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Modified-atmosphere packaging maintains the quality of postharvest whole lettuce (*Lactuca sativa* L. Grand Rapid) by mediating the dynamic equilibrium of the electron transport chain and protecting mitochondrial structure and function

Zhenlong Guo[a,c,1], Huijun Liu[a,1], Xiangning Chen[a,*], Lingling Huang[b], Junfeng Fan[b], Jing Zhou[c], Xiguang Chang[c], Bin Du[a], Xiyuan Chang[a]

[a] Beijing Key Laboratory of Agricultural Product Detection and Control for Spoilage Organisms and Pesticides, Food Science and Engineering College, Beijing University of Agriculture, Beijing 102206, China
[b] Department of Food Science and Engineering, College of Biological Sciences and Technology, Beijing Key Laboratory of Forest Food Process and Safety, Beijing Forestry University, Beijing 100083, China
[c] Beijing Unong High-quality Farm Products Planting Company, Beijing 101400, China

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ABSTRACT

The mechanism by which modified-atmosphere packaging (MAP) protects the quality of whole lettuce (*Lactuca sativa* L. Grand Rapid) was investigated in this study. MAP-treated lettuce exhibited a low respiration rate, weight loss, chlorophyll loss, and relative conductivity, together with improved overall quality. Transmission electronic microscopy (TEM) was used to reveal the integrated mitochondrial morphology in MAP-treated lettuce, and a further analysis found that MAP treatment could increase the calcium (Ca^{2+}) content and Ca^{2+} pump activity. MAP-treated lettuce also displayed low levels of the superoxide anion and hydroxyl radical, which was explained by a stable electron transport chain (ETC), as evidenced by high succinate dehydrogenase and cytochrome c oxidase activity. It was further found that MAP could increase superoxide dismutase and ascorbate peroxidase activity, as well as the ascorbic acid and glutathione content. These findings suggest that MAP maintains the quality of postharvest whole lettuce by protecting the mitochondrial structure and maintaining the ETC balance.

Industrial relevance

This study shows that MAP treatment could maintain the freshness and nutritional quality of postharvest whole lettuce (*Lactuca sativa* L. Grand Rapid) through mediating the dynamic equilibrium of the ETC and protecting mitochondrial structure and function. Therefore, MAP could be utilized in the preservation of vegetable and fresh-cut vegetable, and the results in this study also help the postharvest vegetable industry to improve strategies controlling the deterioration of vegetable.

1. Introduction

Loose-leaf lettuce is a leafy vegetable favoured by consumers because of its pleasant aroma, crispness, tenderness, and high levels of phytochemicals such as phenolic compounds (Llorach et al., 2008; Martínez-Sánchez et al., 2011). However, lettuce is perishable and highly susceptible to browning (Degl'Innocenti et al., 2007; Saltveit, 2000). Leaf decay occurs more rapidly and is more difficult to control in loose-leaf lettuce than other types of lettuce, such as iceberg lettuce. Various strategies have been used to control the decay of postharvest

Abbreviations: APX, ascorbate peroxidase; AsA, ascorbic acid; CAT, catalase; Ca^{2+}, calcium; Ca^{2+}-ATPase, calcium pump; CCO, cytochrome c oxidase; CO_2, carbon dioxide; ETC, electron transport chain; GSH, glutathione; GSH-Px, glutathione peroxidase; MAP, modified-atmosphere packaging; (N_2, nitrogen; $O_2^{\cdot -}$, superoxide radical anion; $OH^{\cdot}$, hydroxyl radical anion; O_2, oxygen; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SOD, superoxide dismutase; TEM, transmission electronic microscopy

* Corresponding author at: Department of Agricultural Product Processing and Storage Engineering, College of Food Science and Engineering, Beijing University of Agriculture, 7Bei Nong Road, Changping District, Beijing, 102206, China.
E-mail address: cxn@bua.edu.cn (X. Chen).

[1] These authors contributed equally to this work.

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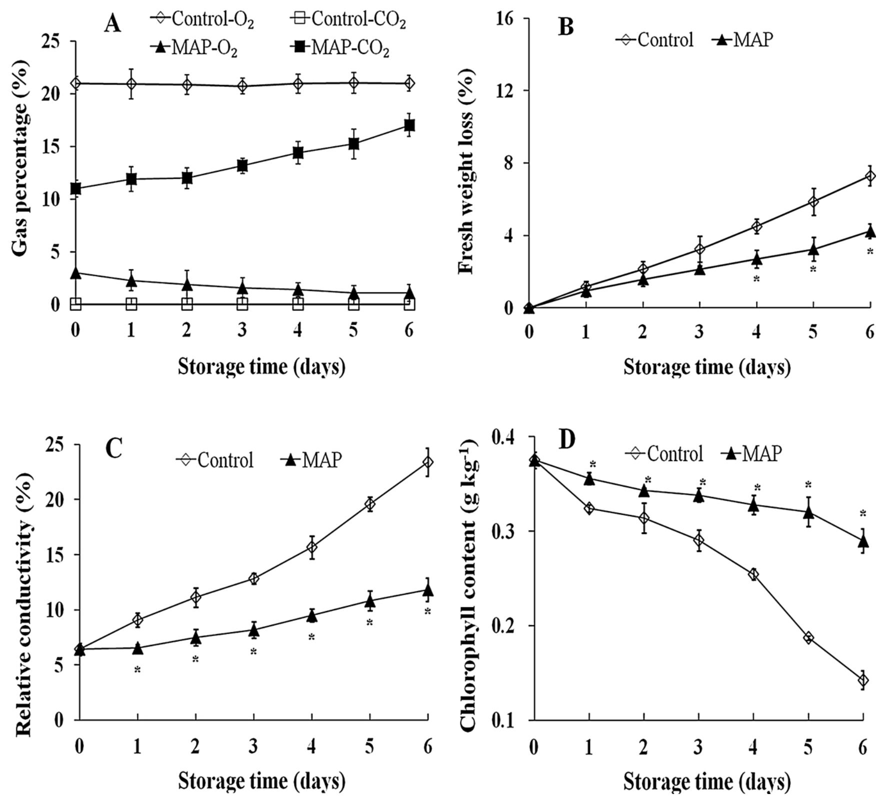

Fig. 1. Gas percentage (A), weight loss (B), relative conductivity (C) and chlorophyll content (D) of postharvest whole lettuce stored at 4 °C for six days. Values are means ± standard error (n = 3). The asterisk (*) denotes a significant difference between MAP treatment and the control ($P < 0.05$).

lettuce, including chemical treatment (Altunkaya and Gökmen, 2009; Chen et al., 2010; Saltveit, 2004); physical treatment (Rico et al., 2008; Zhang et al., 2006); and a combination of chemical and physical treatments (Roura et al., 2008); as well as novel biological treatments, such as allicin (Peng et al., 2014), green tea extracts (Martín-Diana et al., 2008), and whey protein (Altunkaya, 2011). However, these approaches are generally limited by their toxicity, low efficiency, and high cost, and by the reduced organoleptic properties and nutrient content of the produce (Alothman et al., 2009; Chen et al., 2012; Paull and Chen, 2000). Therefore, an inexpensive, safe, and environmentally sustainable method of preventing the oxidative browning and decay of lettuce is required.

Modified-atmosphere packaging (MAP) involves in actively or passively modifying the air in packaging with a mixture of oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2) to regulate the physiological and biochemical responses of the product. It has been widely used to extend the shelf-life of postharvest vegetables and fruits. MAP significantly reduces respiration, bacterial growth, oxidative stress (Gill and Tuteja, 2010; Sharma et al., 2012), and the production of reactive oxygen species (ROS; Moller et al., 2007; Noctor et al., 2007). It therefore delays nutrient decomposition and maintains the original colour and texture (Chen et al., 2013; Fonseca et al., 2002; Rahman et al., 2013; Reis et al., 2016). Respiration occurs primarily in the inner membrane of mitochondria. The balance between ROS production and degradation is regulated by the electron transport chain (ETC; Gill and Tuteja, 2010; Sharma et al., 2012). Excessive ROS levels reduce the quality of vegetables. Vegetables without packaging produce more ROS due to the disruption of mitochondrial intima and reduced levels of ETC components (Gill and Tuteja, 2010; Sharma et al., 2012). However, to the best of our knowledge, the effects of MAP on the dynamic equilibrium of the ETC and mitochondrial intima structure have not been investigated. This information is essential to clarify the mechanism underlying the effects of MAP on the quality of postharvest produce.

We hypothesised that MAP could affect the dynamic equilibrium of ETC and mitochondrial intima structure, thus impacting the quality of whole lettuce (*Lactuca sativa* L. Grand Rapid). Therefore, we explored changes in mitochondria, calcium (Ca^{2+}) levels, and calcium pump (Ca^{2+}-ATPase) activity to assess the structural stability of mitochondria under MAP conditions in this study. We also evaluated mitochondrial structure, ETC state, and ROS level to gain insight into the molecular mechanism underlying the effects of MAP on produce quality.

2. Materials and methods

2.1. Raw material handling and packaging

The lettuce (*Lactuca sativa* L. Grand Rapid) was manually harvested in Beijing in the morning and then transported to the laboratory within 2 h of the experiment, where the lettuce were selected for uniformity of colour and size visually, and cleaned to remove external green leaves. To retain the homogeneity, the lettuce that had a weight of 250 ± 50 g was selected for further processing. After precooling under 4 °C for 2 h, about 250 g of undamaged lettuce was packaged using a high-barrier film (length, 0.35 m; width, 0.29 m; O_2 transmission rate, 2.1×10^{-8} L m^{-2} s^{-1} bar and water vapour transmission, 4.6×10^{-5} g m^{-2} s^{-1}, at 4 °C and 90–95 % relative humidity (RH); Fibosa Packaging S. L., Tordera, Spain), with a 10/3/87% $CO_2/O_2/N_2$ atmosphere and immediately placed in a cold-storage room (4 °C, 90–95 % RH). Lettuce packaged with polyethylene fresh-keeping film (O_2 transmission rate, 8.1×10^{-5} L m^{-2} s^{-1} bar and water vapour transmission, 4.9×10^{-5} g m^{-2} s^{-1}, at 4 °C and 90–95 % RH; Fibosa Packaging S. L.) was stored in the cold-storage room as a control. Before storage, the control film was punctured with about 10 holes using a needle to maintain air circulation. The treatment and control each had 18 replicates, and three bags for each were sampled daily for six days. Samples were collected from the internal, middle and external part of whole lettuce, cut into 0.02 m × 0.02 m cubes, and the cubes were mixed uniformly for analysis.