooling treatments and the untreated lettuce. Texture of leaf lettuce of all treatments increased in firmness over the storage time.

Color

The application of ascorbic acid or water hydrocooling did not affect L^* (bright-dark scale) (Figure 3.7), and b^* (yellow-blue scale) values of leaf lettuce throughout the study ($P > 0.05$) (Appendix VI). Regarding the a^* (red-green scale) values, only the sprayed (ascorbic acid and water) lettuce maintained their a^* values throughout the 21 d of storage (Figure 3.6, Appendix VI), and the other treatments increased over time ($P < 0.05$), since water immersed lettuce increased at d 7, ascorbic acid immersed at d 14, and control lettuce at d 21. Lopez-Galvez and others (1996) stated that a^* value is related to the visual quality of lettuce, and that decreases in L^* values indicate produce darkening

(Bolin and Huxsoll 1991). Bolin and Huxsoll (1991) reported decreases in a^* value of iceberg lettuce presumably attributed to chlorophyll losses. Visible brown spots were observed in samples of all treatments at d 14 of storage, and those brown spots increased in size considerably at d 21. It is possible that browning was promoted by the accumulation of ethylene within the packaging bag. Lettuce produces low amounts of ethylene, but is very sensitive to damage due to this plant hormone (Ke and Saltveit 1988; Saltveit 2004a, 2004c). Wounding due to harvesting and lettuce processing induces ethylene production in this produce (Saltveit 2004a). Ethylene promotes lettuce enzymatic browning and quality deterioration, since it increases respiratory rate and the enzymatic activity of phenylalanine ammonia lyase. Moreover, ethylene causes senescence in lettuce when accumulates in enclosed environments during transport and/or storage of this vegetable (Kim and Wills 1995; Saltveit 1997: Saltveit 2004a, 2004b). Thus, the a^* value of leaf lettuce decreased during storage time in lettuce hydrocooled by water or 1% ascorbic acid, and in control lettuce. Hydrocooling by spraying with water or 1% ascorbic acid solution did not affect the color of leaf lettuce in any value.

CONCLUSIONS

Hydrocooling of leaf lettuce by immersion or spraying using 1% ascorbic acid solution increased total ascorbate content for up to 7 days, with an increase of more than 300% in total ascorbate content on day 1 compared to its initial value before treatments. Hydrocooling of leaf lettuce using water did not prevent the decrease of total ascorbate content in leaf lettuce over storage time. Ascorbic acid immersed lettuce maintained its relative water content throughout the 21 days of storage, and its texture (instrumental

measurement) was firmer than all other treatments on days 14 and 21. The application of water or ascorbic acid during hydrocooling of lettuce did not affect the antioxidant capacity of leaf lettuce. Hydrocooling with ascorbic acid by immersion increased the total phenolic content of leaf lettuce for up to 7 d, but this could be due to the known interference that ascorbic acid has on the Folin-Ciocalteu assay. Meanwhile, hydrocooling with water (immersion or spraying) or with ascorbic acid by spraying did not improve the total phenolic content of leaf lettuce. Application of ascorbic acid during hydrocooling of leaf lettuce by immersion maintained the relative water content of lettuce for 21 d, thereby resulting in a firmer texture compared to the other hydrocooling treatments and the untreated lettuce. Texture of leaf lettuce of all treatments increased in firmness over the storage time. a^* value (instrumental color red-green scale) of leaf lettuce decreased during storage time in hydrocooling with 1% ascorbic acid, and in control lettuce. Hydrocooling by spraying with water or 1% ascorbic acid solution did not affect the color of leaf lettuce in any value. Further experimentation with the application of ascorbic acid during hydrocooling may result in its implementation as a preservation practice to extend shelf life of nutritionally fortified leaf lettuce.

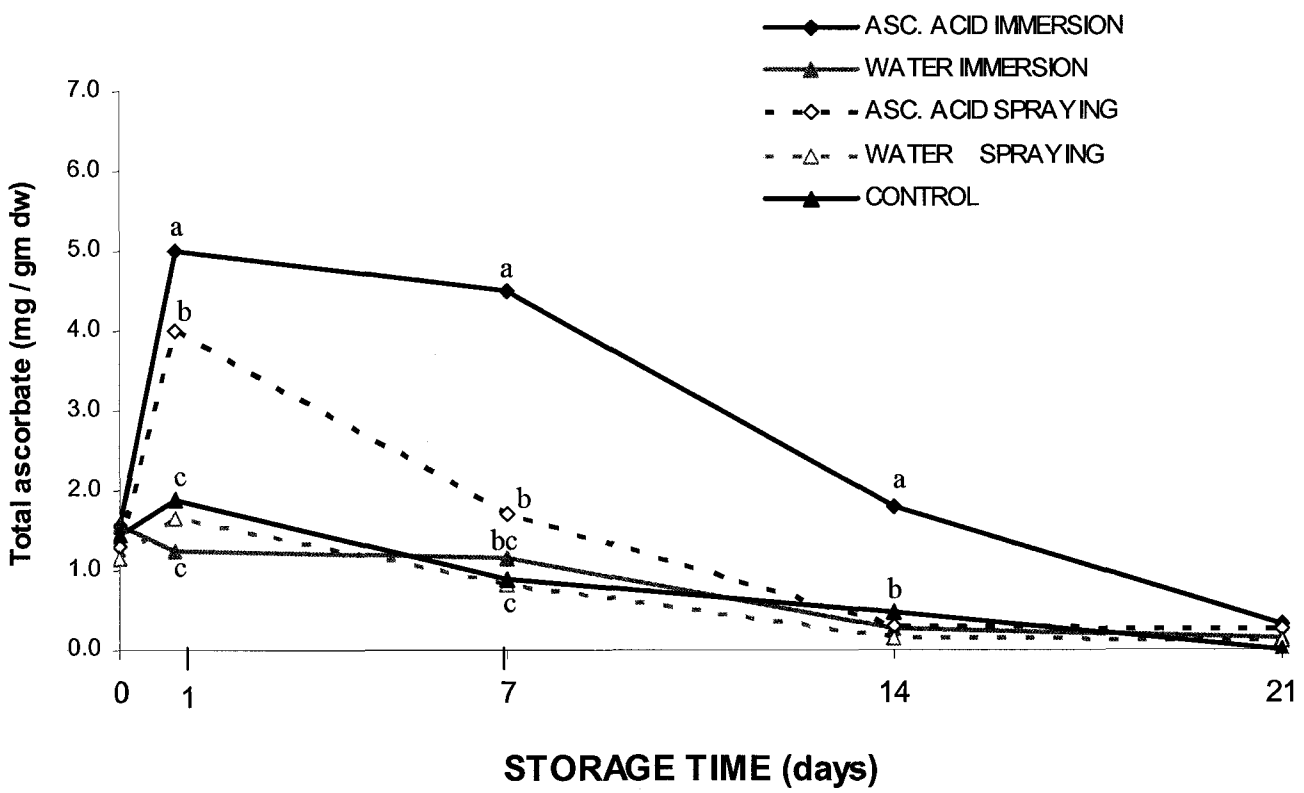


Figure 3.1 Means (mg of total ascorbate/gm dry wt) of the total ascorbate content of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 0.85 mg of total ascorbate/gm dry wt. Different letters within the same time interval are significantly different.

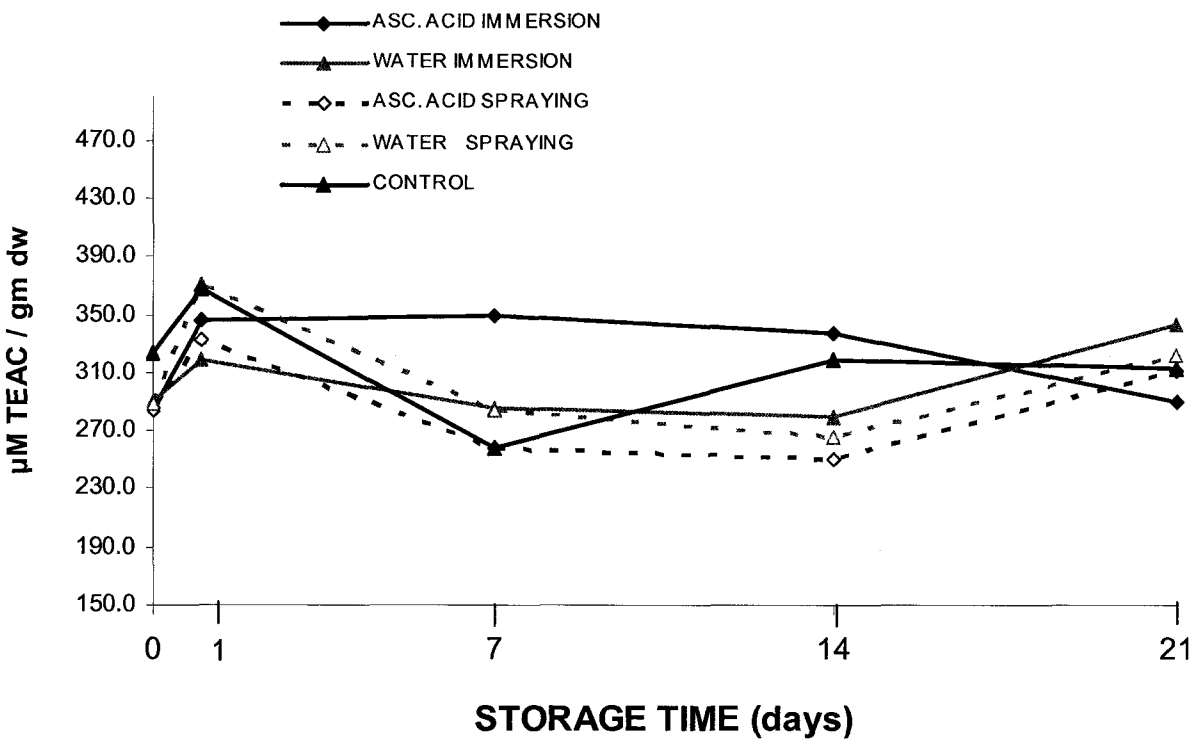


Figure 3.2 Means (μM TEAC/gm dry wt) of the Trolox equivalent antioxidant capacity (TEAC) of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 95.19 µmol TEAC / gm dry wt. Different letters within the same time interval are significantly different.

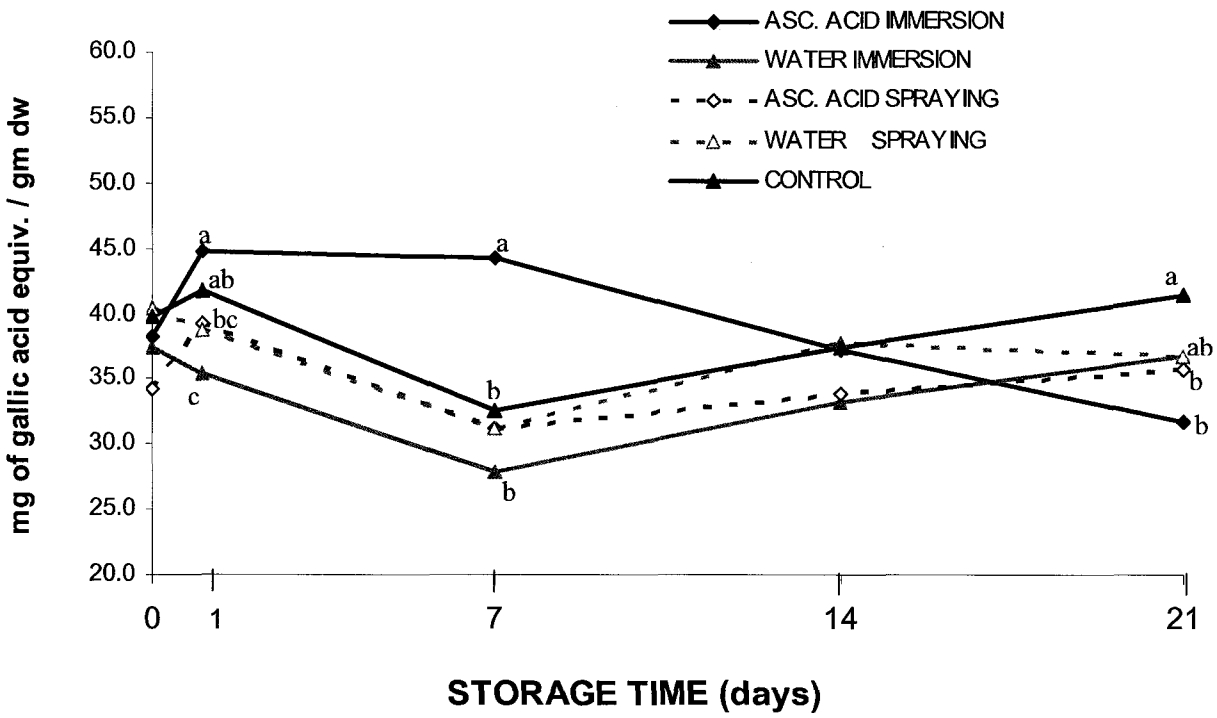


Figure 3.3 Means (mg of gallic acid equivalent/gm dry wt) of the total phenolic content of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 5.19 mg of gallic acid equivalent / gm dry wt. Different letters within the same time interval are significantly different.

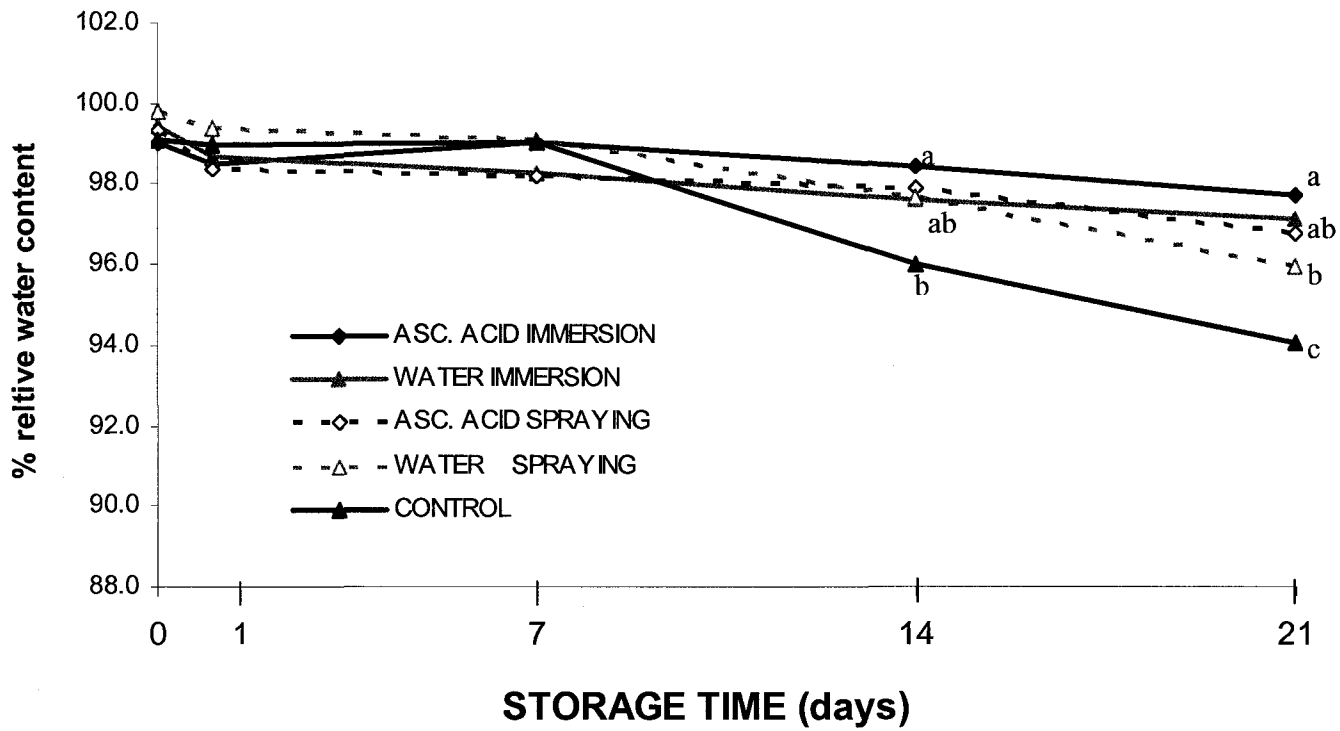


Figure 3.4 Means (% RWC) of the relative water content of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 ^{o}C for 0 (before treatments), 1, 7, 14 and 21 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 1.61 % RWC. Different letters within the same time interval are significantly different ($P < 0.05$).

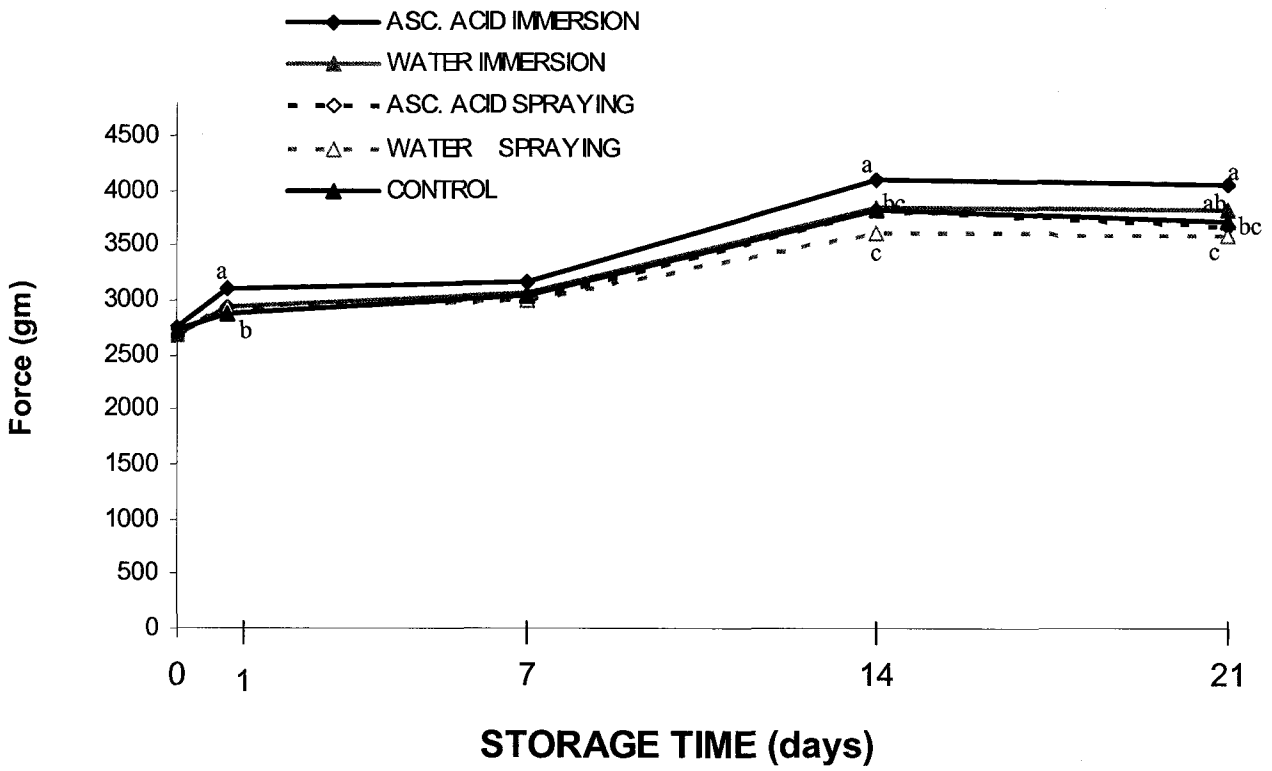


Figure 3.5 Means (gm of force) of the texture of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 221.1 gm. Different letters within the same time interval are significantly different.

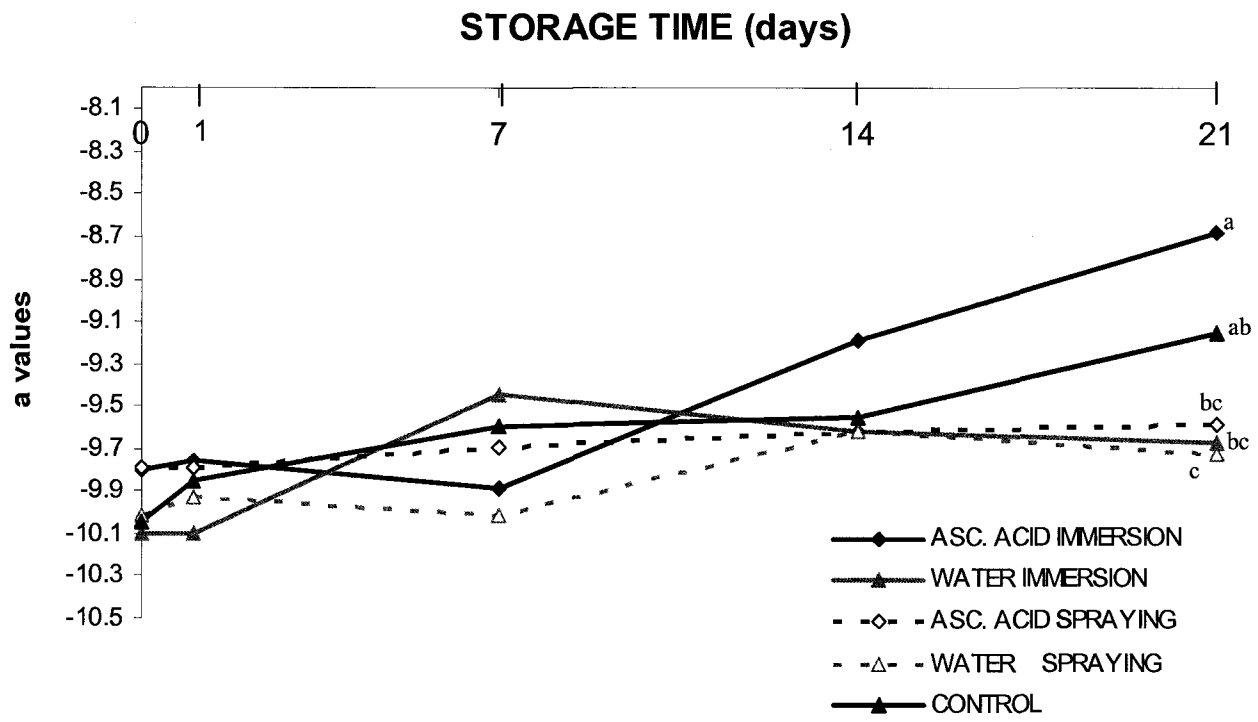


Figure 3.6 Means of the a^* values of instrumental color of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 0.56. Different letters within the same time interval are significantly different.

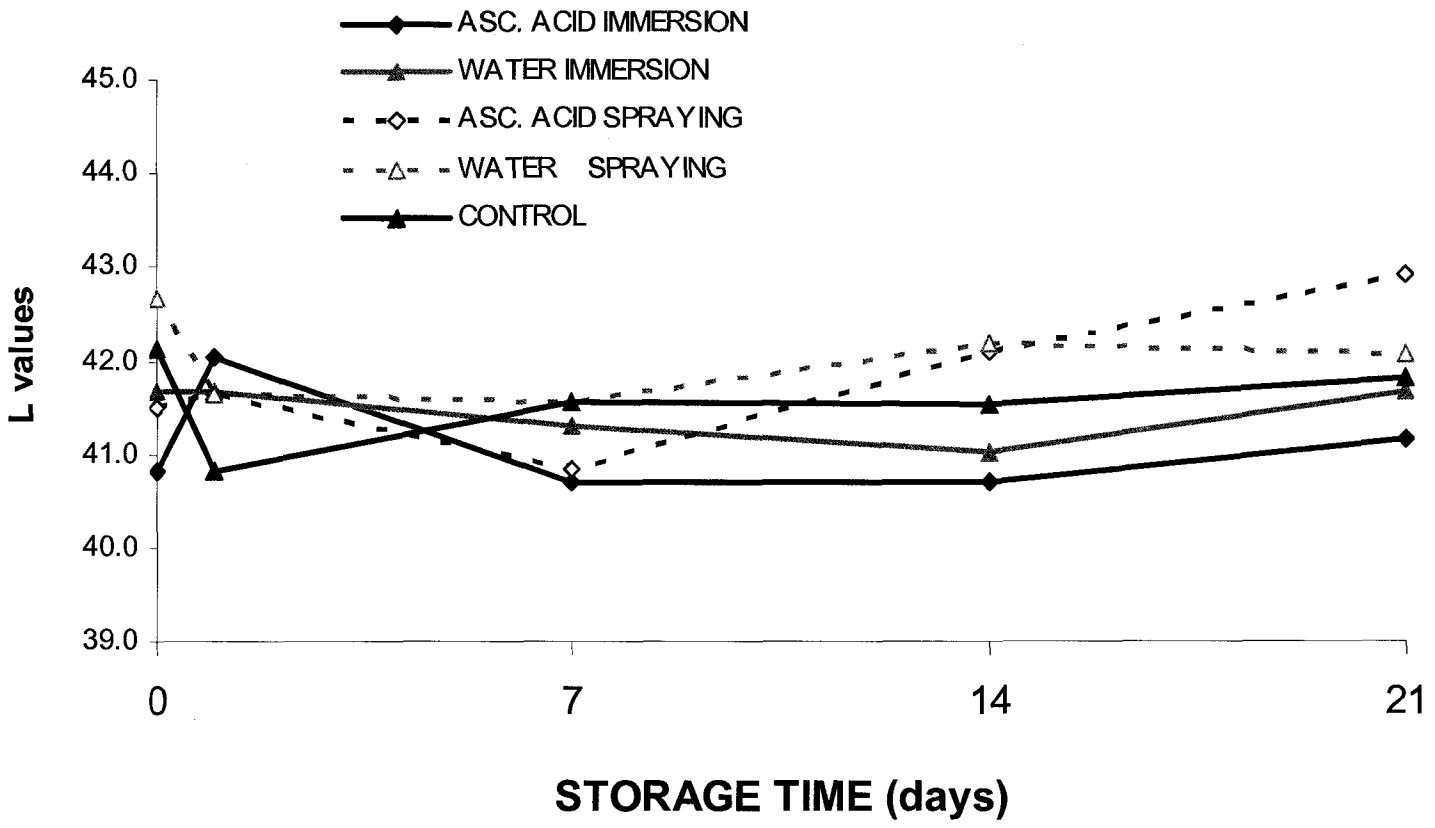


Figure 3.7 Means of the L^* values of instrumental color of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 3.48.

CHAPTER IV

SENSORY ATTRIBUTES OF GREEN LEAF LETTUCE TREATED WITH ASCORBIC ACID BY TWO HYDROCOOLING METHODS AND STORED AT 5 °C

ABSTRACT

The effect of ascorbic acid applied by two hydrocooling methods on sensory attributes of Waldmann's leaf green lettuce was studied. Lettuce was immersed (in 1% ascorbic acid or tap water) or sprayed (with 1% ascorbic acid or tap water) or left untreated (control). Treatment solutions for immersion or spraying were applied at 5C for 2 min. Lettuce then was packaged and stored at 5C for up to 14 days. Lettuce was evaluated at days 1, 7 and 14 of storage for the sensory attributes of appearance, color intensity, flavor, texture, bitterness, tartness and general acceptability. On day 1 ascorbic acid sprayed lettuce was less bitter and had a more acceptable appearance, flavor and overall acceptability than ascorbic acid immersed lettuce. Control lettuce maintained all sensory attributes during the study except color (it was darker on day 14). Lettuce hydrocooled by spraying with 1% ascorbic acid increased in firmness and became less bitter over the storage time, while hydrocooling by immersion using 1% ascorbic acid negatively affected lettuce appearance and resulted in a darker color over storage time. Water spraying was the treatment with the least acceptable appearance and overall acceptability on day 14 of storage time. Further experimentation with the application of

ascorbic acid during hydrocooling is needed to fully assess its usefulness in maintaining the quality of leaf lettuce during storage.

Keywords: lettuce, ascorbic acid, hydrocooling, sensory attributes

INTRODUCTION

One of the most popular vegetables around the world is lettuce *(Lactuca sativa L.)*, which is frequently used in green salads. Lettuce is used as a raw fresh product, and is never canned, dried, or frozen. In recent years the consumption of this vegetable has increased, mainly as a minimally processed lettuce, though fresh-cut or fresh processed lettuce also has increased in commercial importance (Glaser *et al.* 2001). Minimally processed lettuce is the vegetable subjected only to such processing steps as washing and packaging (Glaser *et al.* 2001). Fresh-cut lettuce is the produce sold as bagged lettuce chopped alone or as salad blends (shredded lettuce combined with other fresh vegetables and ingredients) (Glaser *et al.* 2001). Fresh-cut lettuce has several advantages, including less waste disposal, minimum home processing and more product variety (Delaquis *et al.* 2000). However, processing reduces the quality of this vegetable since some lettuce sensory attributes such as color, texture and appearance can be negatively affected. Regardless of variety, a high quality lettuce should be clean, free of brown spots, and its color should be bright green or red depending on the variety. Moreover, the appearance of high quality lettuce should be crisp, turgid and firm without damaged parts (Saltveit 2004). Lettuce flavor is mild and sometimes bitter depending on the variety (Lipton and Ryder 1989).

Two of the most important quality problems that impact the sensory attributes and shelf life of minimally processed and fresh-cut lettuce are browning and textural changes.

Enzymatic browning in lettuce is a quality problem associated with wounding caused by processing steps such as cutting during harvesting and handling (Saltveit 2004). Wounding promotes an increase in the enzymatic activity of phenylalanine ammonia lyase (Peiser *et al.* 1998), polyphenol oxidase (Tomas-Barberan *et al.* 1997) and peroxidase (Prestamo and Manzano 1993). The increase in enzymatic activity of phenylalanine ammonia lyase promotes the production of new phenolic compounds which serve as substrate for oxidative enzymes such as polyphenol oxidase (Cantos *et al.* 2002) in the presence of oxygen, forming melanins (dark pigments) (Castañer *et al.* 1996). Textural changes in vegetable products including lettuce are associated with dehydration, because of the reduction in turgor pressure within cells as well as cellular wall degradation (Alzamora *et al.* 2000). There are differences among lettuce varieties for their tolerance to dehydration. For instance, leaf lettuce is more susceptible to dehydration than other lettuce varieties such as crisphead, since leaf lettuce has a larger exposed surface-to-volume ratio (Lipton and Ryder 1989). These quality problems mostly occur during the post harvesting stages of processing and storage, and result in a need to implement preventive measures to maintain produce quality attributes.

Lettuce is a perishable vegetable, and depending on the variety its storage life is 2-4 weeks when stored at temperatures close to 0C (Saltveit 2004). The most important factor that determines lettuce quality during postharvest stages is storage temperature, since it affects lettuce enzymatic activity and dehydration rate (Saltveit 2004). Rapid cooling after harvesting helps to maintain lettuce sensory attributes such as flavor, color, texture and appearance for longer time periods (Bolin and Huxsoll 1991), because cooling retards respiration, ripening, senescence, dehydration and decay in freshly

harvested commodities (Ferreira *et al.* 1994). Thus, it is critical to cool lettuce as promptly as possible after harvesting, and to store it at a cold temperature close to 0C to reduce its postharvest metabolism, thereby extending lettuce shelf life (Kang and Saltveit 2002). Two methods used to cool leafy vegetables are vacuum-cooling and hydrocooling. Hydrocooling is a procedure used to cool freshly harvested vegetables and fruits using cold water (Thompson 2004). There are several hydrocooling methods, but the most commonly used to cool fresh commodities are hydrocooling by immersion and the shower type or hydrocooling by spraying (Salunhke *et al.* 1984; Thompson 2004). Hydrocooling by spraying consists of spraying cold water over the warmer produce. In hydrocooling by immersion, the commodities are dipped in circulating cold water, thereby reducing product temperature. Immersion hydrocoolers usually cool produce slower than shower coolers because water flows at slower rates past the product (Thompson 2004).

Another treatment to avoid vegetable product deterioration is the use of reducing agents or antioxidants such as sulfites and ascorbic acid (Sapers and Hicks 1989; Sapers 1993). Sulfites are effective browning inhibitory agents, but these compounds were prohibited for use in produce intended to be sold as a raw products in 1986 by the FDA due to reports of adverse reactions attributed to their consumption, mostly in asthmatic persons (Sapers 1993). Ascorbic acid, or vitamin C, is a good alternative as a browning inhibitor for vegetables. This water soluble vitamin is one of the most abundant antioxidant compounds naturally present in many vegetable products (Bauernfeind 1985; Howard *et al.* 1999). The reducing activity of vitamin C is caused by its ease of losing or donating electrons, i.e. as in vivo, where ascorbic acid avoids the oxidation of cellular

components such as lipids by donating electrons to reactive oxidant free radicals (Bauernfeind 1985; Sapers 1993). Ascorbic acid is Generally recognized as safe (GRAS) as a food additive, and has also shown bactericidal properties (Burnham *et al.* 2001).

The purpose of the current study was to evaluate the sensory attributes of green leaf lettuce (Waldmann's dark green variety) treated with ascorbic acid applied by two methods of hydrocooling (immersion and spraying), and stored at 5C. Hydrocooling (immersion or spraying) with tap water and no treatment were used as comparative controls.

MATERIALS AND METHODS

Raw materials

Waldmann's dark green lettuce heads were grown and harvested from a field in Fort Collins, CO. Sixty lettuce seeds (Johnny's Selected Seeds, Albion, ME) were planted in Colorado State University greenhouses during June 2004. Lettuce plants were transplanted to the field four weeks after planting. Fifty five lettuce heads were harvested on September 6, 2004. Harvesting was performed manually using a sharp knife to cut lettuce plants at the soil surface. Lettuce heads were transported to the laboratory in coolers containing ice within 30 min of harvesting, and lettuce heads were prepared for treatments within one h of harvesting by washing them with running tap water (free chlorine residual 0.5 mg/L, pH 7.9) at 20C ± 1C for 5 min (washing step), removing damaged outer leaves and dirt. Entire washed lettuce heads were placed into 68 L plastic containers (Sterilite, Townsend, MA) and allowed to drain for 10 min at room

temperature (~20C) with 10 to 12 heads per container. The 55 washed and drained lettuce heads were distributed randomly across five treatments, with eleven heads per treatment.

Treatments

Five treatments were applied to lettuce heads within 4 hr after harvest. These treatments included: 1) hydrocooling by immersion with 1 % ascorbic acid (Sigma, St. Louis, MO) w/v solution (ascorbic acid immersion), 2) hydrocooling by spraying with 1 % ascorbic acid w/v solution (ascorbic acid spraying), 3) hydrocooling by immersion with tap water (free chlorine residual 0.5 mg/L, pH 7.9) (water immersion), 4) hydrocooling by spraying with tap water (water spraying), 5) no treatment (control). Treatment solutions for immersion or spraying were applied at 5C ± 1C for 2 min. The 1 % ascorbic acid solution was prepared 5-10 min before use diluting the ascorbic acid in tap water at 5 C. The pH of the 1% ascorbic acid solution was 3.0. Hydrocooling by immersion was performed by soaking the washed lettuce heads for 2 min in a 68 L plastic container (Sterilite, Townsend, MA) containing 45 L of cooling solution (tap water or 1% ascorbic acid solution) at 5C. Three or four samples (entire lettuce heads) were treated at the same time. Afterwards, lettuce heads were removed from the cooling solution and placed in plastic containers to drain excess solution for 5 min, then were packaged in 49 L sealed 20 μm thick, moisture impermeable polyethylene bags (Great Value, Wal-Mart®, Bentonville, AR), with one entire lettuce head per plastic bag. Packaged lettuce heads were stored at 5C ± 1C for 14 days. Storage time started when the packaged lettuce heads were placed under storage conditions. Hydrocooling by spraying was performed by placing the samples into a 68 L plastic container (Sterilite, Townsend, MA), and spraying constantly (50 sprays/min for 2 min) using a 24 oz plastic spray bottle (The Bottle Crew®,

West Bloomfield, MI) with the respective solution (tap water or 1% ascorbic acid) at a distance of 35-40 cm from the lettuce head. The samples were turned upside down at least twice during treatment. One lettuce head was treated at a time, and after the spraying step it was drained, packaged and stored as previously described. Lettuce heads were evaluated at 3 different storage time intervals: at days 1, 7 and 14 of storage time. An additional sensory evaluation was planned for day 21 of storage. However, samples of all treatments were too deteriorated at day 16 of storage to present to the sensory panel.

Sample preparation

Twelve hours prior to each sensory evaluation session three lettuce heads per treatment were washed with running tap water (22-25C) for 2 min and blotted dry with paper towels. Samples for visual evaluation were prepared using 2 randomly selected entire untrimmed leaves which were displayed on a white flat plate and covered with transparent plastic. Samples for tasting were prepared by cutting the washed lettuce leaves (excluding colorless leaf parts and midrib) into pieces of approximately 5 cm x 10 cm with a sharp knife. Cut lettuce was packaged into 16 x 9 cm moisture impermeable transparent plastic zip-closure bags (Ziploc®, Racine, WI), with 10-15 g of leaf per bag. All prepared samples were labeled with 3-digit random numbers assigned for respective treatment, and stored at 5C ± 1C until sensory evaluation test.

Sensory evaluation

Sensory evaluation sessions were conducted on lettuce after 1, 7 and 14 days of storage time, with a minimum of 30 untrained consumer panelists per session. On the day of the sensory test the panelists were seated in individual booths in a room with fluorescent lighting. Panelists were given a consent form approved by the Colorado State

University Human Research Committee and score sheets for leaf lettuce sensory evaluation. After signing the consent form, panelists were given a tray containing 5 labeled bags with cut samples for tasting, unsalted crackers and distilled water to rinse the palate between samples. Cut samples for tasting were used to rate color intensity, flavor, texture, bitterness, tartness and overall acceptability. Panelists were asked to first score appearance by evaluating the lettuce leaves displayed on white plates, then they evaluated the samples for tasting. Sensory attributes were rated using a 15-cm semi-structured scale with 0 = lowest score for the attribute and 15 = highest score for the attribute (Table 4.1).

TABLE 4.1
DESCRIPTION OF THE ATTRIBUTES USED FOR SENSORY EVALUATION[1] OF CONTROL (UNTREATED) AND TREATED[2] LETTUCE STORED AT 5C ± 1C FOR 1, 7 AND 14 DAYS

ATTRIBUTE	LOWEST SCORE ON THE SCALE (0)	MIDDLE SCORE ON THE SCALE (7.5)	HIGHEST SCORE ON THE SCALE (15)
Appearance	Unacceptable	Neutral	Acceptable
Color intensity	Extremely dark	Neither dark nor light	Light
Flavor	Unacceptable	Neutral	Acceptable
Texture	Firm	Neither firm nor soft	Soft
Bitterness	Extremely bitter	Neither bitter nor mild	Mild
Tartness	Extremely tart	Neither tart nor mild	Mild
Overall acceptability	Unacceptable	Neutral	Acceptable

[1] A 15-cm semi-structured scale was used
[2] Immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, sprayed with tap water.

Statistical analysis

Data were analyzed using a 2-way ANOVA (5 x 3 factorial design) with 5 treatments x 3 time intervals when sensory panels were conducted, i.e. after 1, 7 and 14 days of storage time. Statistical analysis of the data was conducted using the Mixed Procedure in SAS System (SAS Inc. version 9.1, Cary, NC). Differences between means were calculated using the Least Significant Difference test ($P < 0.05$).

RESULTS AND DISCUSSION

Ascorbic acid sprayed lettuce maintained all its sensory attributes except texture (firmer texture at day 14 than at days 1 and 7; Table 4.2, Figure 4.4) and bitterness (less bitter at day 14, Figure 4.5) during the study. Lettuce treated by hydrocooling with ascorbic acid by immersion maintained all lettuce sensory attributes except the color intensity (darker color intensity at day 1, Figure 4.2) and the appearance (less acceptable at days 7 and 14, Figure 4.7) during the study. Possibly the concentration of ascorbic acid used in the study (1 % w/v, pH = 3.0) caused some damage to the appearance of ascorbic acid immersed lettuce. The control treatment (untreated leaf lettuce) maintained all attributes with the exception of its color intensity (darker color intensity at day 14 than at day 1) during the 14 days of the storage time of the study. Water immersed lettuce maintained all attributes but color intensity (darker color intensity at day 7 compared to that at day 1) and appearance (less acceptable appearance at day 14) during the experiment. Hydrocooling of lettuce by water spraying maintained its color intensity and texture during the study, but had changes in all other sensory attributes over the 14 days

of the storage time (darker color, less acceptable appearance, more acceptable flavor and less bitter).

Sensory attributes of the control and immersed (in ascorbic acid or water) lettuce on day 1 of storage were similar, with the exception of texture ("firmer" texture in the control lettuce). Moreover, on day 1 ascorbic acid sprayed lettuce received the highest scores for flavor (12.00, "acceptable" flavor) and overall acceptability (12.09, "acceptable" overall acceptability).

On day 7 water immersed lettuce was darker, water sprayed lettuce had a higher overall acceptability and a more acceptable flavor, control had a more acceptable appearance, and ascorbic acid immersed lettuce had a less acceptable appearance. The appearance score of the ascorbic acid immersed lettuce decreased (7.63), which was considered as a "neither acceptable nor unacceptable" appearance. Perhaps the use of different concentration levels of ascorbic acid for hydrocooling of leaf lettuce could result in the maintenance of the quality attributes of this vegetable including appearance, thereby extending its shelf life.

On day 14 the ascorbic acid sprayed lettuce had a firmer texture and less bitter flavor than on days 1 and 7 (Figure 4.5), and all its other sensory attributes remained constant throughout the experiment. There were no significant differences among ascorbic acid treated (immersed and sprayed) and control lettuce on day 14 of storage for color intensity, flavor, texture, bitterness, tartness and overall acceptability. Flavor and overall acceptability of all treatments but water spraying were maintained throughout the study, and water spraying was the treatment which produced the least acceptable appearance (8.79, considered as "slightly acceptable" appearance) and overall

acceptability (8.85, considered as "slightly" acceptable) at day 14 of storage time. In addition, the samples of all treatments on day 16 of storage were too deteriorated to be presented to the sensory panelists.

CONCLUSIONS

Lettuce hydrocooled by spraying with 1% ascorbic acid increased in firmness over the storage time (firmer texture on day 14 of storage), while hydrocooling by immersion using 1% ascorbic acid negatively affected the appearance of lettuce over the storage time. Moreover, control (no treatment) had no changes in appearance during the storage time. Lettuce hydrocooled by spraying with any solution (1% ascorbic acid or water) became more bitter over the storage time, while hydrocooling of leaf lettuce by immersion in any solution (1% ascorbic acid or water) did not affect the bitterness of lettuce during the storage time of 14 days. Furthermore, lettuce hydrocooled by water spraying had the lowest overall acceptability of leaf lettuce over the 14 days of storage time. Control lettuce (no treatment) maintained all sensory attributes for 14 days except color intensity (darker color in lettuce over the storage time). Moreover, hydrocooling of leaf lettuce by immersion using any solution (water or 1% ascorbic acid) did not affect the flavor, texture, bitterness, tartness and overall acceptability of lettuce during the 14 days of the storage time. Further experimentation with the application of ascorbic acid during hydrocooling is needed to fully assess its usefulness in maintaining the quality of leaf lettuce during storage.

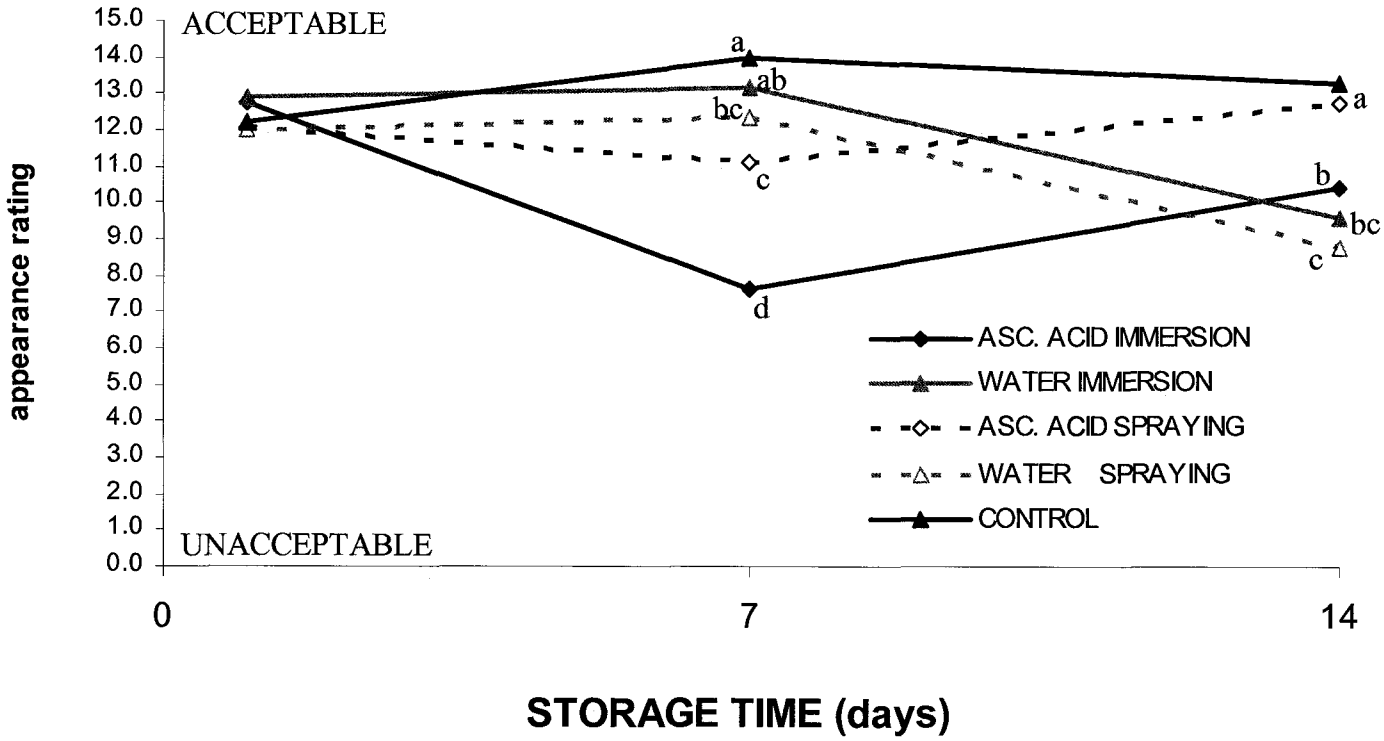


Figure 4.1 Mean scores for appearance of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C at 1, 7 and 14 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 1.44. Different letters within the same time interval are significantly different.

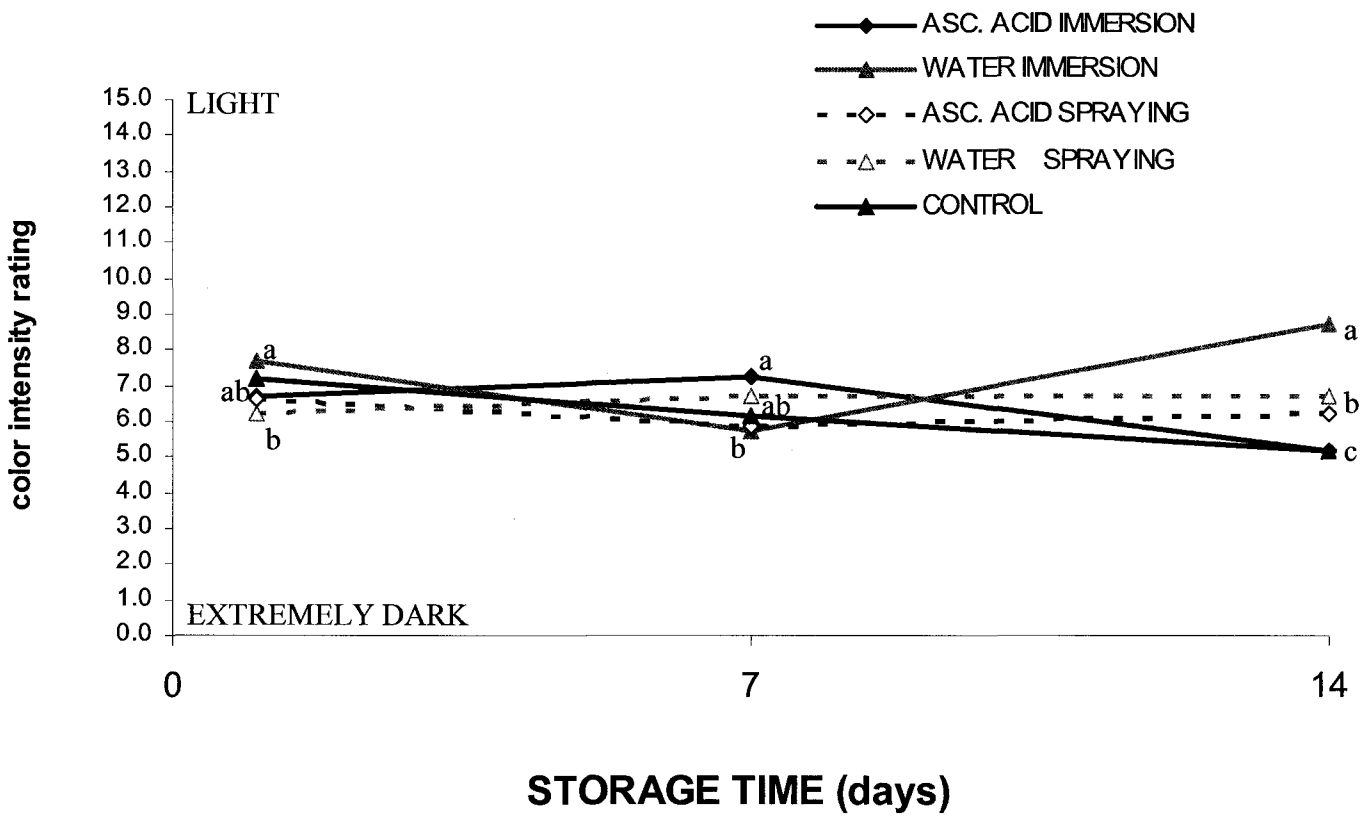


Figure 4.2 Mean scores for color intensity of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C at 1, 7 and 14 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 1.39. Different letters within the same time interval are significantly different.

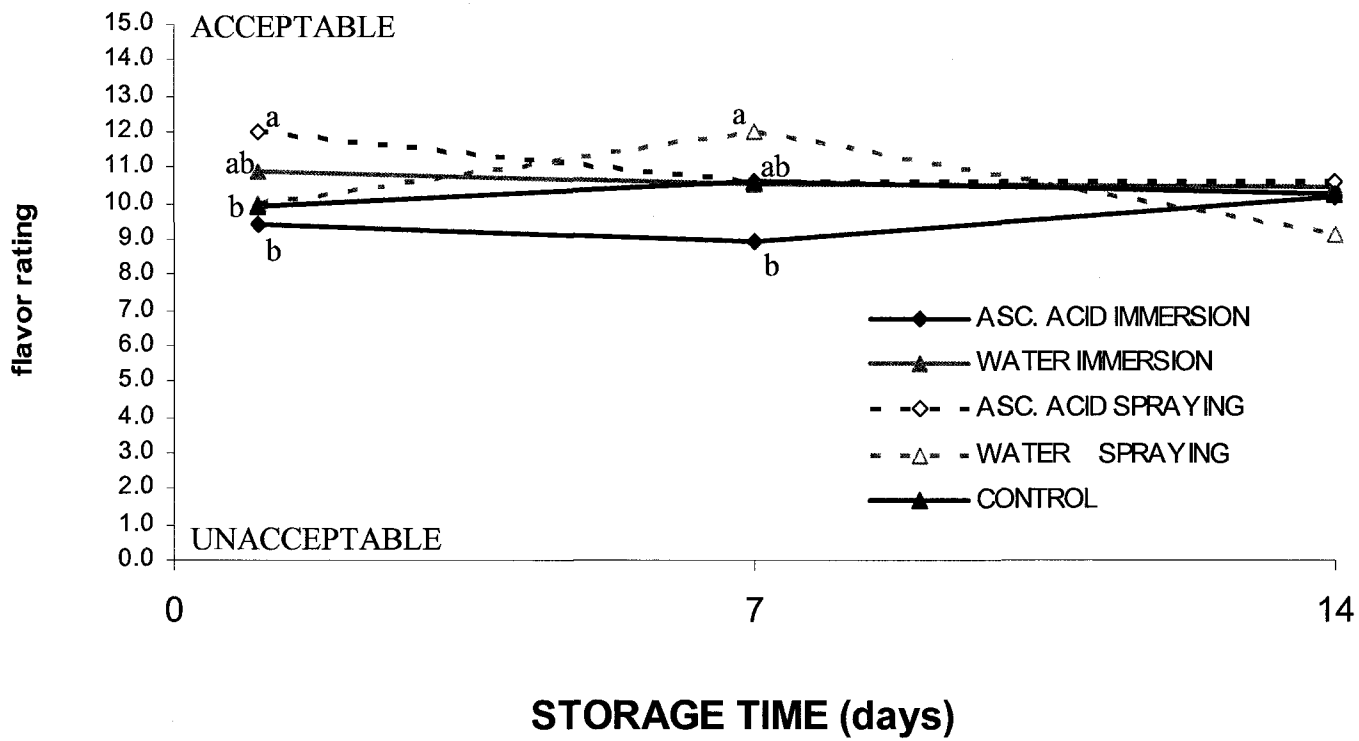


Figure 4.3 Mean scores for flavor of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C at 1, 7 and 14 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 1.80. Different letters within the same time interval are significantly different.

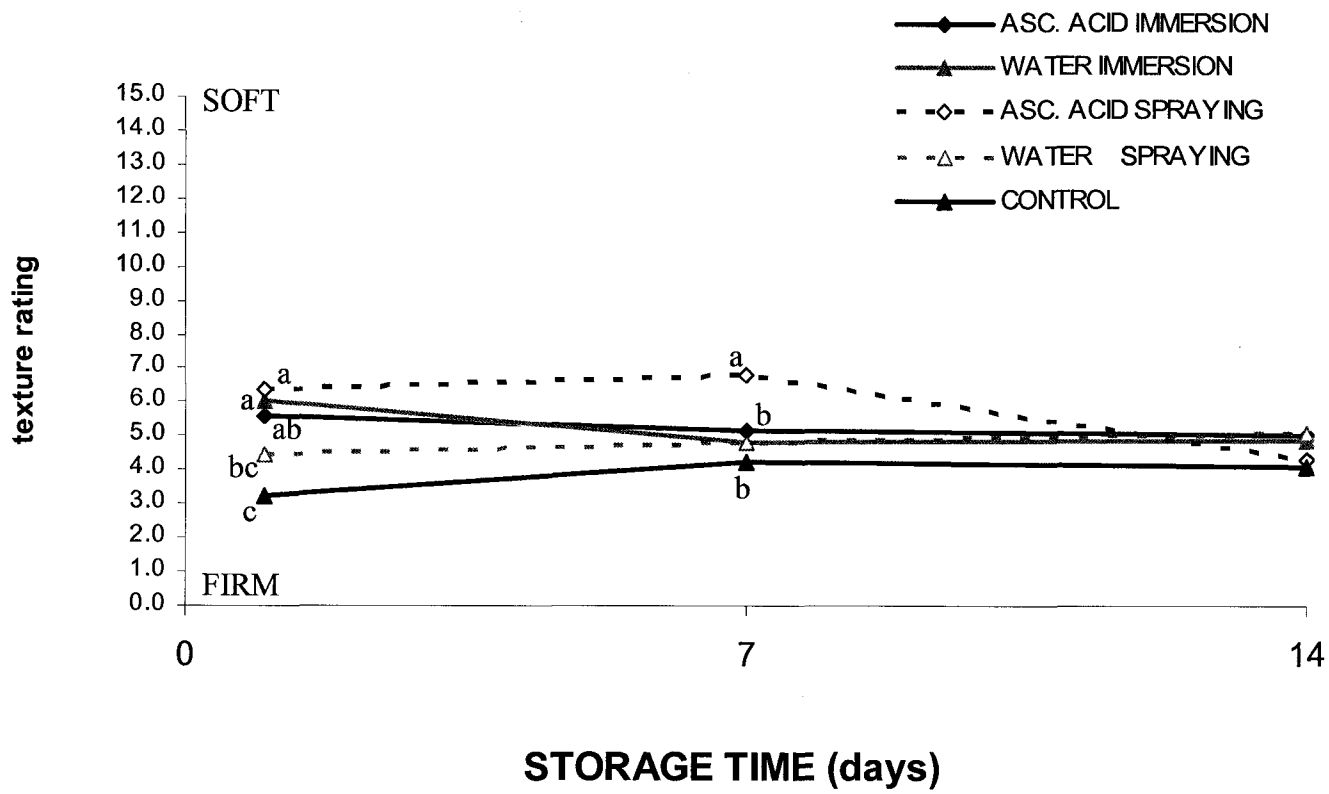


Figure 4.4 Mean scores for texture of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 ^{o}C at 1, 7 and 14 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 1.47. Different letters within the same time interval are significantly different.

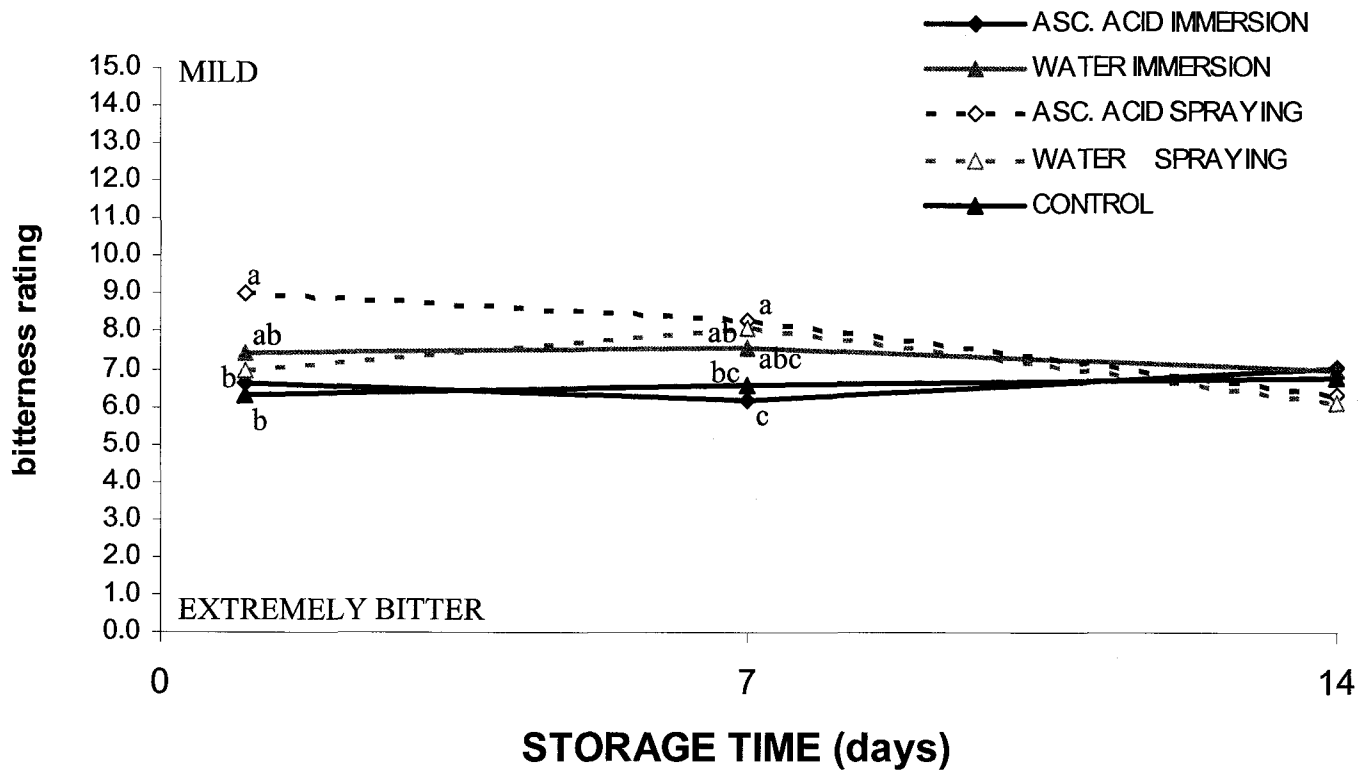


Figure 4.5 Mean scores for bitterness of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C at 1, 7 and 14 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 1.63. Different letters within the same time interval are significantly different.

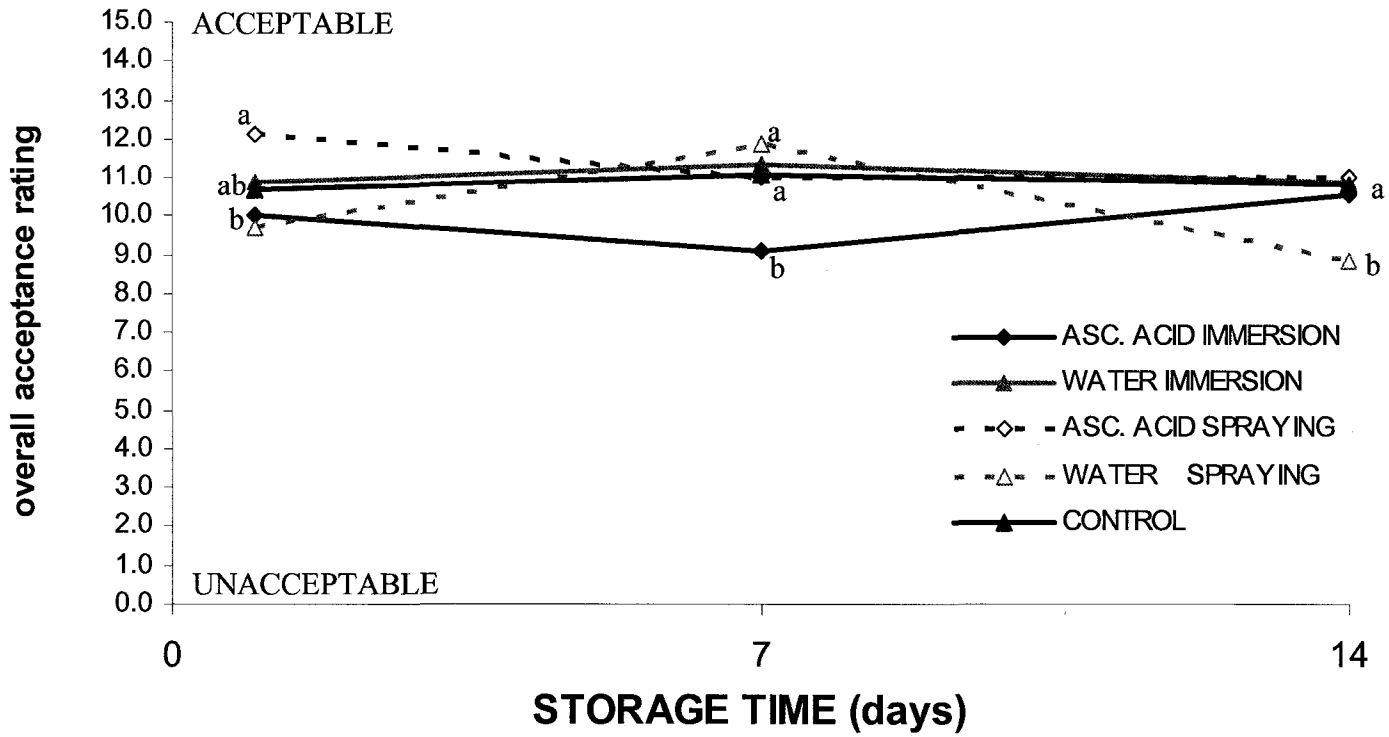


Figure 4.6 Mean scores for overall acceptability of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C at 1, 7 and 14 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 1.69. Different letters within the same time interval are significantly different.

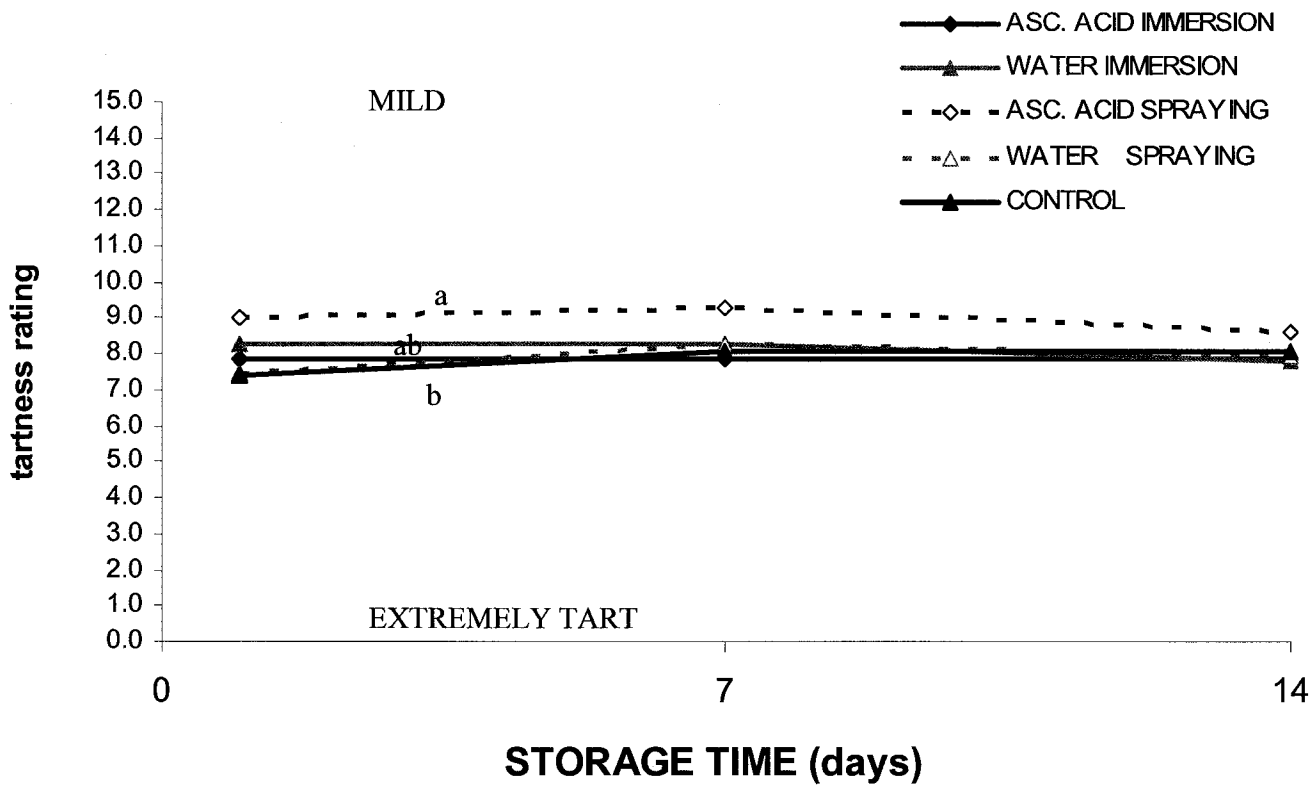


Figure 4.7 Mean scores for tartness of control (untreated) and treated (immersed in 1% ascorbic acid, immersed in tap water, sprayed with 1% ascorbic acid, or sprayed with tap water) lettuce stored at 5 °C at 1, 7 and 14 days of storage time. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$). LSD = 1.47. Different letters within the same time interval are significantly different.

TABLE 4.2
MEAN RATINGS AND SD FOR SENSORY ATTRIBUTES OF CONTROL (UNTREATED) AND TREATED LETTUCE[1] STORED AT 5C ± 1C FOR 1, 7 AND 14 DAYS

	APPEARANCE			COLOR INTENSITY		
TREATMENT	1 day	7 days	14 days	1 day	7 days	14 days
Asc. acid immersion[2]	12.72 ± 2.45 ^{aA}	7.63 ± 4.60 ^{dC}	10.40 ± 3.67 ^{bB}	6.72 ± 2.59 ^{abA}	7.25 ± 3.09 ^{aA}	5.13 ± 2.28 ^{cB}
Water immersion[3]	12.87 ± 2.26 ^{aA}	13.14 ± 2.31 ^{abA}	9.65 ± 3.84 ^{bcB}	7.67 ± 2.92 ^{aA}	5.72 ± 2.85 ^{bB}	8.70 ± 2.71 ^{aA}
Asc. acid spraying[4]	12.06 ± 3.26 ^{aAB}	11.12 ± 3.72 ^{cB}	12.75 ± 2.33 ^{aA}	6.59 ± 3.21 ^{abA}	5.85 ± 3.44 ^{bA}	6.18 ± 2.79 ^{bcA}
Water spraying[5]	11.98 ± 3.08 ^{aA}	12.36 ± 3.02 ^{bcA}	8.79 ± 3.97 ^{cB}	6.23 ± 2.85 ^{bA}	6.71 ± 2.65 ^{abA}	6.71 ± 3.28 ^{bA}
Control[6]	12.23 ± 2.48 ^{aB}	13.99 ± 0.44 ^{aA}	13.29 ± 1.15 ^{aAB}	7.16 ± 3.11 ^{abA}	6.11 ± 2.61 ^{abAB}	5.18 ± 2.38 ^{cB}
	LSD = 1.44			LSD = 1.39		
	FLAVOR			TEXTURE		
TREATMENT	1 day	7 days	14 days	1 day	7 days	14 days
Asc. acid immersion[2]	9.42 ± 3.95 ^{bA}	8.95 ± 4.44 ^{bA}	10.16 ± 3.46 ^{aA}	5.58 ± 2.95 ^{abA}	5.15 ± 3.30 ^{bA}	5.05 ± 2.71 ^{aA}
Water immersion[3]	10.91 ± 3.03 ^{abA}	10.56 ± 3.77 ^{abA}	10.43 ± 4.00 ^{aA}	6.06 ± 3.14 ^{aA}	4.79 ± 3.50 ^{bA}	4.88 ± 3.07 ^{aA}
Asc. acid spraying[4]	12.00 ± 2.49 ^{aA}	10.62 ± 3.65 ^{abA}	10.63 ± 3.62 ^{aA}	6.37 ± 3.39 ^{aA}	6.79 ± 3.73 ^{aA}	4.28 ± 2.58 ^{aB}
Water spraying[5]	9.98 ± 4.18 ^{bB}	12.01 ± 3.27 ^{aA}	9.16 ± 4.01 ^{aB}	4.42 ± 2.95 ^{bcA}	4.79 ± 3.49 ^{bA}	5.07 ± 3.38 ^{aA}
Control[6]	9.88 ± 3.77 ^{bA}	10.62 ± 3.99 ^{abA}	10.29 ± 3.39 ^{aA}	3.20 ± 2.00 ^{cA}	4.23 ± 2.96 ^{bA}	4.08 ± 2.74 ^{aA}
	LSD = 1.80			LSD = 1.47		
	BITTERNESS			OVERALL ACCEPTABILITY		
TREATMENT	1 day	7 days	14 days	1 day	7 days	14 days
Asc. acid immersion[2]	6.61 ± 2.92 ^{bA}	6.15 ± 3.46 ^{cA}	7.06 ± 3.07 ^{aA}	10.00 ± 3.85 ^{bA}	9.08 ± 4.34 ^{bA}	10.58 ± 3.63 ^{aA}
Water immersion[3]	7.41 ± 3.38 ^{abA}	7.55 ± 3.66 ^{abcA}	6.97 ± 2.84 ^{aA}	10.86 ± 3.32 ^{abA}	11.33 ± 3.71 ^{aA}	10.85 ± 3.48 ^{aA}
Asc. acid spraying[4]	8.99 ± 3.31 ^{aA}	8.29 ± 3.71 ^{aA}	6.33 ± 3.19 ^{aB}	12.09 ± 2.52 ^{aA}	10.99 ± 3.47 ^{aA}	11.02 ± 3.12 ^{aA}
Water spraying[5]	6.97 ± 3.38 ^{bAB}	8.08 ± 3.89 ^{abA}	6.09 ± 3.63 ^{aB}	9.70 ± 3.98 ^{bB}	11.87 ± 3.23 ^{aA}	8.85 ± 4.18 ^{bB}
Control[6]	6.30 ± 3.69 ^{bA}	6.57 ± 3.04 ^{bcA}	6.76 ± 2.97 ^{aA}	10.68 ± 3.47 ^{abA}	11.06 ± 3.41 ^{aA}	10.83 ± 2.88 ^{aA}
	LSD = 1.63			LSD = 1.69		

[1] 15 cm semi structured linear scale, with 0 = lowest point of the scale and 15 = highest point in the scale.
Means ± standard deviations (day 1 = 32; day 7 = 35; day 14 = 33) of the replicates using each day as a replicate. Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$).
[2] Immersed in 1% ascorbic acid, [3] immersed in tap water, [4] sprayed with 1% ascorbic acid, [5] sprayed with tap water, or [6] control (untreated).
Values in a column followed by a different lower-case letter are significantly different ($P < 0.05$).
Values in a row followed by a different capital letter are significantly different ($P < 0.05$)

CHAPTER V

GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER STUDIES

The effects of ascorbic acid applied by two hydrocooling methods on chemical, physical and sensory properties of Waldmann's dark green leaf lettuce were evaluated. Lettuce heads were immersed (in 1% ascorbic acid or tap water) or sprayed (in 1% ascorbic acid or tap water) or left untreated (control). Treatment solutions for immersion or spraying were applied at 5 °C for 2 min. After treatment application, lettuce heads were packaged and stored at 5 °C for up to 21 days.

These were the general conclusions obtained from the results in these research studies:

- Hydrocooling of leaf lettuce by immersion or spraying using 1% ascorbic acid solution increased total ascorbate content for up to 7 days, with an increase of more than 300% in total ascorbate content on day 1 compared to its initial value before treatments.
- Application of ascorbic acid during hydrocooling of leaf lettuce by immersion caused the relative water content of leaf lettuce to be maintained for 21 days, thereby resulting in a firmer texture compared to the other hydrocooling treatments and the untreated lettuce.

- The application of water or ascorbic acid during hydrocooling of lettuce did not affect the antioxidant capacity of leaf lettuce.
- Hydrocooling with ascorbic acid by immersion increased the total phenolic content of leaf lettuce for up to 7 d, but this could be due to known interference that ascorbic acid has on the Folin-Ciocalteu assay.
- a* value (instrumental color red-green scale) of leaf lettuce decreased during storage time in lettuce hydrocooled by water or 1% ascorbic, and in control lettuce. Hydrocooling by spraying with water or 1% ascorbic acid solution did not affect the color of leaf lettuce in any value.
- Lettuce hydrocooled by spraying with 1% ascorbic acid increased its firmness over the storage time (firmer texture on day 14 of storage), but this treatment (hydrocooling of leaf lettuce by immersion using 1% ascorbic acid) negatively affected the appearance of lettuce over the storage time. Possibly the concentration of ascorbic acid used in the study (1 % w/v, pH = 3.0) caused some damage to the ascorbic acid immersed lettuce appearance.
- Lettuce hydrocooled by water spraying had the lowest overall acceptability of leaf lettuce over the 14 days of storage time. Control lettuce (no treatment) maintained all sensory attributes for 14 days except color intensity (darker color in lettuce over the storage time).
- Hydrocooling of leaf lettuce by immersion using any solution (water or 1% ascorbic acid) did not affect the flavor, texture, bitterness, tartness and overall acceptability of lettuce during the 14 days of the storage time.

- Further experimentation with the application of ascorbic acid during hydrocooling is needed to fully assess its usefulness in maintaining the quality of leaf lettuce during storage.

The following recommendation can be made for growers and producers of lettuce:

- Hydrocooling of leaf lettuce is a step performed during leaf lettuce processing. Addition of 1% ascorbic acid to the tap water for hydrocooling by immersion or spraying of leaf lettuce will increase its vitamin C content more than 300%.This results in a nutritionally fortified leaf lettuce with a higher vitamin C content when compared to leaf lettuce hydrocooled using tap water as a cooling solution.

Recommendations for further studies are:

- Vary the concentration of ascorbic acid used during hydrocooling of leaf lettuce, to optimize the effect of the ascorbic acid without damaging lettuce sensory attributes such as texture or color.
- Combine the use of ascorbic acid during hydrocooling by spraying or immersion with packaging under modified atmosphere to see if this results in the maintenance of sensory quality of nutritionally enriched leaf lettuce for longer time periods.
- Investigate the effects of using ascorbic acid in combination with other compounds such as citric acid to extend shelf life of lettuce. Some treatments using ascorbic acid combined with other compounds have been proven to be more effective to maintain quality of vegetable produce than the application of ascorbic acid alone.
- Investigate the application of calcium treatments in maintaining lettuce texture during post harvest stages. Calcium stabilizes cell wall in vegetable products, maintaining their texture.

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APPENDIX I

Mean values (mg of total ascorbate content / gm dw) ± standard deviation of the total ascorbate content of control (untreated) and treated lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time

	Storage time				
TREATMENT	**0 days**	**1 day**	**7 days**	**14 days**	**21 days**
Asc. acid immersion[b]	1.57 ± 0.49 aA	5.00 ± 1.49 aB	4.50 ± 1.77 aB	1.80 ± 1.37 aA	0.32 ± 0.65 aC
Water immersion[c]	1.57 ± 0.19 aA	1.22 ± 0.45 cA	1.13 ± 0.23 bcA	0.27 ± 0.31 bB	0.13 ± 0.27 aB
Asc. acid spraying[d]	1.30 ± 0.43 aA	4.01 ± 1.22 bB	1.69 ± 0.81 bA	0.28 ± 0.34 bC	0.25 ± 0.30 aC
Water spraying[e]	1.13 ± 0.19 aA	1.64 ± 0.32 cA	0.83 ± 0.57 cAB	0.14 ± 0.30 bB	0.11 ± 0.23 aB
Control[f]	1.43 ± 0.54 aAB	1.89 ± 0.19 cA	0.87 ± 0.38 bcBC	0.48 ± 0.34 bCD	ND*

[a] Means ± standard deviation (n = 4) of the replicates in mg of total ascorbate / gm dry wt. ND* Not detected. Differences between means found using the Least significant difference (LSD) test ($P < 0.05$). Least Significant Difference = 0.85 mg of total ascorbate / gm dry wt.

[b] Immersed in 1% ascorbic acid, [c]immersed in tap water, [d]sprayed with 1% ascorbic acid, [e]sprayed with tap water, or [f]control (untreated).

Values in a column followed by a different lower-case letter are significantly different ($P < 0.05$)

Values in a row followed by a different capital letter are significantly different ($P < 0.05$).

APPENDIX II

Mean values[a] (μmol TEAC/gm dry wt) ± standard deviation of the Trolox equivalent antioxidant capacity of control (untreated) and treated lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time

	STORAGE TIME				
TREATMENT	**Before treatments**	**1 day**	**7 days**	**14 days**	**21 days**
Asc. acid immersion[b]	283.2 ± 61.6 aA	346.2 ± 51.4 aA	349.3 ± 50.4 aA	337.6 ± 46.7 aA	289.3 ± 116.2 aA
Water immersion[c]	290.7 ± 61.3 aA	319.4 ± 43.6 aA	285.4 ± 30.1 aA	279.1 ± 26.8 aA	343.7 ± 16.2 aA
Asc. acid spraying[d]	284.5 ± 33.3 aA	332.4 ± 47.2 aA	258.7 ± 98.1 aA	250.2 ± 31.2 aA	311.1 ± 82.2 aA
Water spraying[e]	287.8 ± 111 aAB	371.1 ± 15.3 aA	283.6 ± 104 aAB	266.3 ± 42.8 aB	322.1 ± 73.9 aAB
Control[f]	323.6 ± 82.8 aAB	367.0 ± 73.4 aA	258.5 ± 66.9 aB	319.6 ± 86.9 aAB	313.2 ± 55.9 aAB

[a] Means ± standard deviation (n = 4) of the replicates in μmol TEAC (Trolox equivalent antioxidant capacity) / gm dry wt. Differences between means found using the Least significant difference (LSD) test ($P < 0.05$). LSD = 95.19 μmol TEAC / gm dry wt.

[b] Immersed in 1% ascorbic acid, [c] immersed in tap water, [d] sprayed with 1% ascorbic acid, [e] sprayed with tap water, or [f] control (untreated).

Values in a column followed by a different lower-case letter are significantly different ($P < 0.05$)

Values in a row followed by a different capital letter are significantly different ($P < 0.05$)

APPENDIX III

Mean values[a] (mg GAE / gm dry wt) ± standard deviation of the total phenolic content of control (untreated) and treated lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time

	STORAGE TIME				
TREATMENT	**Before treatments**	**1 day**	**7 days**	**14 days**	**21 days**
Asc. acid immersion[b]	38.3 ± 5.8 abB	44.8 ± 6.7 aA	44.2 ± 7.6 aA	37.2 ± 8.7 aB	31.7 ± 9.5 bC
Water immersion[c]	37.5 ± 7.4 abA	35.5 ± 4.1 cA	34.2 ± 6.8 bA	33.3 ± 5.1 aA	36.7 ± 7.5 abA
Asc. acid spraying[d]	34.2 ± 6.1 bAB	39.2 ± 6.6 bcA	37.5 ± 9.1 bAB	33.9 ± 7.2 aB	35.7 ± 6.4 bAB
Water spraying[e]	42.5 ± 10.8 aA	38.7 ± 9.0 bcAB	37.4 ± 8.2 bAB	37.8 ± 6.9 aAB	36.7 ± 8.3 abB
Control[f]	39.7 ± 6.2 aA	41.8 ± 9.2 abA	38.3 ± 8.0 bA	37.5 ± 9.6 aA	41.4 ± 5.4 aA

[a] Means ± standard deviations (n = 4) of the replicates in mg of GAE (gallic acid equivalent) / gm dry wt. Differences between means found using the Least significant difference (LSD) test ($P < 0.05$). LSD = 5.19 mg of GAE / gm dry wt

[b] Immersed in 1% ascorbic acid, [c] immersed in tap water, [d] sprayed with 1% ascorbic acid, [e] sprayed with tap water, or [f] control (untreated).

Values in a column followed by a different lower-case letter are significantly different ($P < 0.05$)

Values in a row followed by a different capital letter are significantly different ($P < 0.05$)

APPENDIX IV

Mean values[a] (% RWC) ± standard deviation of the relative water content of control (untreated) and treated lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time

	STORAGE TIME				
TREATMENT	**Before treatments**	**1 day**	**7 days**	**14 days**	**21 days**
Asc. acid immersion[b]	99.05 ± 1.06 aA	98.51 ± 1.31 aA	99.02 ± 1.49 aA	98.43 ± 1.23 aA	97.73 ± 2.26 aA
Water immersion[c]	99.45 ± 0.71 aA	98.69 ± 1.74 aAB	98.24 ± 1.53 aAB	97.63 ± 1.56 abB	97.11 ± 1.71 abB
Asc. acid spraying[d]	99.35 ± 0.76 aA	98.39 ± 2.26 aAB	98.22 ± 2.18 aAB	97.89 ± 1.41 abAB	96.78 ± 2.16 abB
Water spraying[e]	99.79 ± 0.40 aA	99.40 ± 0.64 aA	99.09 ± 0.86 aAB	97.68 ± 1.45 abB	95.96 ± 3.58 bC
Control[f]	99.07 ± 1.10 aA	99.00 ± 1.43 aA	99.02 ± 1.26 aA	96.03 ± 2.44 bB	94.07 ± 4.10 cC

[a] Means ± standard deviation (n = 3) of the replicates in % RWC (percentage of relative water content). Differences between means found using the Least significant difference (LSD) test ($P < 0.05$). LSD = 1.61 % RWC

[b] Immersed in 1% ascorbic acid, [c] immersed in tap water, [d] sprayed with 1% ascorbic acid, [e] sprayed with tap water, or [f] control (untreated).

Values in a column followed by a different lower-case letter are significantly different ($P < 0.05$)

Values in a row followed by a different capital letter are significantly different ($P < 0.05$)

APPENDIX V

Mean values[a] (gm) ± standard deviation of the texture (firmness) of control (untreated) and treated lettuce stored at 5C for 0 (before treatments), 1, 7, 14 and 21 days of storage time

	STORAGE TIME				
TREATMENT	**Before treatments**	**1 day**	**7 days**	**14 days**	**21 days**
Asc. acid immersion[b]	2746 ± 516 aC	3116 ± 493 aB	3162 ± 468 aB	4112 ± 367 aA	4063 ± 520 aA
Water immersion[c]	2688 ± 496 aC	2950 ± 332 abB	3072 ± 469 aB	3856 ± 480 bA	3818 ± 489 bA
Asc. acid spraying[d]	2693 ± 536 aC	2940 ± 280 abB	3007 ± 435 aB	3827 ± 462 bcA	3673 ± 599 bcA
Water spraying[e]	2707 ± 612 aC	2943 ± 267 abB	3004 ± 494 aB	3611 ± 648 cA	3587 ± 512 cA
Control[f]	2719 ± 570 aC	2884 ± 342 bBC	3038 ± 491 aB	3828 ± 460 bcA	3717 ± 522 bcA

[a] Means ± standard deviation (n = 3) of the replicates in gm. Differences between means found using the Least significant difference (LSD) test ($P < 0.05$). LSD = 221.1 gm

[b] Immersed in 1% ascorbic acid, [c] immersed in tap water, [d] sprayed with 1% ascorbic acid, [e] sprayed with tap water, or [f] control (untreated).

Values in a column followed by a different lower-case letter are significantly different ($P < 0.05$)

Values in a row followed by a different capital letter are significantly different ($P < 0.05$)

APPENDIX VI

Mean ratings[a] and standard error for L^*, a^* and b^* values of control (untreated) and treated lettuce stored at 5 °C for 0 (before treatments), 1, 7, 14 and 21 days of storage time

	STORAGE TIME				
	***L** values*				
TREATMENT	0 days	1 day	7 days	14 days	21 days
Asc. acid immersion[b]	42.41 ± 0.87 aA	42.64 ± 0.30 aA	41.82 ± 0.56 aA	40.45 ± 0.39 aA	41.47 ± 0.20 aA
Water immersion[c]	43.90 ± 0.61 aA	41.15 ± 0.61 aA	42.79 ± 0.81 aA	40.90 ± 0.30 aA	43.81 ± 0.96 aA
Asc. acid spraying[d]	42.20 ± 0.39 aA	42.79 ± 0.58 aA	41.29 ± 0.35 aA	41.14 ± 0.48 aA	43.94 ± 0.55 aA
Water spraying[e]	44.59 ± 0.99 aA	41.38 ± 0.20 aAB	41.36 ± 0.47 aAB	40.89 ± 0.71 aB	42.99 ± 0.48 aAB
Control[f]	42.12 ± 0.78 aA	40.81 ± 0.73 aA	99.02 ± 0.69 aA	41.53 ± 0.57 aA	41.80 ± 1.25 aA
LSD = 3.48					
	***a** values*				
TREATMENT	0 days	1 day	7 days	14 days	21 days
Asc. acid immersion[b]	-9.80 ± 0.20 aA	-9.76 ± 0.14 aA	-9.89 ± 0.21 aA	-9.18 ± 0.11 aB	-8.68 ± 0.22 aB
Water immersion[c]	-10.10 ± 0.20 aA	-10.10 ± 0.22 aA	-9.44 ± 0.19 aAB	-9.62 ± 0.20 aB	-9.67 ± 0.09 bcB
Asc. acid spraying[d]	-9.79 ± 0.23 aA	-9.79 ± 0.11 aA	-9.69 ± 0.12 aA	-9.62 ± 0.09 aA	-9.58 ± 0.09 bcA
Water spraying[e]	-10.02 ± 0.24 aA	-9.93 ± 0.18 aA	-10.01 ± 0.20 aA	-9.62 ± 0.16 aA	-9.72 ± 0.14 cA
Control[f]	-10.05 ± 0.27 aA	-9.85 ± 0.26 aA	-9.59 ± 0.04 aAB	-9.55 ± 0.36 aAB	-9.15 ± 0.12 abB
LSD = 0.56					
	***b** values*				
TREATMENT	0 days	1 day	7 days	14 days	21 days
Asc. acid immersion[b]	23.69 ± 0.43 aA	24.43 ± 0.58 aA	24.17 ± 0.41 aA	24.36 ± 0.19 aA	24.55 ± 0.33 aA
Water immersion[c]	25.50 ± 0.31 aA	25.20 ± 0.72 aA	24.31 ± 0.60 aA	25.13 ± 0.60 aA	25.74 ± 0.91 aA
Asc. acid spraying[d]	25.22 ± 0.51 aA	24.60 ± 0.34 aA	23.97 ± 0.57 aA	24.93 ± 0.60 aA	26.45 ± 0.64 aA
Water spraying[e]	24.89 ± 0.68 aA	25.92 ± 0.25 aAB	25.40 ± 0.81 aA	26.15 ± 0.28 aA	25.83 ± 0.68 aA
Control[f]	24.84 ± 1.17 aA	24.10 ± 0.85 aA	25.23 ± 0.77 aA	25.26 ± 0.76 aA	25.96 ± 0.89 aA
LSD = 2.54					

[a] Means ± standard error (n = 3) of the replicates. Differences between means found by using the Least Significant Difference (LSD) test ($P < 0.05$).

[b] Immersed in 1% ascorbic acid (2 min, 5 °C), [3]immersed in tap water (2 min, 5 °C), [4]sprayed with 1% ascorbic acid (2 min, 5 °C), [5]sprayed with tap water (2 min, 5 °C), or [6]control (untreated).

Values in a column followed by a different lower-case letter are significantly different ($P < 0.05$).

Values in a row followed by a different capital letter are significantly different ($P < 0.05$).

APPENDIX VII

MEAN RATINGS[1] AND STANDARD DEVIATIONS FOR TARTNESS OF CONTROL (UNTREATED) AND TREATED (IMMERSED IN 1% ASCORBIC ACID, IMMERSED IN TAP WATER, SPRAYED WITH 1% ASCORBIC ACID, OR SPRAYED WITH TAP WATER) LETTUCE STORED AT 5 °C FOR 1, 7 AND 14 DAYS

	Storage time		
TREATMENT	**1 day**	**7 days**	**14 days**
Asc. acid immersion	7.86 ± 2.82 ab	7.90 ± 3.35 a	7.90 ± 2.99 a
Water immersion	8.27 ± 2.77 ab	8.29 ± 3.28 a	7.81 ± 3.02 a
Asc. acid spraying	9.01 ± 3.37 a	9.30 ± 3.28 a	8.62 ± 3.28 a
Water spraying	7.48 ± 3.36 b	8.28 ± 3.26 a	7.92 ± 2.58 a
Control	7.41 ± 2.83 b	8.10 ± 2.93 a	8.10 ± 2.90 a

[1] 15 cm 5-point hedonic scale, with 0 = lowest point of the scale and 15 = highest point in the scale. Means ± standard deviations (n = 3) of the replicates using each sensory evaluation panel as a replicate

Differences between means found using Least Significant Difference (LSD) test ($P < 0.05$).

Least Significant Difference = 1.47.

Values in a column followed by a different lower-case letter are significantly different ($P < 0.05$).

Values in a row followed by a different capital letter are significantly different ($P < 0.05$).

APPENDIX VIII

ANALYSIS OF VARIANCE FOR FIXED EFFECTS FOR TOTAL ASCORBATE CONTENT OF WALDMANN'S LEAF LETTUCE USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	**Num DF**	**Den DF**	**F-value**	**P > F**
Treatment	4	5	15.50	0.0008
Storage time	4	40	16.84	< 0.0001
Treatment * storage time	16	40	2.43	0.0117

APPENDIX IX

ANALYSIS OF VARIANCE FOR FIXED EFFECTS FOR TROLOX EQUIVALENT ANTIOXIDANT CAPACITY OF WALDMANN'S LEAF LETTUCE USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	48	0.15	0.9643
Storage time	4	48	5.02	0.0018
Treatment * storage time	16	48	0.58	0.8814

APPENDIX X

ANALYSIS OF VARIANCE FOR FIXED EFFECTS FOR TOTAL PHENOLIC CONTENT OF WALDMANN'S LEAF LETTUCE USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	**Num DF**	**Den DF**	**F-value**	**P > F**
Treatment	4	34.8	4.40	0.0055
Storage time	4	132	3.75	0.0063
Treatment * storage time	16	134	2.12	0.0108

APPENDIX XI

ANALYSIS OF VARIANCE FOR FIXED EFFECTS FOR
TEXTURE (FIRMNESS) OF WALDMANN'S LEAF LETTUCE
USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	**Num DF**	**Den DF**	**F-value**	**P > F**
Treatment	4	28.9	4.30	0.0075
Storage time	4	1776	270.78	<0.0001
Treatment * storage time	16	1776	1.34	0.1648

APPENDIX XII

ANALYSIS OF VARIANCE FOR FIXED EFFECTS
FOR RELATIVE WATER CONTENT OF LETTUCE
USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	75	2.80	0.0317
Storage time	4	75	22.65	<0.0001
Treatment * storage time	16	75	1.82	0.0433

APPENDIX XIII

ANALYSIS OF VARIANCE FOR FIXED EFFECTS FOR TOTAL COLOR DIFFERENCE OF WALDMANN'S LEAF LETTUCE USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	**Num DF**	**Den DF**	**F-value**	**P > F**
Treatment	4	20	2.02	0.1298
Storage time	3	20	2.19	0.1209
Treatment * storage time	12	20	1.21	0.3393

APPENDIX XIV

ANALYSIS OF VARIANCE FOR FIXED EFFECTS FOR *L* VALUES (LIGHTNESS-DARKNESS) OF WALDMANN'S LEAF LETTUCE USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	5	0.34	0.8429
Storage time	4	20	3.26	0.0327
Treatment * storage time	16	20	0.65	0.8069

APPENDIX XV

ANALYSIS OF VARIANCE FOR FIXED EFFECTS
FOR *a* VALUES (COLOR) WALDMANN'S LEAF LETTUCE
USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	**Num DF**	**Den DF**	**F-value**	**P > F**
Treatment	4	5	4.87	0.0564
Storage time	4	20	11.23	<0.0001
Treatment * storage time	16	20	2.31	0.0391

APPENDIX XVI

ANALYSIS OF VARIANCE FOR FIXED EFFECTS
FOR *b* VALUES (COLOR) WALDMANN'S LEAF LETTUCE
USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	**Num DF**	**Den DF**	**F-value**	**P > F**
Treatment	4	5	2.05	0.2258
Storage time	4	20	3.19	0.0353
Treatment * storage time	16	20	0.46	0.9376

APPENDIX XVII

ANALYSIS OF VARIANCE FOR FIXED EFFECTS
FOR APPEARANCE OF WALDMANN'S LEAF LETTUCE
USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	481	12.92	<0.0001
Storage time	2	481	8.47	0.0002
Treatment * storage time	8	481	11.89	<0.0001

APPENDIX XVIII

ANALYSIS OF VARIANCE FOR FIXED EFFECTS FOR
COLOR INTENSITY OF WALDMANN'S LEAF LETTUCE
USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	478	2.89	0.0219
Storage time	2	478	1.77	0.1710
Treatment * storage time	8	478	4.31	<0.0001

APPENDIX XIX

ANALYSIS OF VARIANCE FOR FIXED EFFECTS
FOR FLAVOR OF WALDMANN'S LEAF LETTUCE
USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	473	2.32	0.0561
Storage time	2	473	0.57	0.5659
Treatment * storage time	8	473	1.89	0.0590

APPENDIX XX

ANALYSIS OF VARIANCE FOR FIXED EFFECTS FOR TEXTURE (SENSORY EVALUATION) OF WALDMANN'S LEAF LETTUCE USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	482	5.70	0.0002
Storage time	2	482	1.25	0.2870
Treatment * storage time	8	482	2.10	0.0340

APPENDIX XX1

ANALYSIS OF VARIANCE FOR FIXED EFFECTS
FOR BITTERNESS OF WALDMANN'S LEAF LETTUCE
USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	482	2.60	0.0354
Storage time	2	482	2.10	0.1233
Treatment * storage time	8	482	1.86	0.0649

APPENDIX XXII

ANALYSIS OF VARIANCE FOR FIXED EFFECTS
FOR TARTNESS OF WALDMANN'S LEAF LETTUCE
USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	481	2.34	0.0541
Storage time	2	481	0.68	0.5074
Treatment * storage time	8	481	0.27	0.9745

APPENDIX XXI11

ANALYSIS OF VARIANCE FOR FIXED EFFECTS FOR OVERALL ACCEPTABILITY OF WALDMANN'S LEAF LETTUCE USING THE MIXED MODEL PROCEDURE OF SAS

ANOVA TABLE OF FIXED EFFECTS

Source of variation	Num DF	Den DF	F-value	P > F
Treatment	4	480	3.03	0.0175
Storage time	2	480	0.65	0.5219
Treatment * storage time	8	480	2.19	0.0267

APPENDIX XXIV

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Effects of ascorbic acid applied by two hydrocooling methods on browning, texture, antioxidant capacity and sensory properties of green leaf lettuce

NAME OF PRINCIPAL INVESTIGATOR: Martha Stone, Ph.D.

NAME OF CO-INVESTIGATOR: Patricia A. Kendall, Ph.D.; Juan Ramon Esparza Rivera

CONTACT NAME AND PHONE NUMBER FOR QUESTIONS/PROBLEMS: Martha Stone, 970-491-6772

SPONSOR OF PROJECT:

PURPOSE OF THE RESEARCH:
This study involves research into the effects of ascorbic acid applied by two different methods (hydrocooling and spraying) on reducing browning and textural changes on fresh cut green leaf lettuce. This research study is designed to develop a method to extend shelf life of fresh cut lettuce.

PROCEDURES/METHODS TO BE USED:
You will taste lettuce samples that have been treated with an ascorbic acid or vitamin C solution in order to avoid browning and textural changes in this vegetable. You will taste food products containing ingredients all found to be safe by the Food and Drug Administration. Green leaf lettuce samples will be provided by the Horticulture Department of Colorado State University. Lettuce samples will be treated in a Food Laboratory in the Gifford Building at Colorado State University. You will evaluate the samples for appearance, texture, bitterness, sourness, color, flavor, and overall acceptability. The sample testing will not take more than 30 minutes. You will not be videotaped or audiotaped during any tastings.

RISKS INHERENT IN THE PROCEDURES:
There are no known risks involved in this research. It is not possible to identify all potential risks in an experimental procedure, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

BENEFITS:
There are no known benefits to participate in this study, but we hope the outcome of this research will be to extend the shelf life of lettuce.

CONFIDENTIALITY:
Strict confidentiality of information will be maintained by recording data using numbers to identify the participants. Resulting data will be reported in research materials in aggregate. Only the investigators and necessary personnel (graduate student) will have access to the participant's identification.

LIABILITY:
The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury. Questions about subjects' rights may be directed to Celia S. Walker at (970) 491-1563.

PARTICIPATION:
Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 2 pages.

_____
Participant name (printed)

_____ _____
Participant signature Date

_____ _____
Witness to signature (project staff) Date

PARENTAL SIGNATURE FOR MINOR

As parent or guardian you authorize _____ (print name) to become a participant for the described research. The nature and general purpose of the project have been satisfactorily explained to you by _____ and you are satisfied that proper precautions will be observed.

_____
Minor's date of birth

_____
Parent/Guardian name (printed)

_____ _____
Parent/Guardian signature Date

APPENDIX XXV

Sample # _____
Panelist # _____
Date _____

Leaf Lettuce Sensory Evaluation

Please complete scoring for each sample and score using the attributes listed below. Make a vertical pencil mark to indicate your opinion of the sample along the given line. Please eat the entire sample and cleanse palate with water between samples.

Appearance:

Unacceptable	Somewhat Unacceptable	Neutral	Somewhat Acceptable	Acceptable

Color intensity

Extremely Dark	Somewhat Dark	Neither Dark nor light	Somewhat light	Light

Flavor:

Unacceptable	Somewhat Unacceptable	Neutral	Somewhat Acceptable	Acceptable

Texture:

Firm	Somewhat Firm	Neither Firm nor Soft	Somewhat Soft	Soft

Bitterness:

Extremely Bitter	Somewhat Bitter	Neither Bitter nor Mild	Somewhat Mild	Mild

Tartness:

Extremely Tart	Somewhat Tart	Neither Tart nor Mild	Somewhat Mild	Mild

Overall Acceptability:

Unacceptable	Somewhat Unacceptable	Neutral	Somewhat Acceptable	Acceptable

Comments:_____

APPENDIX XXVI

CITY OF FORT COLLINS DRINKING WATER QUALITY SUMMARY 2004

CITY OF FORT COLLINS DRINKING WATER QUALITY SUMMARY 2004									
Parameter	MCL	Poudre River	Horsetooth Reservoir	SS#2	Overland Foods	Service Center	Poudre Valley Hospital	1 Drake	PRPA
Free Chlorine Residual, mg/L	4	-	-	0.52	0.31	0.31	0.26	0.40	0.19
Temperature, °C	-	8.4	7.1	8.3	10.9	12.5	12.6	10.7	15.4
Total Coliforms / 100mL	<1	76	288	0	0	0	0	0	0
Fecal Coliforms / 100mL	<1	4	0	0	-	-	-	-	-
Fecal Strep / 100mL	-	12	0	0	-	-	-	-	-
Heterotrophic Plate Cnt / 1.0mL	-	237	57	0	2	2	0	3	3
Alkalinity, mg/L as $CaCO_3$	-	23.0	26.8	37.0	36.9	37.1	36.9	36.9	37.9
Ammonia,i mg/L as N	-	<0.02	<0.02	<0.02	-	-	-	-	-
Calcium, mg/L as $CaCO_3$	-	17.9	23.0	42.4	42.7	42.7	42.6	41.6	40.3
Chlorate, mg/L	-	-	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Chloride, mg/L	-	1.6	1.3	2.5	-	-	-	-	-
Chlorite, mg/L	-	-	-	0.1	0.1	0.1	0.1	0.1	0.2
Color, units	(15)	16.0	15.9	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Conductivity, µmhos/cm	-	60.4	67.2	113	113	113	113	112	111
Fluoride, mg/L	(2)	0.16	0.14	0.92	0.92	0.91	0.91	0.91	0.86
Hardness, mg/L as $CaCO_3$	-	24.2	28.1	48.8	49.3	49.2	49.2	48.9	46.9
Langlier Larson Saturation Index	-	-2.26	-2.17	-1.30	-1.21	-1.15	-1.28	-1.26	-1.00
Nitrate, mg/L as N	10	<0.2	<0.2	<0.2	-	-	-	-	-
Nitrite, mg/L as N	1	<0.1	<0.1	<0.1	-	-	-	-	-
pH, units	6.5 - 8.5	7.58	7.40	7.91	7.96	8.02	7.98	7.93	8.19
Silica, mg/L	-	7.8	4.1	7.3	-	-	-	-	-
Sulfate, mg/L	(250)	<5.0	<5.0	12.0	-	-	-	-	-
Total Dissolved Solids, mg/L	(500)	45	46	71	68	70	66	69	66
Turbidity, NTU	-	2.0	3.0	0.2	0.1	0.1	0.1	0.1	0.1
Aluminum, µg/L	(50 - 200)	177	485	69.8	71.4	66.3	64.8	61.7	78.7
Antimony, µg/L	6	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Arsenic, µg/L	10	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Barium, µg/L	2000	14.6	15.9	14.8	14.4	14.7	15.3	15.4	14.3
Beryllium, µg/L	4	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Cadmium, µg/L	5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Chromium, µg/L	100	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Copper, µg/L	[1300](1000)	<3.0	5.2	1.5	7.5	7.7	28.7	7.8	10.5
Iron, µg/L	(300)	187	220	18.0	17.6	21.1	16.9	15.7	25.8
Lead, µg/L	[15]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Magnesium, mg/L	-	1.6	1.5	1.6	-	-	-	-	-
Manganese, µg/L	(50)	8.4	15.0	1.1	<1.0	<1.0	<1.0	<1.0	<1.0
Mercury, µg/L	2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Nickel, µg/L	100	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Selenium, µg/L	50	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Silver, µg/L	(100)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Sodium, mg/L	(20)	2.9	2.4	2.9	2.9	2.9	3.0	3.1	4.0
Thallium, µg/L	2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Zinc, µg/L	(5000)	<100	<100	<100	<100	<100	<100	<100	<100
Total Organic Carbon, mg/L	-	2.9	3.1	1.2	1.2	1.2	1.1	1.2	1.2
Total Trihalomethanes, µg/L	80	<0.4	<0.4	17.7	28.9	27.9	29.2	25.1	30.3

Notes: mg/L = milligram per liter or part per million
µg/L = microgram per liter or part per billion
Poudre River = Cache la Poudre River Raw Water at Plant
Horsetooth Res = Horsetooth Reservoir Raw Water at Plant
SS #2 = Sample Station 2, Official dist. System entry point
MCL = Maximum Contaminant Level ( ) = Secondary level (asthetic) [ ] = 90th %tile action level < = less than

Overland Foods, N. Hwy 287, Laporte CO
Service Center, 700 Wood St.
PVH = Poudre Valley Hospital, 1024 Lemay Ave
1 Drake Park, 373 W Drake Rd.
PRPA = Platte River Power Authority Office

APPENDIX XXVII

DISTRIBUTION OF LETTUCE HEADS WITHIN TREATMENT

EACH HARVEST LOT

TEST or ASSAY	First harvest	Second harvest	Third harvest	Fourth harvest
FREEZE-DRYING (for total ascorbate content, ABTS antioxidant activity assay and total phenolic content	2 lettuce heads/treatment	2 lettuce heads/treatment	2 lettuce heads/treatment	
Relative water content	2 lettuce heads/treatment	2 lettuce heads/treatment	2 lettuce heads/treatment	
Instrumental texture measurement	3 lettuce heads/treatment	3 lettuce heads/treatment	3 lettuce heads/treatment	
Instrumental texture measurement	2 lettuce heads/treatment	2 lettuce heads/treatment	2 lettuce heads/treatment	
Extra samples	2 lettuce heads/treatment	2 lettuce heads/treatment	2 lettuce heads/treatment	
Sensory Evaluation				11 lettuce heads/treatment
TOTAL LETTUCE HEADS PER TREATMENT	**11** lettuce heads/treatment	**11** lettuce heads/treatment	**11** lettuce heads/treatment	**11** lettuce heads/treatment

APPENDIX XXVIII

DISTRIBUTION OF LETTUCE HEADS ACROSS TREATMENTS

EACH HARVEST LOT

TEST or ASSAY	First harvest	Second harvest	Third harvest	Fourth harvest
ASCORBIC ACID IMMERSION	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment
ASCORBIC ACID SPRAYING	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment
WATER IMMERSION	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment
WATER SPRAYING	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment
CONTROL	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment	11 lettuce heads/treatment
TOTAL LETTUCE HEADS PER TREATMENT	**55** lettuce heads/treatment	**55** lettuce heads/treatment	**55** lettuce heads/treatment	**55** lettuce heads/treatment

APPENDIX XXIX

EXPERIMENTAL DESIGN FOR ALL ASSAYS FOR

THE ANALYSIS OF LEAF LETTUCE

STORAGE TIME

TREATMENT	Before treatments	1 day	7 days	14 days	21 days
Ascorbic acid immersion					
Water immersion					
Ascorbic acid spraying					
Water immersion					
Control					

APPENDIX XXX

EXPERIMENTAL DESIGN FOR SENSORY EVALUATION OF LEAF LETTUCE

STORAGE TIME

TREATMENT	1 day	7 days	14 days
Ascorbic acid immersion			
Water immersion			
Ascorbic acid spraying			
Water immersion			
Control			