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Changes in Headspace Gas Concentration Including Volatiles as Affected by Post-Harvest Treatments in Packaged Fresh-Cut Lettuce

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A B S T R A C T

The changes in headspace gas concentration including volatiles; ethylene, acetaldehyde and ethanol in the packages of fresh cut lettuce cv. GKL-2, as effected by the post-harvest treatments at 5 ± 1^0C storage were studied at Postharvest Laboratory of Horticulture Section, College of Agriculture, Kolhapur. Eight different post-harvest treatments viz., ozonated water (1.2 mgL^{-1}),sodium hypochlorite (150 ppm free chlorine for 15 min), Calcium lactate (15 gL^{-1} at 50^0C) alone or combined with ozonated water, Calcium chloride (1%), Allicin (1%) and UV-C radiations (for 30 min) along with control were used for pretreatment of fresh-cut lettuce. The changes in headspace gas concentrations including volatiles were minimum in fresh cut lettuce treated with $15gL^{-1}$calcium lactate at 50^0C followed by ozonated water treatment 1.2 mgL^{-1} for 1 minute and stored at 5 ± 1^0C. Maximum changes in oxygen and carbon dioxide concentrations were recorded in control lettuce packages followed by calcium chloride (1%) treated fresh-cut lettuce leaves. The increase in ethylene concentrations (ppm) was maximum in control packages followed by UV-C radiations treated fresh-cut lettuce leaves. The pretreatments with calcium lactate (15 gL^{-1} at 50^0C) and Allicin (1%) were the potential tool to control the off-odor caused by excess concentrations of ethylene and ethanol in fresh cut lettuce packages. The headspace concentration of acetaldehyde in the packaged fresh cut lettuce was significantly influenced by treatments under study. Among the entire chemical washing as well as irradiation treatments, pretreatment of fresh cut lettuce with $15gL^{-1}$ calcium lactate at 50^0C followed by ozonated water @1.2 mgL^{-1} registered the minimum volatiles concentration in the headspace of fresh-cut lettuce packages when stored at 5 ± 1^0C.

Introduction

Fresh-cut fruits and vegetables emerged to fulfill consumer's new demands of healthy palatable and easy to prepare plant food (Allende *et al.,* 2006).Fresh-cut products due to their convenience are commonly consumed directly from the bags without any further rinse (Altunkaya *et al.,* 2009). The fresh cut ready to use fruit and vegetable industry is constantly growing at faster rate of 10 per cent per annum mainly due to the consumer's tendency of health consciousness and their increasing interest in the role of fresh, healthy and convenient food for maintaining and improving human well-being (Gilbert, 2000). This beneficial effect has been attributed to non-essential food constituents,

phytonutrients that pose a relevant bioactivity when frequently consumed as a part of regular diet (Steinmetz and Potter, 1996).

Among the nutritious salad vegetable, lettuce is highly demanded commodity in big hotels and restaurants in India and abroad. Lettuce and other leafy vegetables are perceived to be healthier by consumers, resulting in increased consumption due to nutraceutical properties (Altunkaya *et al.,* 2009). As salad vegetable, it is mainly marketed in minimally processed form such as fresh-cut and shredded form depending on the variety and type of lettuce. Large scale processing for distant markets and for long duration requires good shelf life for getting profit and to encourage the cultivation of exotic but highly nutritious vegetables in India. The post-harvest losses recorded in lettuce is 20-30% (Serrato *et al.,* 2014) which includes losses from the physiochemical factor, microbial contamination and changes in sensorial attributes of the products over the time. To be desirable, the fresh-cut lettuce salad should be good in appearance and better aroma. Different physiochemical and sensorial attributes including the off-odor are most responsible to reduce the overall acceptability of the fresh-cut lettuce. This off-odor over the storage period is contributed by different volatiles such as ethanol, ethyl acetate, acetaldehyde, methyl acetate, and acetone present in fresh-cut lettuce package during anaerobic respiration and thus decrease the consumer preference. Therefore, new technique and methods are required to retain the original aroma and flavor of the products during the supply chain. Storage temperature is the single most important factor affecting spoilage of fresh-cut products. An increased storage temperature enhances the metabolism and respiration rate, which may lead to a decreased O_2 concentration and an increase in the CO_2 level inside the package which may result in the accelerated deterioration of plant tissue and may induce off-odors (Ballantyne *et al.,* 1988). However, numbers of many other preservation techniques are currently being used by the fresh-cut industry such as antioxidants, chlorines and modified atmosphere packaging (MAP) (Allende and Artes, 2003) different packaging material and cold storage, MAP, controlled atmospheric storage, UV-C illumination, ozone bubbling as antimicrobial and anti-browning agents, calcium as firming agent and Allicin as antimicrobial agent, which could be a solution to delay such quality losses and extend shelf life and freshness of minimally processed lettuce(Rico *et al.,* 2006; Allende *et al.,* 2009; Karaca *et al.,* 2014 and Escalona *et al.,* 2007). This research compared the effect of post-harvest treatments on head space gas concentrations including volatiles of fresh-cut lettuce stored under refrigerated conditions (5 ± 1^0C).

Materials and Methods

Sample preparation

The healthy and fresh lettuce leaves cv. GKL-2 obtained from the Instructional-cum-research farm of Horticulture section, College of Agriculture, were used for experiment. Lettuce leaves were hand-harvested using disinfected scissors. On the same day, the lettuce leaves were transported to the laboratory in a thermocole boxes maintaining the temperature. The leaves were washed in 200 μLL^{-1} chlorine solution and were surface dried under ambient conditions. Defective and damaged leaves were discarded. After weighing, the lettuce leaves were gently cut in pieces of 5 cm wide using a sharp knife. The cut lettuce was held for 1 min in running tap water immediately, and then excess water drained on a stainless-steel mesh for 5 minutes. The healthy and fresh-cuts leaves were divided into lots for further treatments of chemicals viz. ozonated water @ 1.2 mgL^{-1} for1 minutes, sodium hypochlorite @ 150

ppm free chlorine for 15 minutes, calcium lactate (15 gL^{-1} at 50^0C) alone or combined with ozonated water @ 1.2 mgL^{-1} for one minutes, calcium chloride (1%), Allicin (1%) and UV-C irradiations (100 to 280 nm) for 30 minutes in laminar air flow system. The treated fresh-cut lettuce leaves weighing 250g were filled in each presterlized polypropylene bag and were flushed with 100% nitrogen gas. Pretreated fresh-cut lettuce leaves samples sealed in polypropylene bags were kept in refrigerated storage at 5 ± 1^0C.

Determinations of head space gas concentrations

The in-package atmosphere (O_2, CO_2) was measured with a portable headspace O_2 and CO_2 gas analyzer drawing up to 2 mL of air samples. Sampling was done with a hypodermic needle through a silicon septum pasted on the packaging. The headspace volatiles ethylene, ethanol and acetaldehyde were sampled from the package on the initial and end of storage period. The headspace volatiles were determined by injecting 1 mL gas sample into a gas chromatograph equipped with alumina 60/80/100 mesh packed column.

Ethylene, CO_2 and O_2 standards were obtained as a mixture of 1 ppm ethylene, 1 % O_2 and 5 % CO_2. A 98 ppm acetaldehyde standard was made at 2°C by pipetting 1 ml of pure acetaldehyde on a piece of filter paper and moved to 23°C and allowed to come to equilibrium.

Statistical Analysis

The data was reported as an average value of replicates with standard deviation. Analysis of variance (ANOVA) was performed using IBM SPSS statistics 22 (Windows 8.1, Statistical analysis). The level of significance for all the tests was $\alpha=0.05$. Followed by Duncan's Multiple Range Test ($P\leq0.05$) was carried out to evaluate significant statistical difference of data. For the data expressed as proportions arcsine transformation was applied before analysis.

Results and Discussion

Head space gas concentration

Oxygen and carbon dioxide

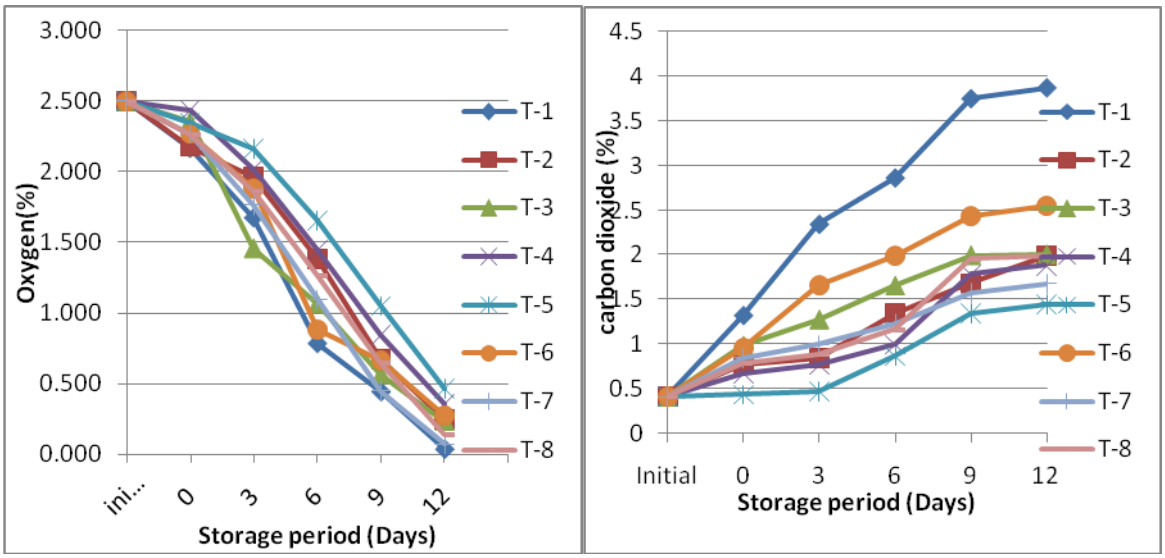
The packaging of the fresh cut lettuce was done in nitrogen enriched environment with low oxygen concentration (0.5 to 2.5 %).At all the stages (days) of the storage at 5^0C, oxygen concentration in the headspace of fresh cut lettuce packages was significantly influenced by chemical washing treatments.

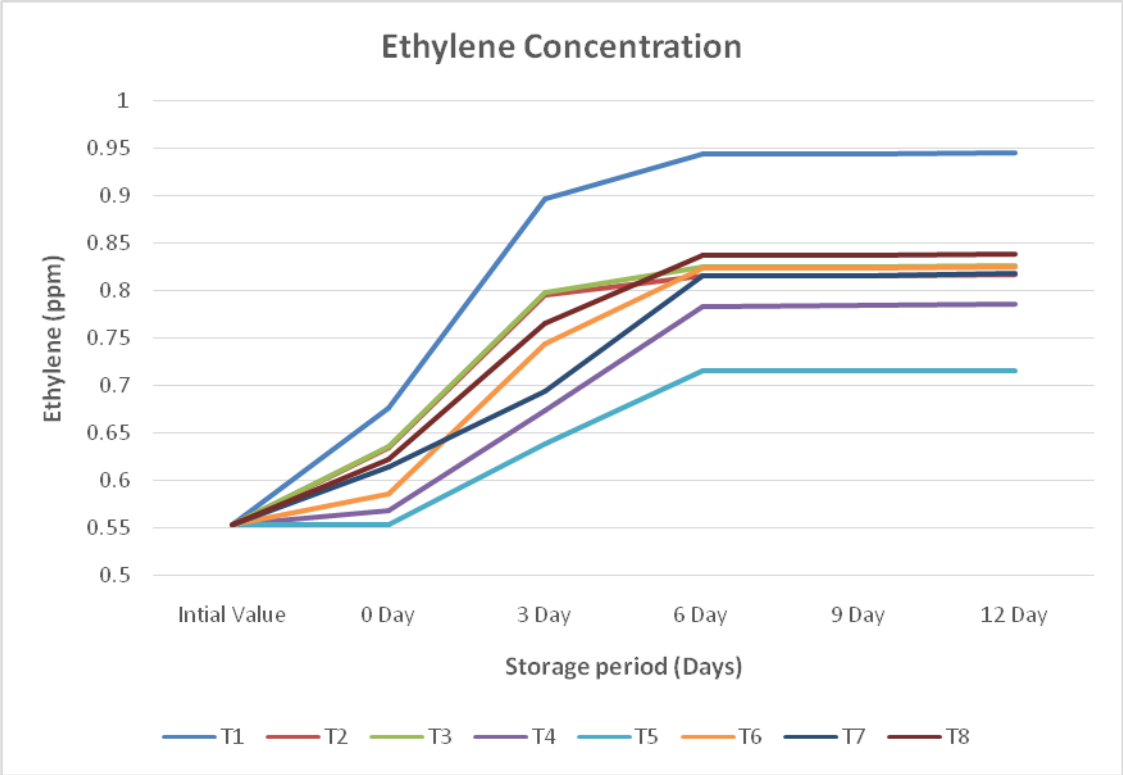
The oxygen concentration in headspace of packages had shown decreasing trend irrespective of chemical treatments (Fig. 1). During entire storage period of up to 12 days at 5^0C, T_5 ($15gL^{-1}$ calcium lactate at 50^0C followed by ozonated water @1.2 mgL^{-1} for 1 minute)treatment showed minimum consumption of oxygen (0.465%) while treatment T_1(Control) recorded minimum concentration of 0.035 percent oxygen in its headspace followed by T_7 (1 percent Allicin) (0.07%).

As the storage period advanced, the carbon dioxide concentration in the headspace of fresh cut lettuce packages increased gradually. The increase was maximum in control (T_1) packages.

After 12 days storage at 5^0C, among all the chemical washing treatments, T_5 ($15gL^{-1}$ calcium lactate at 50^0C followed by ozonated water @ 1.2 mgL^{-1}) treatment registered the minimum accumulation of carbon dioxide (1.446%) in the headspace of fresh cut lettuce packages (Fig. 1).

Fig.1 Effect of post-harvest treatments on the headspace gas concentrations of packaged fresh-cut lettuce stored at 5±1^{0}C





T_1= Control

T_2= Ozonated Water (1.2mgL^{-1} for 1 min)

T_3= Sodium hypochlorite (150mg L^{-1} free chlorine for 15 min)

T_4= Calcium lactate (15gL^{-1} at 50^{0}C)

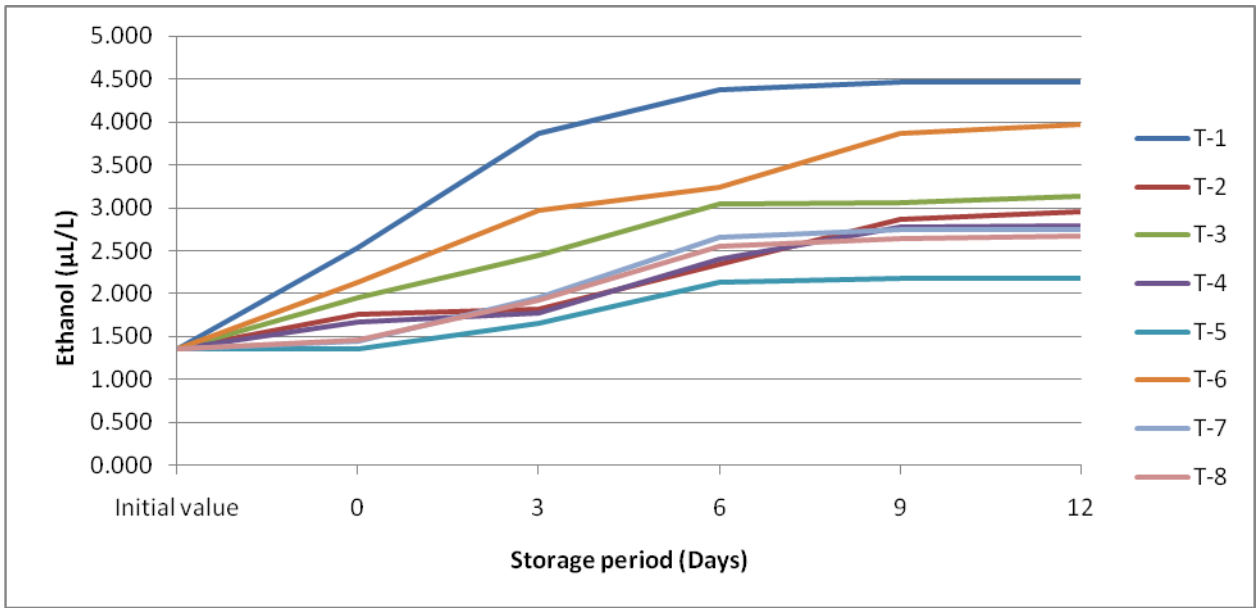
T_5= 15 gL^{-1} Calcium lactate at 50^{0}C followed by ozonated water @ 1.2 mg L^{-1}for 1 minute

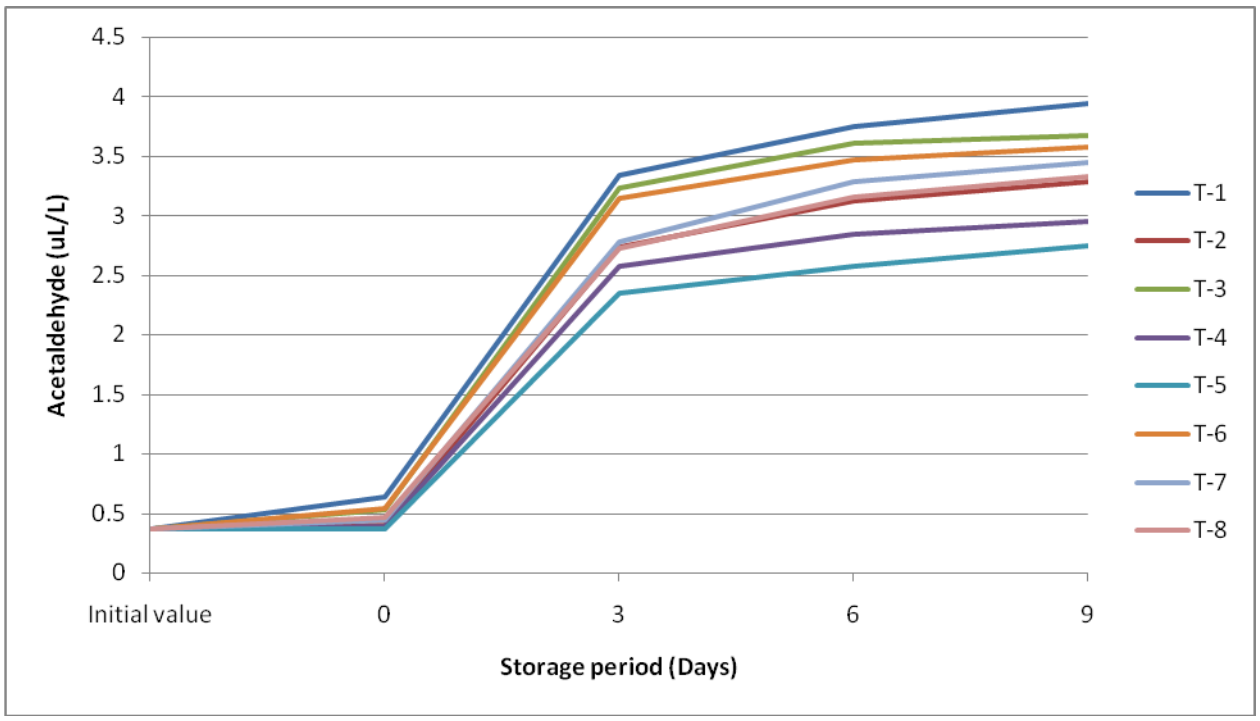
T_6= Calcium chloride (1%)

T_7= Allicin (1%)

T_8= UV-C radiations

Fig.2 Effect of post-harvest treatments on the headspace gas concentrations of volatiles (Ethanol and Acetaldehyde) in the packaged fresh-cut lettuce stored at 5±1^{0}C





T_1= Control

T_2= Ozonated Water (1.2mgL^{-1} for 1 min)

T_3= Sodium hypochlorite (150mg L^{-1} free chlorine for 15 min)

T_4= Calcium lactate (15gL^{-1} at 50^{0}C)

T_5= 15 gL^{-1} Calcium lactate at 50^{0}C followed by ozonated water @ 1.2 mg L^{-1} for1 minute

T_6= Calcium chloride (1%)

T_7= Allicin (1%)

T_8= UV-C radiations

The modified atmosphere composition in the headspace of package of fresh-cut lettuce during storage changed and increases in carbon dioxide (CO_2) and decrease in oxygen (O_2) was recorded. This might be due to barrier properties of used packaging films as reported by Krasnova *et al.,* (2012) in fresh-cut mixed salad quality; Anon. (2000) and Kim *et al.,* (2005) in defatted soybean meal quality during storage. Percent oxygen content in all treatments decreased (Fig. 1) as a result of fresh-cut lettuce leaves breathing and generation of mass losses as water evaporates from fresh-cut lettuce and forming of equilibrium modified atmosphere as reported by Smyth *et al.*,(1998). The decrease in O_2 content and corresponding gas composition in different treatments packed in polypropylene bag was desperate at the end of storage (Fig. 1). Equilibrium among O_2 and CO_2 concentrations developed within 8 days of storage in treatment T_5 ($15gL^{-1}$ calcium lactate at 50^0C followed by ozonated water @1.2 $mgL^{-1)}$ which was found substantially different from other treatment due to low rate of respiration, less ethylene evolution which was supplemented with inhibitory effect of ozone on oxidase enzyme activities as reports by Zhang *et al., (*2005) in fresh-cut celery and Rico *et al.,* (2006) in fresh-cut lettuce.

Ethylene

At the beginning of the storage, ethylene concentration in the headspace of lettuce was 0.553 percent which was significantly influenced by various chemical dipping treatments and irradiations.

With the advancement of the storage period at 5^0C, the ethylene concentration in the headspace of fresh-cut lettuce packages increased gradually but increase was minimum in the packages treated with T_5 ($15gL^{-1}$ calcium lactate at 50^0C followed by ozonated water @1.2 mgL^{-1}) treatment (Fig. 1). The ethylene biosynthesis of fresh-cut lettuce was found relatively scarce in this experiment. It might be due to the prevailing low storage temperature conditions (5^0C) maintained throughout the storage period.

Volatiles like ethylene, ethanol and acetaldehyde

The maximum increase in the headspace ethanol concentration (4.463 μLL^{-1}) was recorded by control (T_1) packages. Among all the chemical washing and irradiation treatments, T_5 ($15gL^{-1}$ calcium lactate at 50^0C followed by ozonated water@1.2 mgL^{-1}) registered the minimum ethanol concentration (2.183μLL^{-1}) in the headspace of lettuce packages in refrigerated storage (Fig. 2). After chemical washing and irradiation treatments, significant increase in headspace acetaldehyde concentration was recorded in all treatments. Maximum concentration (4.023 μLL^{-1}) of acetaldehyde was recorded in control packages (T_1) whereas T_5 ($15gL^{-1}$ calcium lactate at 50^0C followed by ozonated water @1.2 mgL^{-1}) treatment registered the minimum acetaldehyde release (2.88μLL^{-1}) in headspace of fresh cut lettuce packages up to 12^{th} day of storage at 5^0C (Fig. 2).

A very low (~1kpa) concentration of ethanol and acetaldehyde may have a floral-fruity or otherwise pleasant odor and had a beneficial antimicrobial effect as reported by Anon. (2003) and Kim *et al.*,(2005) in fresh-cut lettuce. Ethanol and acetaldehyde concentration was affected by storage time and post-harvest treatments (Fig. 2). The increase in volatiles might be due to the low oxygen concentration in headspace which might have led to anaerobic respiration in fresh-cut lettuce as reported by Lopez-Galvez *et al.,* (1997), Cameron *et al.,* (1995) and Smyth *et al.,* (1998) in minimally processed lettuce. Acetaldehyde gas was accumulated gradually in all samples irrespective of

treatment. The ethanol concentration in headspace of fresh-cut lettuce treated with T_5 ($15gL^{-1}$ calcium lactate at 50^0C followed by ozonated water @ 1.2 mgL^{-1} for 1 minute) was 2.183 μLL^{-1} (Fig. 2). This was in conformity with results reported by Lopez-Galvez *et al.,* (1997) in fresh-cut lettuce.

The pre-treatment of fresh cut lettuce with $15gL^{-1}$ calcium lactate at 50^0C followed by ozonated water @ $1.2mgL^{-1}$ for 1 minute and packaged in 150gauge polythene bag effectively controlled headspace gas concentrations of packages and maintained or enhanced taste, flavor, retained freshness, crispness and maintained fresh like quality upto 12 days at 5^0C. The pretreatments with calcium lactate (15 gL^{-1} at 50^0C) and Allicin (1%) were the potential tool to control the off-odor caused by excess concentrations of ethylene and ethanol in fresh cut lettuce leaves packagings.

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