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Certain Changes in Chemical Composition of Lettuce (Lactuca Sativa L.) Stored in Controlled Atmosphere

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CERTAIN CHANGES IN CHEMICAL COMPOSITION OF LETTUCE (*LACTUCA SATIVA* L.) STORED IN CONTROLLED ATMOSPHERE

by

Don Jeng Wang

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ABSTRACT

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Utah State University, 1971

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This study was undertaken to determine the effect of four different combinations of treatments and storage; i.e., controlled atmosphere (2.5 percent O_2 and 2.5 percent CO_2), controlled atmosphere in combination with phaltan (1,000 ppm), controlled atmosphere with packaging in polyethylene bags, controlled atmosphere with phaltan and packaging in polyethylene bags, as well as conventional refrigeration, on the chemical composition of lettuce heads (Cultivar "Great Lakes") during 75 days of storage. Analyses of total sugars, starch, reducing sugars, total organic acids, free amino acids, soluble proteins, pH, titratable acidity, and total carotenes were made on the fifteenth, thirtieth, forty-fifth, sixtieth, and seventy-fifth days of storage at 35 F.

There were no significant differences in total organic acids and free amino acids between the conventional refrigeration (35 F) and other treatments during the early stage of storage. They were, however, higher after 45 days of storage with phaltan treatments, although still not changed in controlled atmosphere and controlled atmosphere in combination with packaging.

The soluble proteins and the reducing sugars were lower in the controlled atmosphere lettuce than in the conventional refrigeration lettuce. The lettuce treated with phaltan or phaltan in combination with polyethylene packaging had higher amounts of soluble proteins.

Although pH of the lettuce heads was not changed throughout 75 days of storage, the titratable acidity was higher in all treatments during the storage.

(38 pages)

INTRODUCTION

Lettuce, like most leafy vegetables, deteriorates after harvest. The loss of quality can be minimized by rapid handling and the best possible storage conditions (Pratt, Morris, and Tucker, 1954). Since producing areas tend to be remotely located from consuming areas, conditions which provide a fresh product that can remain in market channels or in storage for longer times are desirable.

Respiration is a major physiological function contributing to deterioration of lettuce during storage. In temperature ranges of 32 F to 86 F, the respiration-induced degradation can be limited (Burgheimer et al., 1967). During respiration, carbohydrate is broken down by O_2 and CO_2 is produced. The reaction suggests that the respiration can be controlled by regulating the amount of O_2 and CO_2 in the environment around the produce. The increased concentration of CO_2 tends to reduce the respiration rate and the reduction may be extended by decreasing the O_2 concentration. Control of the concentration of the two gases combined with refrigeration is referred to as controlled atmosphere (CA) storage.

In recent years, the industry associated with handling and conveyance of lettuce has become increasingly interested in CA storage. Watada, Morris, and Rappaport (1964) reported that higher concentrations of CO_2 can be harmful to lettuce. In addition, many studies concerning the transportation of lettuce with CA have been conducted by Lipton (1967), Stewart, Ceponis, and Beraha (1970), and Harvey (1969); however, a proper O_2 and CO_2 concentration for continuous storage has not yet been established. The optimum condition for lettuce storage has been investigated in our laboratory (Yang, 1971). When stored at 35 F and 95 percent relative humidity with 2.5 percent O_2 and 2.5 percent CO_2, lettuce maintained its quality for 60 days of storage.

Besides CA storage, chemical and packaging treatments were proved to be effective in extending the shelf-life of fruits and vegetables (Do et al., 1966). The increase in storage life was due to the anti-microbial and senescence-inhibiting action of the chemicals tried. Thus, El-Mansy et al. (1967) recommended the application of N^6-benzyladenine and 6-furfurylaminopurine as a pre- and post-harvest treatment for lettuce. Do et al. (1966) succeeded in extending the shelf-life of sweet cherries by treating with antifungal chemicals; viz., captan (N-Trichloromethylthio)-4-cyclohexene-1,2,-dicarbonimide) and phaltan (N-Trichloromethylthiophthalimide) and packaging with poly-ethylene bags.

The recent work of Yang (1971) on the combined effects of chemical, packaging, and CA showed the possibility of formulating a commercially applicable technique for extending the storage life of lettuce.

In order to determine the effects of CA (2.5 percent O_2 and 2.5 percent CO_2 at 35 F) on nutritive composition of leaves of lettuce, changes in the amount of total sugars, starch, reducing sugars, total organic acids, free amino acids, pH, total titratable acidity, and total carotenes after 15, 30, 45, 60, and 75 days of storage were followed.

REVIEW OF LITERATURE

Control of atmosphere with respect to CO_2 and O_2 is used to retard deterioration of apples, cherries, and other fruits during storage or transit to market. This practice, however, has seldom been employed for vegetables. In many cases, CA has been injurious to vegetables (Nelson, 1926; Platenius, 1943). Nelson (1926) found some external and internal injuries in lettuce held in 0 percent and 0.5 percent O_2 at room temperature. But Parsons, Gates, and Spalding (1964), on the other hand, found the flavor, color, and keeping quality were not damaged when lettuce was held in atmospheres of 99 percent or 100 percent N_2 for 10 days at 33 F. Watada, Morris, and Rappaport (1964) found that higher CO_2 concentrations (20, 40, or 60 percent) produced injuries in lettuce when stored at 41 F for a period of eight days. Lipton (1967) found substantial reduction in russet spotting in letuce stored in 0.5 percent to 8 percent O_2 at 50 F. However, the young heart leaves were sensitive and injured in such low O_2 conditions.

Stewart, Ceponis, and Beraha (1970), working on transportation of fresh lettuce in CA storage, found the same beneficial effect of reduction of russet spotting at 2 to 3 percent O_2 storage. They also found that lettuce in vehicles that had 1.9 percent or less CO_2 at the termination of transportation were free of russet spotting, but not in vehicles with 2.4 percent or more CO_2 at 37 F.

Harvey (1969) suggested that 3 percent O_2 atmosphere at 32 F also reduced browning of lettuce; but when reduced to 0.5 percent O_2, most lettuce was as brown as some of the controls (in a refrigerator at 32 F).

Respiration appears to be one of the metabolic processes influenced by CA. McKenzie (1931) studied the effect of temperature on the respiration rate of lettuce. He noted that as the CO_2 was increased, there was a decrease in rate of CO_2 evolution. Platenius (1943) found that lowering the O_2 concentration to 1.2 percent at 35.6 F could reduce the respiration rate of asparagus by 55 percent. Decreasing the O_2 below the ambient atmospheric level was also found to be effective for other vegetables, such as snapbeans and peas. Claypool and Allen (1948) also observed low respiration rate in apricots, plums, and peaches stored in low oxygen atmosphere. Lieberman and Hardenburg (1954) observed a reduction of respiration rate by decreasing the O_2 concentration from 21 percent to 0 percent in broccoli stored at 75 F. McGill, Nelson, and Steinberg (1966) found in the CA storage of spinach at 34 F, increasing CO_2 in the atmosphere around the product reduced the rate of respiration; as the oxygen level was decreased to 4 percent, respiration was slightly reduced, but was less effective than with increased CO_2.

Several investigators have found that many of the changes or alterations occurring in CA-stored fruits and vegetables have a direct relation to the retarded rate of respiration. Kidd and West (1930) investigated many aspects of biochemical changes investigated by the use of CA. When comparing the changes taking place in apples at 50 F in air, and in 9 percent CO_2 plus 11 percent O_2, they noted that the average loss of carbohydrate by respiration was 1.2 to 1.4 times as fast in the absence of carbon dioxide than in its presence. They also reported that the inversion of sucrose was accelerated in apple at 4 F in 3 percent CO_2 and 3 percent O_2 (Kid and West, 1933). Singh, Littlefield, and Salunkhe (1970) reported that CA did not produce any significant effect on the content of total sugars of cherries. Wankier, Salunkhe, and Campbell (1970) also found the content of total sugars in Elberta peaches and Moorpark apricots was not affected by the conditions of 0 to 10 percent CO_2 and 5 percent O_2 at 32 F. By contrast, total sugars in the "Large Early Montagamet" apricots were influenced by both temperature in storage and CA treatment. The higher the concentration of CO_2, the lower the accumulation of free reducing sugars within the fruit tested. Therefore, CO_2 appeared to influence the processes that degraded non-reducing sugars to free reducing sugars, probably by inhibition of invertase enzyme.

In the studies of the effect of CA on vegetables, McGill, Nelson, and Steinberg (1966) found that in CA the starch content of all spinach samples varied with storage time, but showed no definite trends. The amount of reducing sugars varies with no consistent relation to storage time, atmosphere, or temperature. This variation may be caused by the formation of other reducing substances.

The total titratable acidity and pH of the CA-stored vegetables have been reported by many investigators. The titratable acid and total acid anion content of green beans stored in CA with 9.5 percent CO_2 and 3.3 percent O_2 at 45 F for 15 days were found to be decreased, as compared to that of normal air control samples. Burgheimer et al. (1967) recorded the decrease in titratable acidity in spinach stored in 9.2 percent CO_2 and 4 percent O_2 at 34 F for eight to nine days. In the study on the CA storage of tomato, Parsons, Anderson, and Penney (1970) found that mature-green tomatoes stored at 55 F for six weeks in 3 percent O_2 with 3 to 5 percent CO_2 tended to have 5 to 10 percent more acid after ripening than those stored without CO_2. Apparently, the effects on titratable acidity are dependent on the species under investigation and storage times.

Many of the investigators of CA storage recognized that this new type of storage caused changes in basic metabolic processes. Bendall, Ranson, and Walker (1960); Bonner (1950); and Ranson, Walker, and Clarke (1957) have reported the possible effects of increased CO_2 concentrations on the activities of several enzymes. The accumulation of succinic acid after CA storage could have been related to the inhibitory or toxic action of CO_2 on succinic oxidase enzyme. Ranson (1953) found no major changes in acid composition of Kalanchoe leaves, carrot root, and oat caleoptiles after storage for 12 hours to 3 days in atmospheres containing 20 to 90 percent CO_2 at 68 F, but succinate was accumulated and malate was depleted. Frenkel and Patterson (1969) reported that in pears, high CO_2 concentration inhibited succinic dehydrogenase in pears stored in CA. Wankier, Salunkhe, and Campbell (1970) found that the increased concentration of CO_2 accelerated accumulation of succinic acid and depletion of malic acid.

Li (1963), working on pears in CA storage, noted that total alcohol soluble nitrogen and protein nitrogen decreased at a slower rate in CA, resulting in a higher concentration at the end of the storage period. Groeschel, Nelson, and Steinberg (1966) found more soluble nitrogen in the conventional refrigeration samples than in the samples of green beans stored at 45 F up to 15 days in a 9.5 percent CO_2 and 3 percent O_2 atmosphere.

Salunkhe et al. (1962) suggested that potential success in extending the storage life and marketable quality of fruit depends upon the kind of packaging film and the chemical used, such as phaltan, captan, and

N^6-benzyladenine. Parsons (1959), working on the storage of cabbage, found that use of polyethylene liners eliminated the signs of wilting and the weight loss in cabbage. Hardenburg, Schomer, and Uota (1958) reported that cherries in polyethylene bags may even be held in cold storage at 31 F for two weeks.

The use of antifungal chemicals and packaging treatments on the shelf-life of sweet cherries has been investigated by Do et al. (1966). They suggested that antifungal chemicals yield a zone of inhibition that prevents fungal spore germination and mycelical development. The predominant decay-causing fungi were inhibited for 30 days in over 90 percent of the marketable fruits studied.

N^6-benzyladenine, a senescence inhibitor, has been used in pre- and post-harvest treatment for lettuce by Salunkhe et al. (1962) and Lipton and Ceponis (1962). El-Mansy et al. (1967) noted that in general the inhibition of CO_2 evolution, as well as the stimulation of O_2 consumption, during storage was directly related to the concentration of N^6-benzyladenine. The lettuce with higher values of moisture content, total chlorophyll, and total and soluble nitrogen was obtained during the storage, whether the chemical was applied as pre- or post-harvest treatment. Recently, the work of Yang (1971) on lettuce suggested that 1,000 ppm of phaltan, an antifungal chemical, used in combination with CA storage, had detrimental effects compared to conventional and CA storage. N^6-benzyladenine did not appear to have any significant effect on shelf-life of lettuce. Other chemicals had either no effect or a harmful effect on lettuce.

EXPERIMENTAL

Source of Lettuce

Lettuce, *Lactuca sativa* L. (Cultivar "Great Lakes"), was obtained in the summer of 1970 from a local grower. The lettuce was cut and packed by field crews; but to reduce injury, only 18 heads were placed in each box, instead of the customary 24. Lettuce was placed in a refrigerated trailer after harvest and transported to the laboratory.

Treatments of Lettuce

Since the atmosphere containing 2.5 percent O_2 and 2.5 percent CO_2 at 35 F was found to be the best for prolonging shelf-life of lettuce (Yang, 1971), in this experiment each of the following four groups of lettuce was maintained at 2.5 percent O_2, 2.5 percent CO_2, and 95 percent N_2 at 35 F and 95 percent relative humidity. Group 5 was held under normal air.

Group 1. The lettuce was dipped for five minutes in a solution containing 1,000 ppm phaltan (Phaltan 50, California Spray-Chemical Company) and 1,000 ppm Triton-B 1956 (Rohm and Haas, Pennsylvania). After draining and packaging in polyethylene bags ("Hefty," Mobil Chemical Company), the bags were tied with rubber bands.

Group 2. The lettuce was only treated with 1,000 ppm phaltan.

Group 3. The lettuce was packed in polyethylene bags without chemical treatment.

Group 4. The lettuce was held without any treatment (CA).

Group 5. The lettuce was stored in air without any treatment (conventional refrigeration).

The concentrations of gases were maintained by a continuous flow of the appropriate mixtures of O_2, CO_2, and N_2 dispensed from pressure cylinders (Figure 1) at a rate of 800 ml per minute. A bottle of water and wet cheesecloth were used at the bottom of each barrel to maintain a high relative humidity in the container. The concentration of gas mixture was checked routinely with a "Fyrite" gas analyzer.

Chemical Analyses

Lettuce heads were taken from each group of samples at 15, 30, 45, 60, and 75 days of storage. The lettuce heads were trimmed; all leaves were cut into small pieces and then stored at -20 F. The leaves, for the determination of total carotene, were steam blanched at 212 F for three minutes, cooled immediately to 70 F, and stored at -20 F. For the analyses of total sugars, reducing sugars, starch, total organic acids, amino acids, and soluble proteins, the leaves were quickly frozen (Buicher Boy Freezer, Harvard, Illinois) at -60 F for 30 minutes, and then transferred immediately into the chamber of the Hull Freeze Dehydrator (Hull Corporation, Halboro, Pennsylvania) under 20-inch vacuum for three days. The dried leaves were ground to pass a 40-mesh sieve for chemical analysis.

pH and total titratable acidity

The samples were blended in a Waring Blender for three minutes. The homogenate was filtered through four layers of cheesecloth. pH was determined with a Beckman Expandomatic SS-2 model pH meter. The glass

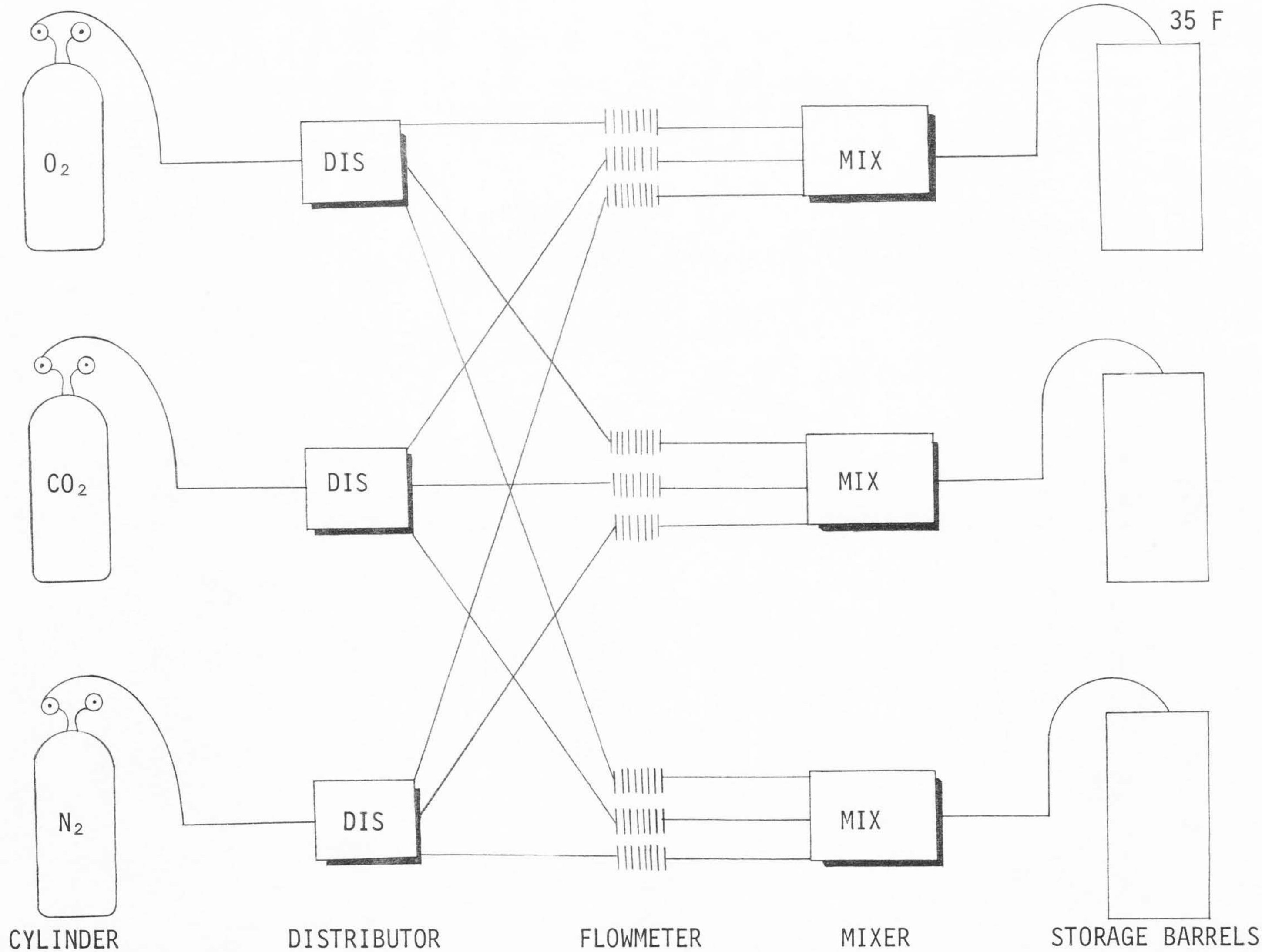

Figure 1. Schematic diagram of controlled atmosphere storage facilities.

electrode method described by the Association of Official Agricultural Chemists (1965) was used for titratable acidity.

Total sugars and starch

Total sugars and starch were determined colorimetrically by means of the sugar-anthrone-sulfuric method of McCready et al. (1950).

Free amino acids

A modified ninhydrin colorimetric analysis was used for free amino acid determination (Rosen, 1957).

Total organic acids

The method used for organic acid analysis was that of Williams (1961). An alcohol extract of freeze-dried leaf powder was passed through a thoroughly washed cation exchange column, Dowex 50 W-x8 (H^+ form, 200-400 mesh), to remove amino acids, proteins, and other alcohol-soluble materials. The eluant was then passed through an anion exchange column, Dowex 1 x 8 (Cl^- form, 400 mesh). The organic acids were then eluted from resin with 6N formic acid until the phosphate ion appeared in the eluant. The eluant was concentrated until almost dry, then diluted with deionized water and titrated with 0.01N NaOH, using phenolphthalein as an indicator.

Soluble proteins

Soluble proteins were determined by using the Folin-Phenol reagent (Lowry et al., 1951).

Reducing sugars

Reducing sugars were determined by the arsenomolybdate reagent method of Nelson (1944).

Total carotenes

The Association of Official Agricultural Chemists' method (1965) was used in analyses of total carotenes.

Statistical Analysis

The analysis of variance was conducted and the means were compared according to Tukey's ω-procedure (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The effects of CA alone, CA with pre-storage treatment of phaltan, packaging, phaltan in combination with packaging, and conventional refrigeration on the appearance of lettuce after 45 days of storage are shown in Figure 2. Lettuce in CA and CA combined with packaging were on par with conventional refrigeration up to 45 days. However, at the end of 60 and 75 days, deterioration started in CA combined with packaging and conventional refrigeration. CA was found to be effective in retaining the quality even up to 60 days of storage (Figure 3).

The chemical treatment with phaltan had a detrimental effect even in the CA and CA with packaging after 45 days of storage (Figure 2). The concentration of phaltan used was 1,000 ppm, which has been found to be beneficial in cherry storage by Do et al. (1966). However, this does not appear to be an appropriate concentration for lettuce.

Starch, Total Sugars, and Reducing Sugars

Although starch and total sugars of all treatments decreased during storage, CA and CA in combination with packaging showed a higher retention of starch and total sugars throughout the storage period than the conventional refrigeration (Table 1, Figure 4). Lettuce treated with phaltan and phaltan combined with packaging, on the contrary, showed lower starch and total sugar than that of conventional refrigeration. The statistical analysis, however, indicated significantly lower total sugars only on the fifteenth and the thirtieth days of storage in phaltan-treated lettuce. This is consistent with the stimulatory effects

Figure 2. Effect of controlled atmosphere and other treatments at 35 F after 45 days of storage.

1. Conventional refrigeration (control).
2. Controlled atmosphere (2.5 percent O_2 and 2.5 percent CO_2).
3. Controlled atmosphere with phaltan (1,000 ppm).
4. Controlled atmosphere with phaltan and packaging in polyethylene bags.
5. Controlled atmosphere with packaging in polyethylene bags.

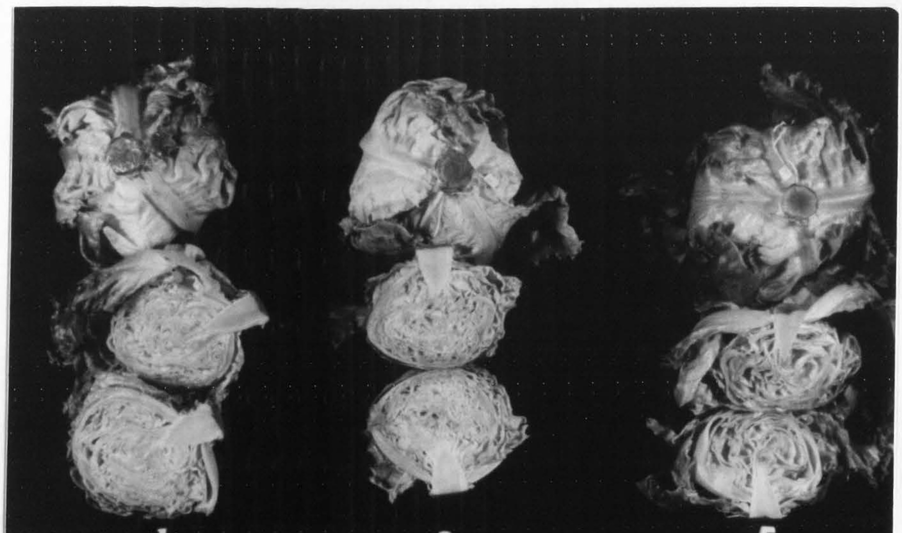

Figure 3. Effect of controlled atmosphere and other treatments at 35 F after 60 days of storage.

1. Conventional refrigeration (control).
2. Controlled atmosphere (2.5 percent O_2 and 2.5 percent CO_2).
5. Controlled atmosphere combined with polyethylene bag packaging.

Table 1. Effects of controlled atmosphere (CA) and CA in combination with phaltan and polyethylene packaging on total starch, total sugars, and reducing sugars of lettuce[a]

Day of storage	Treat- ment	Total starch mg/g	Total sugars mg/g	Reducing sugars mg/g
0	CR	5.38 ± 0.01[b]	132.07 ± 0.32	49.36 ± 0.20
15	CR	4.81 ± 0.06	100.72 ± 8.25	51.90 ± 0.72
	CA	5.09 ± 0.22[ns]	103.24 ± 6.45[ns]	48.41 ± 0.00[ns]
	CPh	4.04 ± 0.05[ns]	83.08 ± 3.24*	39.67 ± 2.24**
	CPP	4.55 ± 0.14[ns]	90.04 ± 4.50[ns]	39.55 ± 0.30**
	CPk	5.25 ± 0.16[ns]	102.61 ± 3.50[ns]	46.44 ± 1.83[ns]
30	CR	4.05 ± 0.04	103.34 ± 3.00	57.15 ± 0.74
	CA	4.07 ± 0.08[ns]	96.18 ± 4.05[ns]	46.29 ± 0.57[ns]
	CPh	4.08 ± 0.04[ns]	85.88 ± 3.95*	42.63 ± 1.85*
	CPP	4.15 ± 0.03[ns]	99.14 ± 7.70[ns]	44.42 ± 2.65*
	CPk	4.40 ± 0.32[ns]	109.79 ± 2.01[ns]	52.60 ± 0.36[ns]
45	CR	2.67 ± 0.04	69.16 ± 0.30	53.95 ± 1.07
	CA	2.61 ± 0.13[ns]	68.73 ± 0.10[ns]	46.48 ± 0.73[ns]
	CPh	2.67 ± 0.09[ns]	68.81 ± 1.00[ns]	38.99 ± 2.18**
	CPP	2.95 ± 0.15[ns]	65.77 ± 0.05[ns]	40.96 ± 0.74**
	CPk	2.72 ± 0.02[ns]	79.56 ± 1.15**	50.24 ± 1.32[ns]
60	CR	2.81 ± 0.09	85.02 ± 0.05	55.67 ± 0.61
	CA	2.90 ± 0.04[ns]	87.43 ± 1.60[ns]	48.99 ± 0.39*
	CPk	3.33 ± 0.14*	86.40 ± 1.00[ns]	46.80 ± 0.43**
75	CR	2.64 ± 0.21	77.99 ± 4.15	50.06 ± 1.09
	CA	2.64 ± 0.89[ns]	83.47 ± 1.80[ns]	45.56 ± 0.37*

*Significant at 0.05 level.
**Significant at 0.01 level.
[ns]Not significant at 0.05 level compared to controls (CR).
[a]Results expressed on dry weight basis.
[b]Mean + standard error.
[c]Each value in the table is a mean of duplicate determination within a given experiment.
Note: CR = conventional refrigeration; CA = controlled atmosphere (2.5 percent O_2 and 2.5 percent CO_2); CPh = CA + phaltan (1,000 ppm); CPP = CA + phaltan (1,000 ppm) + packaging in polyethylene bags; CPk = CA + packaging in polyethylene bags.

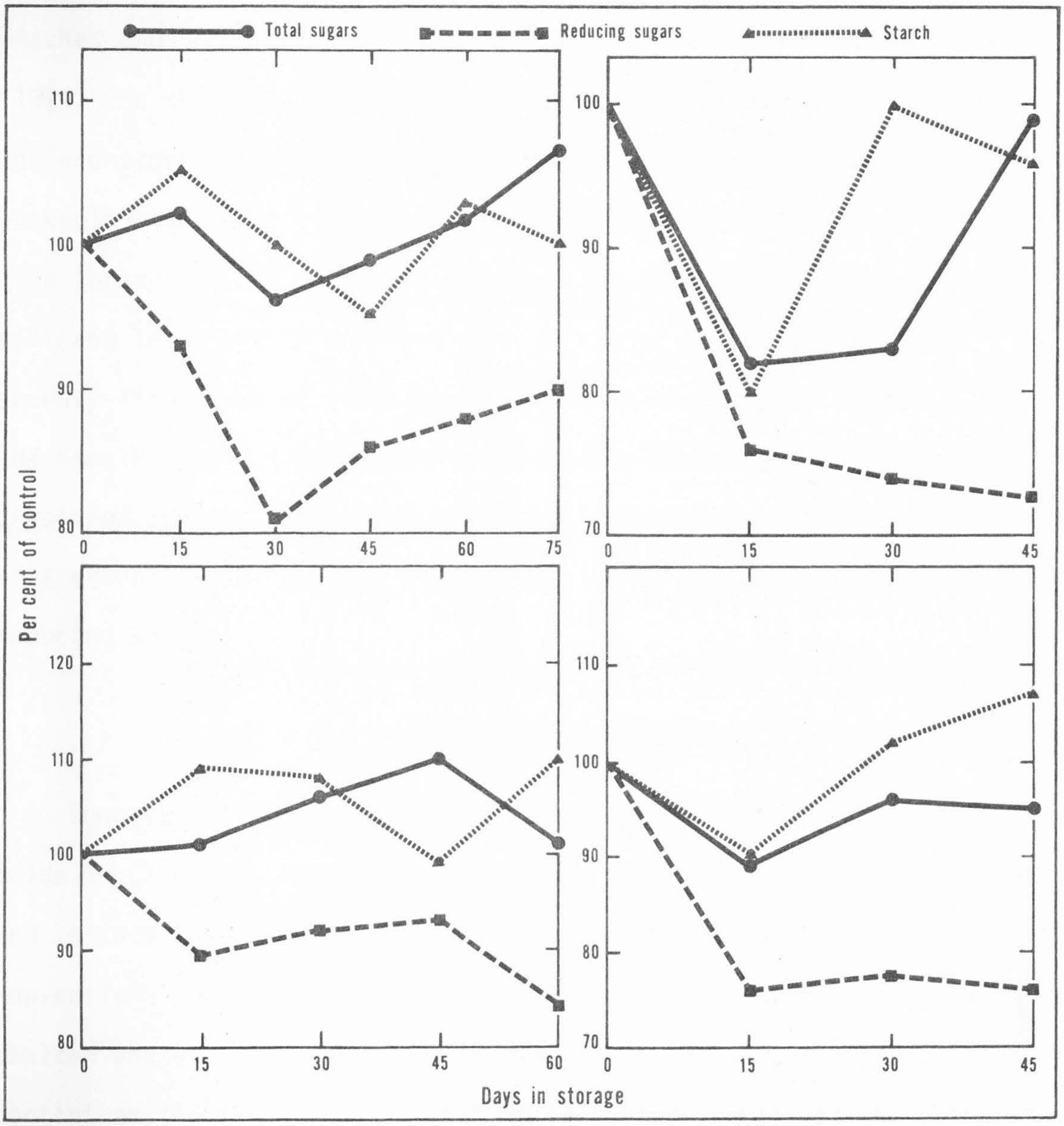

Figure 4. Effects of controlled atmosphere in combination with other treatments on total sugars, reducing sugars, and starch. (A) CA, (B) CA + phaltan, (C) CA + polyethylene packaging, (D) CA + phaltan + polyethylene packaging.

of phaltan on the rate of respiration of lettuce which has been reported by Yang (1971). The nonsignificant changes in starch and total sugars noted in these experiments concur with those of McGill, Nelson, and Steinberg (1966) for spinach; Wankier, Salunkhe, and Campbell (1970) for peaches and "Moorpack" apricots; and Singh, Littlefield, and Salunkhe (1970) for cherries. Although the lettuce in CA and CA with packaging and pre-storage treatment with phaltan and phaltan combined with packaging had lower reducing sugars than that of conventional refrigeration throughout the period of storage, the differences were only significant in CA and CA combined with packaging on the sixtieth and seventy-fifth days of storage. Wankier, Salunkhe, and Campbell (1970) and Karnik et al. (1971) also found a lower amount of reducing sugars in CA-stored apricots, peaches, and sugar beets. Probably, higher concentrations of CO_2 inhibit the degradation of nonreducing sugars to reducing sugars.

Total Organic Acids

Compared to conventional refrigeration storage, the total organic acids in CA and CA in combination with packaging showed 5 percent higher and lettuce in CA alone 6 percent higher total organic acid content than conventional refrigeration (Table 2, Figure 5). Lettuce treated with phaltan and phaltan combined with packaging had a lower organic acid content on the fifteenth day, but it increased rapidly on the thirtieth day of storage and it was 10 percent higher than conventional refrigeration on the forty-fifth day of storage. The statistical data, however, indicated that in most cases the differences in the contents of organic acids were nonsignificant. The accumulation of organic acids in

Table 2. Effects of controlled atmosphere (CA) and CA in combination with phaltan and polyethylene packaging on organic acids, amino acids, and soluble proteins of lettuce[a]

Day of storage	Treat-ment	Organic acids mg/g	Amino acids mg/g	Soluble proteins mg/g
0	CR	30.61 ± 0.7[b]	48.85 ± 1.02	62.77 ± 0.80
15	CR	33.67 ± 0.58	49.97 ± 1.26	63.54 ± 2.34
	CA	31.69 ± 0.97[ns]	44.97 ± 0.69[ns]	54.20 ± 0.44*
	CPh	27.69 ± 2.00**	43.56 ± 0.38**	62.86 ± 2.00[ns]
	CPP	25.00 ± 2.05**	40.71 ± 1.27[ns]	49.09 ± 0.93**
	CPk	32.54 ± 2.08[ns]	47.96 ± 1.81[ns]	54.05 ± 0.38*
30	CR	38.08 ± 0.49	60.85 ± 0.29	71.65 ± 0.58
	CA	34.73 ± 0.30[ns]	52.98 ± 0.26**	68.86 ± 0.39[ns]
	CPh	39.74 ± 1.38[ns]	59.07 ± 0.45[ns]	69.27 ± 0.00[ns]
	CPP	37.49 ± 0.40[ns]	57.37 ± 0.31[ns]	70.47 ± 0.20[ns]
	CPk	37.91 ± 0.43[ns]	57.81 ± 2.78[ns]	65.66 ± 0.00[ns]
45	CR	35.49 ± 0.59	73.62 ± 0.38	64.14 ± 0.26
	CA	34.26 ± 1.56[ns]	76.25 ± 1.97[ns]	55.82 ± 3.14*
	CPh	39.14 ± 0.95*	76.98 ± 1.71[ns]	84.32 ± 2.78**
	CPP	41.21 ± 0.10**	81.04 ± 2.14*	75.87 ± 3.57**
	CPk	36.20 ± 0.62[ns]	75.15 ± 0.98[ns]	58.48 ± 1.61[ns]
60	CR	34.78 ± 0.63	77.48 ± 0.99	64.29 ± 2.14
	CA	35.88 ± 0.45[ns]	79.04 ± 1.46[ns]	53.79 ± 2.17**
	CPk	36.93 ± 0.99[ns]	82.13 ± 1.61*	55.22 ± 3.73**
75	CR	36.32 ± 0.73	77.84 ± 0.77	66.58 ± 0.00
	CA	38.50 ± 0.29[ns]	80.98 ± 0.87[ns]	53.56 ± 0.65**

*Significant at 0.05 level.
**Significant at 0.01 level.
[ns]Not significant at 0.05 level compared to controls (CR).
[a]Results expressed on dry weight basis.
[b]Mean + standard error.
[c]Each value in the table is a mean of duplicate determination within a given experiment.

Note: CR = conventional refrigeration; CA = controlled atmosphere (2.5 percent O_2 and 2.5 percent CO_2); CPh = CA + phaltan (1,000 ppm); CPP = CA + phaltan (1,000 ppm) + packaging in polyethylene bags; CPk = CA + packaging in polyethylene bags.

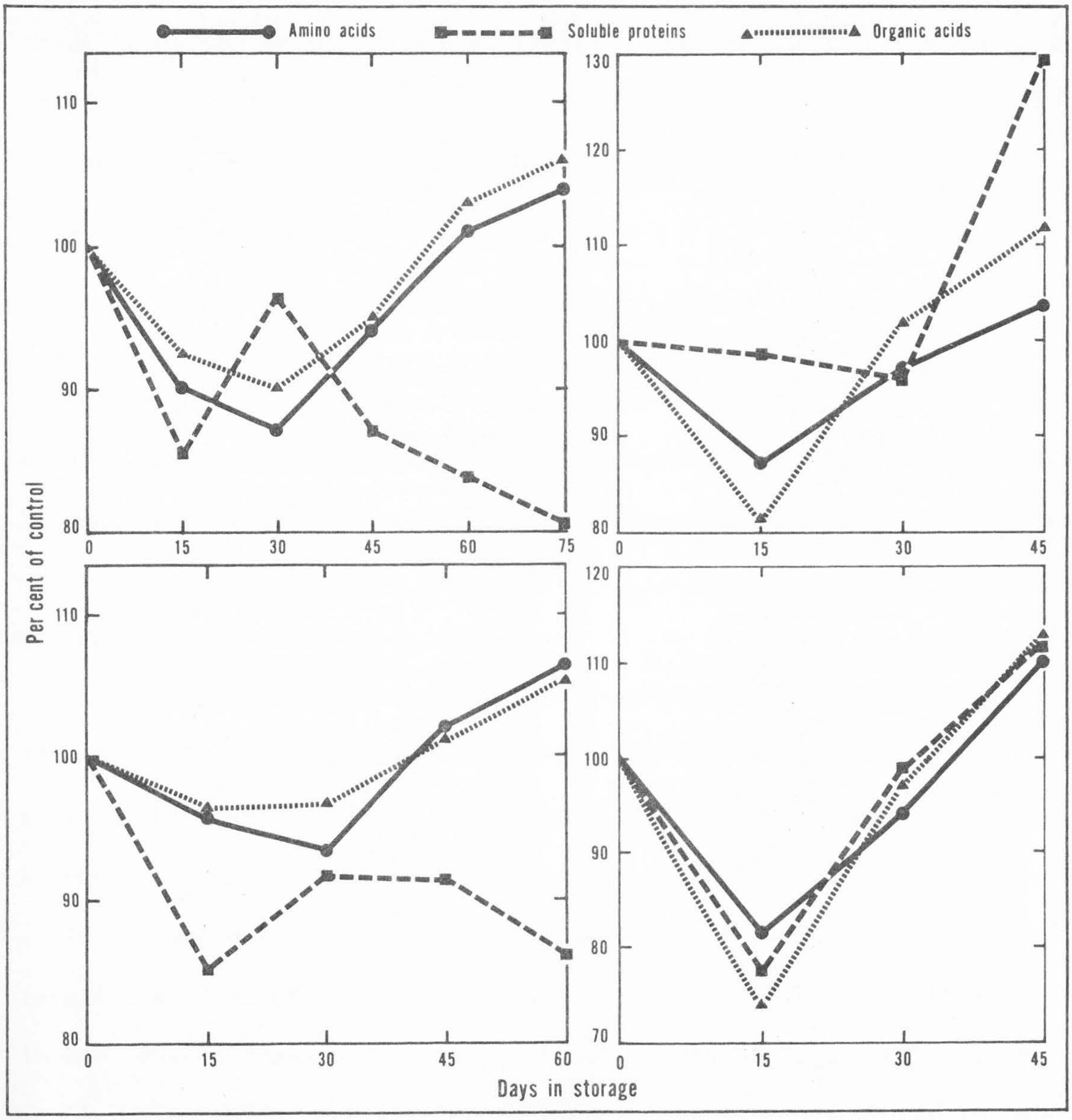

Figure 5. Effects of controlled atmosphere in combination with other treatments on amino acids, soluble proteins, and organic acids of lettuce stored at 35 F.
(A) CA, (B) CA + phaltan, (C) CA + polyethylene packaging, (D) CA + phaltan + polyethylene packaging.

CA-stored samples has been noted by several investigators (Ranson, Walker, and Clarke, 1957; Littlefield, 1968; Singh, Littlefield, and Salunkhe, 1970; Wankier, Salunkhe, and Campbell, 1970; Karnik et al., 1971). It is generally considered that the accumulation of organic acids is caused by the slower utilization rate of TCA cycle acids of the fruits and vegetables stored in CA. This was supported by Frenkel and Patterson (1969), who found that higher CO_2 concentrations inhibited succinic acid dehydrogenase in pears.

Free Amino Acids

There were no significant differences in the content of free amino acids between the controls and those stored under CA (Table 2, Figure 5). Lettuce from CA combined with packaging showed a higher amount of amino acids on the sixtieth day. In lettuce of CA with pre-storage treatment of phaltan, the amino acids were lower on the fifteenth day but were not significantly affected on the subsequent days. It appears that effects of CA on amino acids vary according to the species. For example, Littlefield (1968) found that amino acids in general were higher in pears and apples stored in CA. Singh, Littlefield, and Salunkhe (1970) reported that sweet cherries stored in CA had a lower amount of tyrosine and a higher amount of α-aminobutyric acid than fruits stored in the conventional refrigerator. In the roots of sugar beets stored in CA, amino acids were smaller in amount than in those stored in conventional refrigeration (Karnik et al., 1971).

Soluble Protein

Similar to the reducing sugars, when compared with conventional refrigeration storage, the soluble proteins of lettuce were significantly lower in CA and CA combined with packaging throughout the storage period (Table 2, Figure 5). The lettuce treated with phaltan and phaltan combined with packaging had a higher content of soluble proteins by the forty-fifth day of storage when it started to decay. The lower soluble protein observed in CA indicated less destruction of the membrane system, chloroplasts, or mitochondria in the cells of CA-stored lettuce. On the other hand, the soluble protein was increased significantly in lettuce that was stored in CA combined with phaltan for 45 days. This suggests that the membrane systems of chloroplast, mitochondria, or other organalles were disrupted which resulted in an increase in soluble proteins.

pH and Total Titratable Acidity

The results (Table 3, Figure 6) indicate that there was but a slight increase (statistically nonsignificant) in the pH of the lettuce stored in CA. Wankier et al. (1970) also found that the pH of the stored fruit was not affected by CA storage. In CA-stored lettuce, the titratable acidity was lower than the control until the thirtieth day of storage. On the forty-fifth day, the differences between the CA and the conventional refrigeration storage were not significant; and by the sixtieth day, the titratable acidity became higher than the conventional refrigeration and remained higher up to the end of the storage period. Storage experiments have been conducted for most of the

Table 3. Effects of controlled atmosphere (CA) and CA in combination with phaltan and polyethylene packaging on pH, titratable acidity, and total carotenes of lettuce[a]

Day of storage	Treat-ment	pH	Titratable acidity meW acid/g	Total carotenes µg/g
0	CR	6.41 ± 0.00[b]	0.17 ± 0.00	33.39 ± 0.58
15	CR	6.35 ± 0.00	0.17 ± 0.01	22.95 ± 2.35
	CA	6.42 ± 0.00[ns]	0.16 ± 0.01**	25.52 ± 0.01[ns]
	CPh	6.36 ± 0.01[ns]	0.17 ± 0.01[ns]	25.96 ± 0.51[ns]
	CPP	6.43 ± 0.00[ns]	0.15 ± 0.04**	25.28 ± 1.11[ns]
	CPk	6.42 ± 0.01[ns]	0.16 ± 0.01**	25.91 ± 0.44[ns]
30	CR	6.40 ± 0.03	0.17 ± 0.01	24.79 ± 0.44
	CA	6.41 ± 0.00[ns]	0.16 ± 0.02**	27.00 ± 0.14[ns]
	CPh	6.37 ± 0.00[ns]	0.18 ± 0.08**	29.95 ± 0.30[ns]
	CPP	6.33 ± 0.00[ns]	0.18 ± 0.01**	30.78 ± 0.75*
	CPk	6.42 ± 0.00[ns]	0.17 ± 0.00**	27.06 ± 0.14[ns]
45	CR	6.47 ± 0.00	0.17 ± 0.17	18.49 ± 0.19
	CA	6.49 ± 0.00[ns]	0.17 ± 0.02[ns]	20.51 ± 0.74[ns]
	CPh	6.30 ± 0.00[ns]	0.20 ± 0.83**	18.50 ± 0.44[ns]
	CPP	6.15 ± 0.00**	0.20 ± 0.06**	18.17 ± 0.29[ns]
	CPk	6.44 ± 0.00[ns]	0.18 ± 0.00**	19.57 ± 0.30[ns]
60	CR	6.45 ± 0.01	0.17 ± 0.00	18.41 ± 0.00
	CA	6.42 ± 0.02[ns]	0.18 ± 0.18**	20.46 ± 0.22[ns]
	CPk	6.47 ± 0.01[ns]	0.18 ± 0.05**	20.34 ± 0.29[ns]
75	CR	6.40 ± 0.01	0.18 ± 0.04	26.34 ± 0.72
	CA	6.41 ± 0.01[ns]	0.20 ± 0.00**	29.00 ± 0.62[ns]

*Significant at 0.05 level.
**Significant at 0.01 level.
[ns]Not significant at 0.05 level compared to controls (CR).
[a]Results expressed on dry weight basis.
[b]Mean + standard error.
[c]Each value in the table is a mean of suplicate determination within a given experiment.

Note: CR = conventional refrigeration; CA = controlled atmosphere (2.5 percent O_2 and 2.5 percent CO_2); CPh = CA + phaltan (1,000 ppm); CPP = CA + phaltan (1,000 ppm) + packaging in polyethylene bags; CPk = CA + packaging in polyethylene bags.

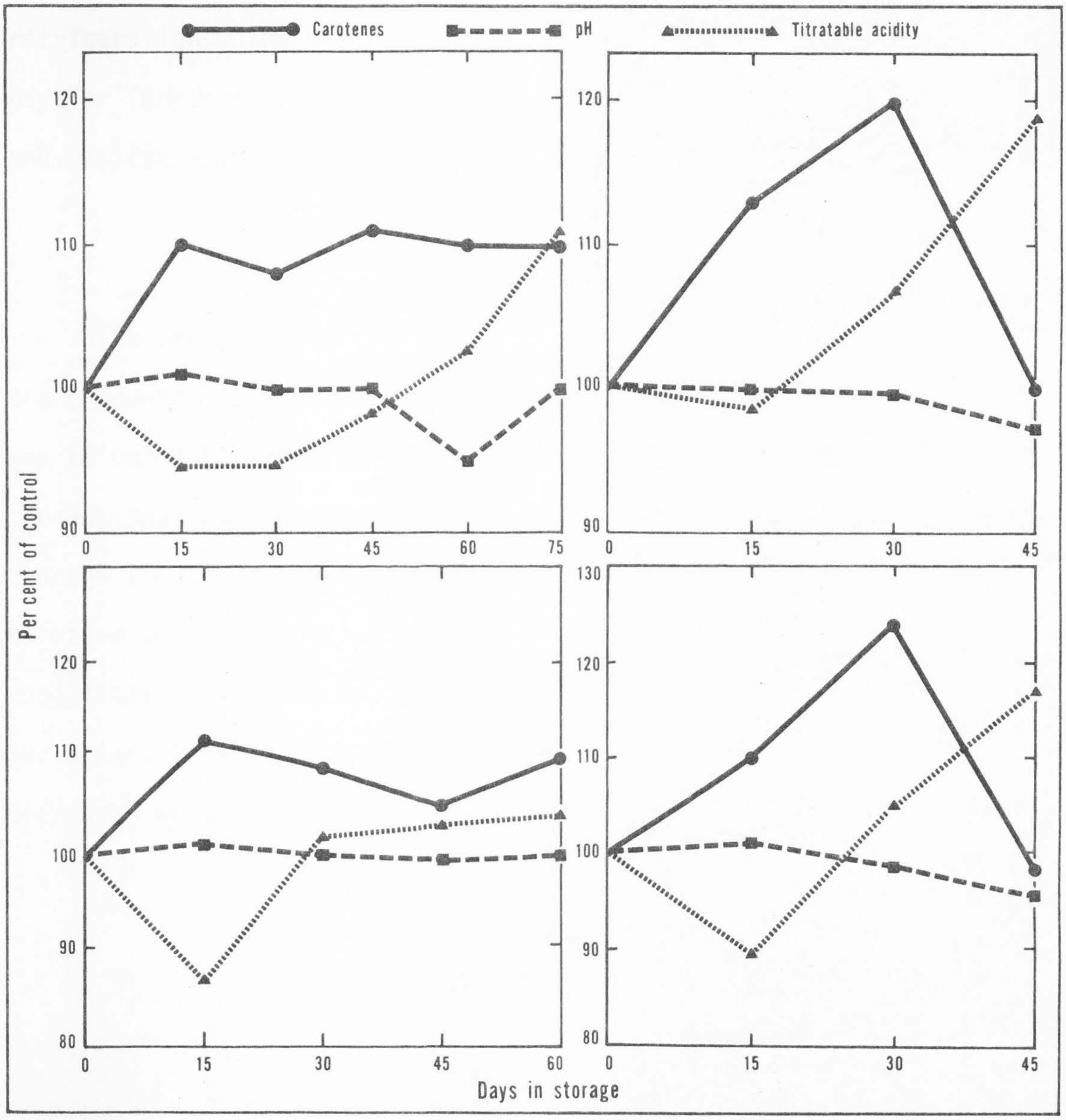

Figure 6. Effects of controlled atmosphere in combination with other treatments on carotenes, pH, and titratable acidity. (A) CA, (B) CA + phaltan, (C) CA + polyethylene packaging, (D) CA + phaltan + polyethylene packaging.

vegetables for short durations. It has been noted that titratable acidity decreases in the CA-stored samples (Groeschel, Nelson, and Steinberg, 1966; Burgheimer et al., 1967; Lebermann, Nelson, and Steinberg, 1968; Wang, Haard, and Dimarco, 1971). Compared to the conventional refrigerations, the titratable acidity was higher in lettuce stored 30 days or longer in CA with pre-storage treatments of phaltan, packaging, and phaltan with packaging.

Total Carotenes

With one exception, none of the treatments affected significantly the concentration of total carotenes. However, the content of carotenes was 10 percent higher in the CA and CA combined with packaging than the conventional refrigeration on the sixtieth and seventy-fifth days of storage (Table 3, Figure 6). This may be due to the lower rate of carotene destruction of lipoxidase in the lettuce stored under these conditions. Compared to the conventional refrigeration, total carotenes in lettuce treated with phaltan and phaltan combined with packaging decreased after 30 days of storage.

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate the changes in chemical composition in lettuce (Cultivar "Great Lakes") when stored in controlled atmosphere (CA); CA in combination with phaltan, a microbe-inhibiting chemical; and packaging in polyethylene bags for a period of 75 days.

The results indicated that lettuce heads maintained a better marketable quality than the conventionally refrigerated when stored in atmospheres of 2.5 percent O_2 and 2.5 percent CO_2 at 35 F for 75 days. Lettuce stored in CA combined with packaging in polyethylene bags or stored in conventional refrigeration retained a good quality for 60 days of storage. The phaltan treatment at 1,000 ppm showed a deteriorating effect even in the presence of CA and packaging in polyethylene bags.

Compared with conventional refrigeration storage, CA, CA in combination with phaltan, CA with packaging, and CA with phaltan and packaging did not alter the starch and total sugars content in lettuce, but lowered the reducing sugars content throughout the 75 days of storage.

Total organic acids and free amino acids content in lettuce were not significantly affected by CA and CA combined with packaging in polyethylene bags. However, lettuce treated with phaltan had a higher total organic acids and free amino acids at the end of 45 days storage when lettuce started to decay.

Soluble proteins content in lettuce was lower in CA and CA in combination with packaging throughout the storage. Storage of lettuce in CA combined with phaltan (1,000 ppm) treatments resulted in a lower soluble protein content on the fifteenth day, but higher on the forty-fifth day of storage.

The pH of lettuce was not affected by CA, phaltan, and packaging treatments. The total titratable acidity was higher in lettuce stored for 30 days or longer in all treatments, compared with the conventional refrigeration storage.

Total carotene content in lettuce was not significantly affected by any treatments during the storage period.

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