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1998-04-01

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Barry-Ryan, C., Beirne, D.:Ascorbic Acid Retention in Shredded Iceberg Lettuce as Affected by Minimal Processing. Journal of Food Science, 64, pp.498-500.

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Ascorbic Acid Retention in Shredded Iceberg Lettuce as Affected by Minimal Processing

Journal of Food Science, 64, 498-500.

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ABSTRACT

The effects of slicing method, packaging atmosphere and storage temperature were determined on total ascorbic acid (TAA) content of modified atmosphere packaged shredded lettuce. TAA was extracted from the lettuce samples and levels were monitored using either titration with 2,6-dichlorophenolindophenol solution or an assay kit. Slicing affected ($p<0.05$) ascorbic acid retention in the order manual tearing > manual slicing > machine slicing. Flushing with 100% nitrogen increased retention (~5%, $p<0.05$) over packages with product modified atmospheres. Storage at 3°C increased retention (~20%, $p<0.05$) compared with storage at 8°C. Scores for product appearance generally followed losses of TAA in the lettuce ($p<0.05$).

Key Words: ascorbic acid, shredded lettuce, slicing, modified atmosphere

INTRODUCTION

FRUITS AND VEGETABLES UNDERGO VARIOUS HANDLING, STORAGE AND PROCESSING STEPS before they are consumed. Because it is widely believed that processing results in significant losses of nutrients, fresh produce is perceived as being more nutritious than canned or frozen products (Klein, 1987). Thus, novel mild processing treatments such as minimal processing, appear desirable to the consumer. Minimal processing typically involves peeling, slicing, dicing or shredding prior to packaging and storage. All of these steps have an effect on nutrients, shelf-life and quality of the prepared produce (McCarthy and Mathews, 1994). Modifying the atmosphere generally results in reduced respiration rates and better sensory attributes (Kader et al., 1989). Packaging of vegetables retards deterioration and enhances maintenance of nutrients (Barth et al., 1993b). Temperature influences many deteriorative processes (Esteve et al., 1995).

Ascorbic acid is labile and its retention is often followed when evaluating postharvest storage effects on nutritional quality of vegetables (Barth et al., 1993b). It is readily affected by light, oxygen, heat, enzymes and metals (Albrecht et al., 1991). Ascorbic acid level is often considered equivalent to Vitamin C content; however, dehydro-ascorbic acid has Vitamin C activity as well, and its utilization in humans is almost the same (Petersen and Berends, 1993). Therefore, the measurement of both is required to determine the effects of processing and storage conditions on total Vitamin content.

The objective of this study was to determine the effects of slicing method, packaging atmospheres and storage temperature on ascorbic acid retention and quality of shredded lettuce. Substantial loses of ascorbic acid could be nutritionally important for those consuming large amounts of green salads. In addition, loss of ascorbic acid provides a useful index of oxidative deterioration.

MATERIALS & METHODS

Processing and packaging procedures

Spanish Iceberg lettuce (cultivar Salodin) was used for the production of modified atmosphere packaged shredded lettuce. Before processing, the outer leaves and any excess dirt were removed from the heads of lettuce. Heads were then cored using a sharp knife. Samples were shredded into 6mm wide pieces, either manually or by machine. Manual shredding was carried out using a sharp knife or tearing by hand into strips. Mechanical slicing was carried out using a Sammic CA300 vegetable processing machine (Barcelona, Spain), equipped with either a sharp or blunt cutting disk. A pair of flat straight blades ($3 \times 9cm^2$) were mounted on the cutting disks in parallel. The sharp blades were unused before this experiment. The blunt blades were rendered blunt through use on a coleslaw production line for 1 yr.

The shredded lettuce samples were dipped for 5 min with agitation in a 100 ppm chlorine solution. The solution was prepared by adding 7.14 mL sodium hypochlorite (14% chlorine) to 10L of distilled water. The pH was adjusted to 6.9 using 1M HCl. Dipping was followed by a water rinse to remove residual chlorine. Shredded lettuce (100g) was then spun at 200 rpm for 1 min in a salad spinner to remove excess moisture.

Shredded lettuce strips (20g) were packaged in bags (280×18 mm^2) made from 35 µm thick oriented polypropylene (OPP-Propafilm, ICI), which had a permeability to O_2 of 1,200 $mL/m^2/day/atm$ and to CO_2 of 4,000 $mL/m^2/day/atm$. Bags were either left unsealed, sealed in air or flushed with nitrogen before sealing using a Multivac A300 packaging machine (Multivac, Germany). Packaged products were stored at 3°C and 8°C.

Gas analysis of package atmospheres

The atmospheric gases within the stored packs were sampled to determine the levels of CO_2 and O_2 during storage. A CTR1 column (Alltech, USA) fitted in a Gow-Mac gas chromatograph equipped with a thermal conductivity detector was used. The carrier gas used was helium (Grade A; BOC) at a flow rate of 60 mL/min. Using an airtight syringe gas (10 mL) was drawn from the pack through the 1 mL sampling loop and allowed to equilibrate before injection.

Ascorbic acid content

Titration Ascorbic acid levels were determined in shredded lettuce samples using the method described by Albrecht et al. (1990). Samples of lettuce (20g) were ground with a mortar and pestle. Metaphosphoric acid (2.5%) in glacial acetic acid was added to make the volume up to 60 mL. This solution was then filtered through fluted filter paper (Whatman No. 1, 7 cm dia) using a rapid flow rate. Aliquots of the filtrate (10 mL) were transferred to 50 mL Erlenmeyer flasks, and titrated rapidly with 2,6-dichlorophenolindophenol solution. The volume used to reach a permanent pink color was proportional to the amount of ascorbic acid in the filtrate (determined from a standard curve). The total ascorbic acid (TAA) content was determined by first reducing dehydro-ascorbic acid (DHAA) back to ascorbic acid (AA) by the addition of 2 mL homocysteine (0.5%) to 10 mL of filtrate.

Colorimetric assay: Samples of lettuce (10g) were homogenized

with 20 mL water for 5 min. The volume was then brought to 100 mL and filtered. The ascorbic acid assay (Boehringer Mannheim) was carried out by adding 0.1 mL of the prepared sample into plastic cuvettes at room temperature, adding 1mL of 7.5 mM 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide solution (MTT) and 1.5 mL distilled water. In the presence of 0.1 mL of 15 mM 5-methylphenazine methosulfate at pH 3.5, the MTT was reduced by the AA. The resulting formazan was measured at 578 nm after 15 min incubation at 37°C. A blank was prepared by initially removing ascorbic acid present by the addition of ascorbate oxidase (17 units). This assay was repeated after reducing the DHAA acid to AA by the addition of 0.1 mL 1,4-dithiothreitol solution (1.3 mM, pH7.5) to determine TAA levels.

Sensory evaluation

Analytical sensory evaluation was used to discriminate between the appearance of the shredded lettuce prepared by different slicing methods, packaged under different atmospheres and stored at different temperatures. A panel of ten judges, aged 22-30 years (eight female and two male, all members of the UL Food Science Research Centre) with sensory evaluation experience, were trained in discriminative evaluation of shredded lettuce. The shredded lettuce used during the training sessions, every second day for 1 month, had been subjected to various storage treatments and times. Fresh lettuce was used as the control (score=9). The training panel members were shown the effects of storage over 10 days in air vs a range of modified atmospheres (achieved using different films). The effects of storage temperature (3, 8 and 20°C) and time (10 days) on the shredded lettuce was also shown during the training sessions. The products were presented in groups, by sample day, to a single sensory judge at a time on a white lab bench in an odor-free fluorescent lit food laboratory. The products were scored for appearance, on a scale of one to nine (ranked), where 1=very poor, 4-5=fair and 9=excellent, at regular intervals during storage. Products were coded using geometric symbols to avoid bias. Sensory evaluation was also used to determine the shelf-life of these products, as scores of 5 or below were taken to indicate the end of shelf-life.

Statistical analysis

All experiments were carried out in duplicate and replicated three times. All data were analyzed by two-way analysis of variance (ANOVA) and a least significant difference multicomparison test to determine significant differences between treatments (Shamaila et al., 1992). Significance of differences was defined as $p < 0.05$.

RESULTS & DISCUSSION

There were no differences between titration with 2,6-dichlorophenolindophenol solution and the colorimetric assay used to determine AA, DHAA, and hence TAA in the shredded lettuce samples. Data presented are those recorded using the titration method. TAA levels fell in all packs during storage. Albrecht (1993) reported that whole lettuce lost ascorbic acid during storage. Some of the AA loss was due to its being oxidized to DHAA. Levels of DHAA rose in all packs up to Day 6, after which it began to level off or fall slightly. These data confirmed trends reported by Petersen and Trolle (1996) for DHAA levels in packaged peas and potatoes and for broccoli spears (Barth et al., 1993b).

Products prepared by the different slicing methods had been packaged directly into OPP bags without nitrogen flushing, in order to examine the effects of slicing without any possible masking benefits of an immediate low oxygen atmosphere. Oxygen (O_2) levels fell in all packs, slower initially in packs prepared by hand slicing steps, and had dropped to ~1% by Day 6, irrespective of slicing method (Fig. 1). These trends in O_2 levels were similar to those reported by Bolin and Huxsoll (1991) for packs of salad-cut lettuce prepared by different slicing methods. Carbon dioxide (CO_2) levels rose as O_2 levels fell and reached ~30% by Day 6, irrespective of slicing method.

TAA content fell in packs prepared by each of the slicing methods. The TAA content in these packs depended on the nature of the slicing method used (Fig. 2). Higher levels of TAA were retained (p 0.05) in samples which had been prepared by manually tearing the lettuce into strips (6-8 mg/100g FW higher). Lettuce shredded using a sharp knife retained initially 18% less TAA than the torn samples, and this difference in losses had risen to ~50% by Day 10. The retention of TAA by the products sliced by machine was 25-63% lower than for lettuce shredded by manual tearing. Using the blunt machine blade resulted in ~10% lower TAA levels than when a sharp blade was used ($p<0.05$).

Sealing the OPP bags and allowing a product modified atmosphere to develop (Fig. 3) increased TAA retention over that in unsealed bags ($p<0.05$) (Fig. 4). TAA retention levels were increased further by flushing packs with nitrogen, levels were ~10% higher from Day 6. The beneficial effects of modified atmosphere (MA) storage on TAA retention and shelf-life extension had been reported

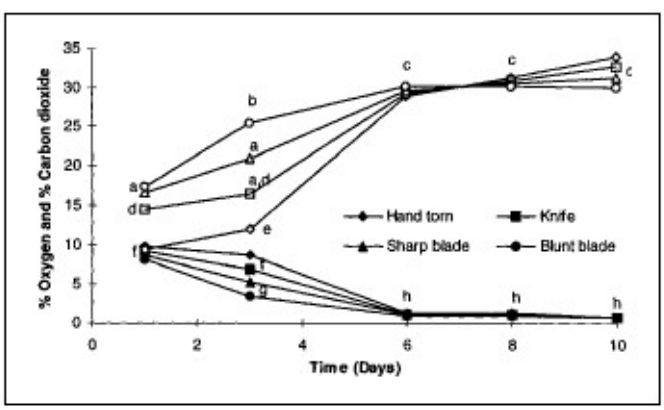

Fig. 1—Percentage of CO_2 (open symbols) and O_2 (closed symbols) in the atmosphere of packs containing shredded lettuce prepared by different slicing methods packaged in OPP (8°C).

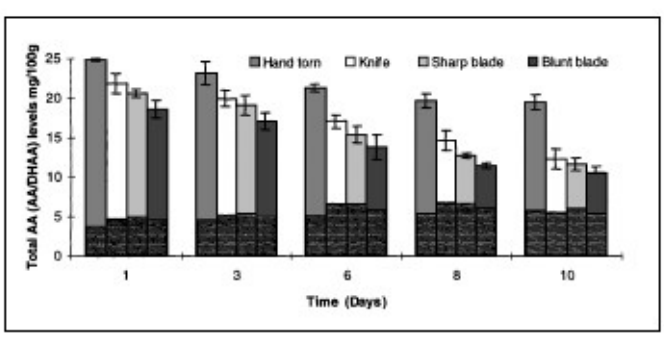

Fig. 2—Effects of slicing method on the TAA (mg/100g fresh weight) retention in shredded lettuce stored in OPP sealed bags at 8°C (AA: upper section and DHAA: lower striped section).

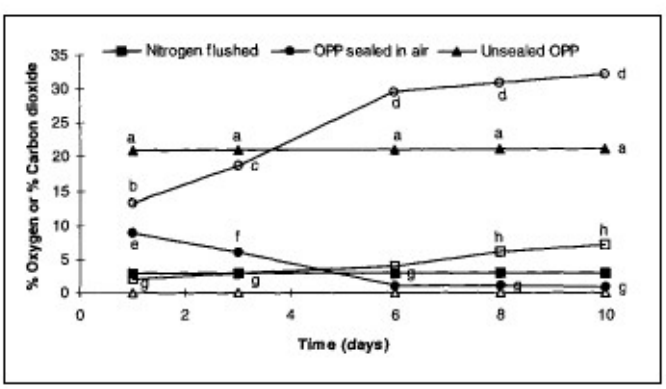

Fig. 3—Percentage of CO_2 (open symbols) and O_2 (closed symbols) in the atmosphere of packs containing lettuce shredded by knife and packaged under different atmospheres (8°C).

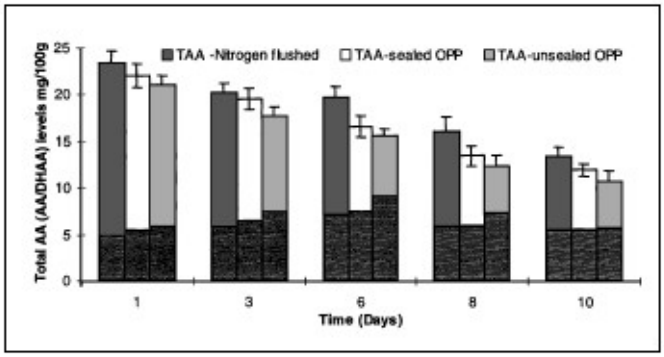

Fig. 4—Effects of packaging method on the TAA (mg/100g fresh weight) retention in lettuce shredded by knife, 8°C (AA: upper section and DHAA: lower striped section).

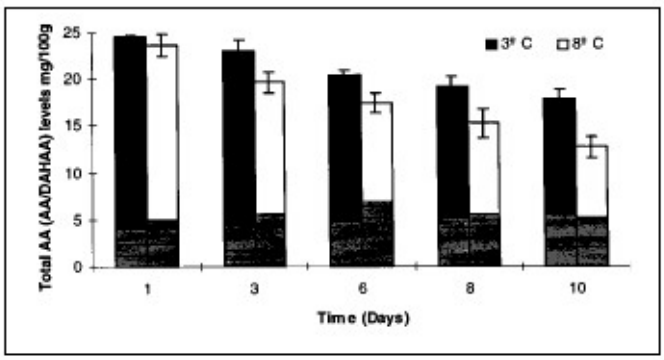

Fig. 5—Effects of storage temperature on the TAA (mg/100g fresh weight) retention in lettuce shredded by knife packaged in OPP bags (AA: upper section and DHAA: lower striped section).

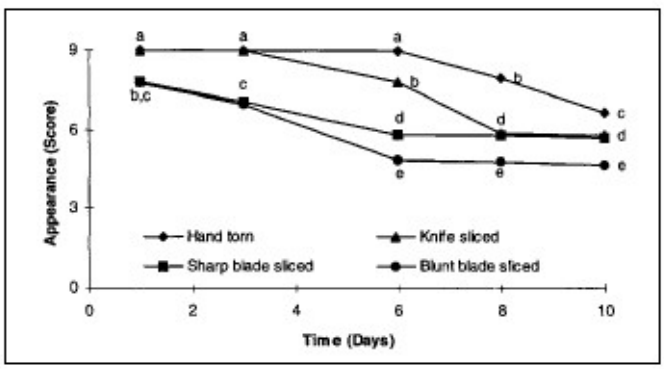

Fig. 6—Effects of slicing method on the appearance scores for shredded lettuce stored in OPP bags at 8°C. Samples were scored on a scale of 1 to 9, where 1 = very poor, 4-5 = fair and 9 = excellent. Values are the means for six determinations.

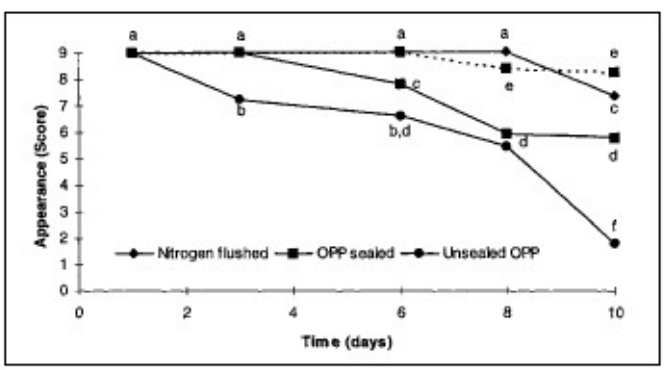

Fig. 7—Appearance scores for lettuce shredded by knife packaged under different atmospheres and stored at 8°C (3°C broken line). Samples were scored on a scale of 1 to 9, where 1=very poor, 4-5=fair and 9=excellent. Values are the means for six determinations.

for fruits and vegetables (Kader, 1986) and for broccoli spears (Barth and Zhuang, 1996 and Barth et al., 1993a).

Petersen and Trolle (1996), reported that the TAA retention levels for peas and potatoes were increased by reducing the O_2 levels in the packs to 1%. However, the MA that developed within the unflushed packs of shredded lettuce contained high CO_2 levels (30%, Fig. 3). McCarthy and Mathews (1994) reported that high levels of CO_2 (30-40%) increased AA losses. Our results demonstrated that considerable amounts of AA were converted into DHAA, especially in the open packs, due to availability of oxygen. Petersen and Berends (1993) had reported that DHAA levels increased in sweet green peppers with exposure to increasing oxygen levels.

Storage temperature affected TAA retention ($p < 0.05$). TAA levels were reduced at the higher storage temperature (8°C); they were ~25% lower than at 3°C by Day 10 (Fig. 5).

The sensory panel examined all shredded lettuce samples during the 10 days storage and found that declines in appearance scores generally followed losses of TAA. Slicing method affected appearance scores (p 0.05, Fig. 6). Manually prepared lettuce samples were more acceptable ($p<0.05$), from Day 1 with manual tearing as compared with slicing improving visual quality from Day 6 onwards. Lettuce prepared using a sharp machine blade received higher appearance scores than samples prepared using a blunt blade, from Day 6 onwards. Bolin et al. (1977) had long ago reported that the cutting method used for shredding lettuce had significant effects on quality.

Flushing with nitrogen also improved acceptability ($p<0.05$) (Fig. 7). Ballantyne et al. (1988) showed that gas flushing shredded lettuce was effective in increasing shelf-life. The MA that developed in the product modified atmosphere packs had very high levels of CO_2 (~30%) which may have initiated the browning that was observed from Day 6.

CONCLUSION

MODIFIED ATMOSPHERE PACKAGING, NITROGEN FLUSHING, manual tearing or slicing and low storage temperatures were effective postharvest storage treatments for conserving ascorbic acid and visual quality in shredded lettuce.

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