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# Effects the mechanism of micro-vacuum storage on broccoli chlorophyll degradation and builds prediction model of chlorophyll content based on the color parameter changes

Yanan Sun, Wenxiang Li*

*Key Laboratory of Modern Agricultural Quality and Safety Engineering of Qingdao, Qingdao Agricultural University, Qingdao 266109, China*

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ABSTRACT

The objective of this study was to investigate the mechanism of micro-vacuum (MV) storage on broccoli chlorophyll degradation and builds prediction model of chlorophyll content based on the color parameter changes. The freshly harvested broccoli were subjected to MV storage (70 ± 5 kPa) and atmospheric pressure storage at 3 ± 1 °C for 49 d; The relationship between broccoli chlorophyll content and its surface color parameters (-a/b value) was studied to build broccoli chlorophyll content prediction model based on the color parameter changes under MV conditions. The results showed the degradation of chlorophyll could be inhibited, activities of CAT, SOD, POD could be improved, superoxide anion ($O_2\cdot$) release rate, hydrogen peroxide ($H_2O_2$) content, malondiadehyde (MDA) content and the increase of membrane permeability could be decreased significantly in MV storage conditions; the correlation analysis showed that relationship between chlorophyll degradation rate and activities of CAT, SOD, POD, the relationship between chlorophyll content and $O_2^{-}\cdot$ release rate, $H_2O_2$ content, MDA content, membrane permeability showed a significant negative correlation; The changes of chloroplast ultrastructure could be inhibited and degradation of chlorophyll could be delayed in MV conditions, yellowing mechanisms of broccoli was revealed by the cellular level in MV conditions. Validation showed that the prediction model could reflect the changes of chlorophyll content during storage, provide theoretical basis for the further application of non-destructive testing technology.

## 1. Introduction

Broccoli (*Brassica oleacea*) is a very popular and healthy horticultural produce with a high nutritional value, due to its significant content of carbohydrates, protein, carotenoids, dietary fiber, niacin and vitamins, antioxidants and anticarcinogenic compounds. For this reason, broccoli is a vegetable which production has increased rapidly to satisfy consumer demand in China. It is easy ageing that postharvest respiration strongly with water loss under the room temperature condition in air (20 °C), the color from green to yellow which should not exceed one week of shelf-life. Chlorophyll (Chl) is an important pigment in green plant chloroplast, and plays an important role in energy transfer and energy capture for photosynthesis (Ding et al., 2016). The degree of greenness, due to chlorophyll pigments, is one of the major quality indicators for postharvest vegetable products character (Ma et al., 2009; Ma et al., 2007). In the process of vegetables mature with the degradation of chlorophyll, it is an important symbol of quality reduction that green vegetables yellowing.

During vegetables ripening and senescence, it has higher endogenous antioxidant enzyme catalases (CAT), peroxidases (POD) and superoxide dismutases (SOD) activity at the beginning of the storage which can maintain the balance of vegetable metabolism, inhibit the accumulation of reactive oxygen species(ROS) (Xu et al., 2016). But with the extension of storage time, the endogenous antioxidant enzyme activities of vegetables decline gradually that cannot clear of excessive reactive oxygen species. The cell membrane system will be damaged when the accumulation of reactive oxygen species above a certain threshold, so that to accelerate vegetables senescence and promote the degradation of chlorophyll. The balance between ROS production and their removal by the antioxidant defense systems determines the speed of ripening and senescence and, therefore, the vegetables shelf-life (Fan et al., 2016). Raseetha et al. (2013) has evaluated the antioxidant enzymes activity and volatile release during storage of processed broccoli (Brassica oleracea L. italica); Fernández-León et al. (2013) studied that different postharvest strategies to preserve broccoli quality during storage and shelf life controlled atmosphere and 1-MCP. The atmosphere packaging (MAP), refrigeration storage and controlled atmosphere storage (CA) have been used to extend the shelf-life of different

* Corresponding author.
*E-mail address:* 956551609@qq.com (W. Li).

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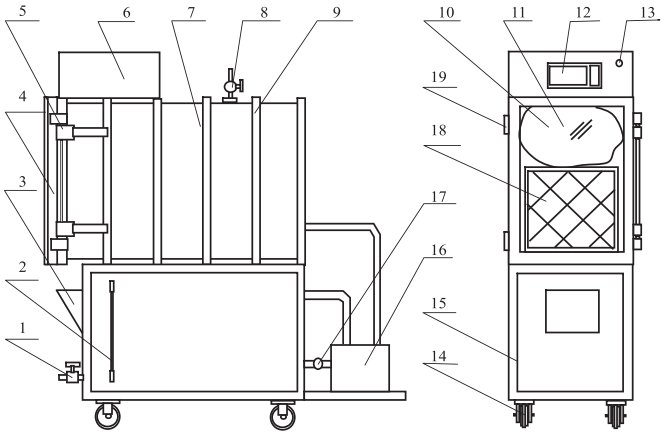


**Fig. 1.** Structure schematic diagram of Micro-vacuum storage experimental equipment 1 blow-down valve, 2 water level gauge, 3 filling pipe end, 4 air-tight door, 5 hinge, 6 control box, 7 storage chamber, 8 inlet or outlet air valve, 9 stiffener, 10 air bag, 11glass, 12 PLC display screen, 13 indicator lamp, 14 return pulley, 15 water tank, 16 vacuum pump, 17 electromagnetic valve, 18 turnover box, 19 fastening handle.

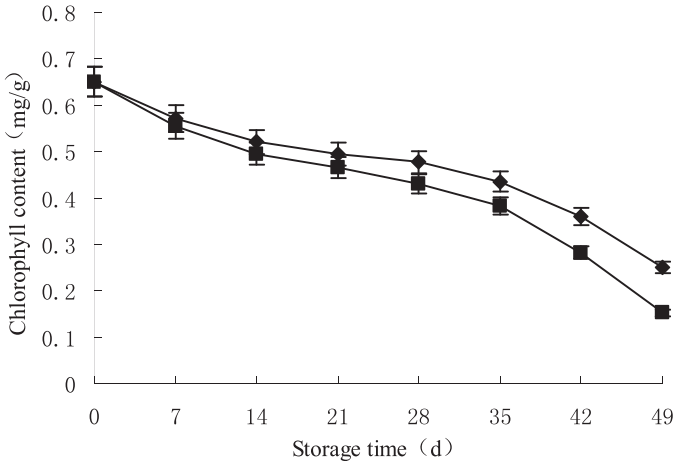


**Fig. 2.** Changes of chlorophyll content with storage time. Control(■): atmospheric pressure storage; MV(◆): micro-vaccum stoage.

broccoli cultivars.

Modified atmosphere packaging (MAP) delayed the ageing process of broccoli by adjusting temperature, $O_2$ content, $CO_2$ content, and maximized the inhibition of respiration in order to maintain normal physiological activities of broccoli on the storage conditions. With the decreasing concentration of $O_2$ content, the physiological activities of the broccoli will be suppressed accordingly, at the same time, the decay of the broccoli was delayed by adjusting the growth of aerobic microorganisms. However, $O_2$ concentration is lower than the threshold, aerobic respiration was inhibited causing the accumulation of anaerobic respiration metabolites. $CO_2$ inhibited respiration of broccoli as a regulator produced by respiration, the dehydrogenase system of broccoli was damaged by higher $CO_2$ concentration (Lange, 2000); The refrigeration storage delayed the ageing process of broccoli by using mechanical cooling system to restrain the growth of the bacteria breeding, reducing to enzymes activity of respiration and extending broccoli post-harvest shelf-life (EI-Hilali et al., 2003); This study used MV storage experimental equipment (Fig. 1), designed and manufactured based on the invention patent CN1530290A (Wang, 2004). Compare with hypobaric storage, MV storage setted a flexible airbags in the storage room to reduced stress which can effectively inhibit vegetable moisture loss. A special emphasis was placed on determining the effects of this storage equipment on degradation of chlorophyll and the capacity of enzymatic systems to maintain the equilibrium between oxidation and antioxidant activity.

Nowadays, many physical and chemical characteristics of vegetables have been determined nondestructively so that nondestructive techniques for quality evaluation have gained in popularity. These techniques are easy and quick to use for vegetables particularly. It was reported that some efforts using near-infrared (NIR) spectroscopy, visual spectral analysis, acoustic and ultrasound techniques for vegetable, but these instruments are costly and difficult to carry (Siva, et al). Godoy et al. (2008) have reported that highbush blueberry (Vaccinium corymbosum L.) fruit growth with exponential mixed models; Diao et al. (2010) have reported that kinetics of colour changes of dehydrated spinaches during storage; Liu et al. (2011) have reported a significant correlation between degradation kinetics of color and chlorophyll for edamame, which gives an idea of maturity. A calibration equation related to maturity level based on some chemicals at harvest and color values must be developed for use with the portable colorimeter to replace the typical subjective method and reduce the problem of carrying the instruments to the field (Tijskens et al., 2001).

## 2. Materials and methods

### 2.1. Experimental apparatus

The MV storage experimental equipment consisted of a vacuum pump, compressor, moistener, soft air bag, storage chamber (100 cm × 75 cm × 65 cm), and a control box (Fig. 1). This equipment was placed in a refrigeration house (3 ± 1 °C, 85–95% relative humidity [RH]).

### 2.2. Plant materials and treatments

Broccoli were harvested from plantations in Chengyang of Qingdao and transported to the laboratory of Qingdao Agricultural University, within 4 h of harvesting. Broccoli of uniform size and maturity were selected and pre-cooled at 4 °C for 24 h. The broccoli were packaged using low-density polyethylene bags. Broccoli were divided into two groups (20 kg each). In the experimental group, broccoli were placed in the MV experimental equipment storage chamber, and stored for 49 d at 70 ± 5 kPa (3 ± 1 °C; 85–95% RH). In the control group, broccoli was stored under atmospheric pressure in a cold storeroom under the same conditions of temperature and RH. Freshly harvested broccoli were used as the 0 d sample. Two broccoli were selected randomly to determine the vegetable characteristics every week; this process was repeated three times.

### 2.3. Determination index

#### 2.3.1. Determination of chlorophyll content

1.0 g of broccoli pulp was grinded in 10 mL of 80% acetone solution until homogenate remove green completely. After a period of time, the supernatant was filtered to 50 mL brown volumetric flask, residue was washed with acetone for many times until it become white completely. With 80% acetone as blank reference zero, filtrate were measured at 663 nm and 645 nm wavelength absorbance values separately. Thus makes the quality of the chlorophyll a and chlorophyll *b* concentrations (mg $L^{-1}$) relationship, deduces the formulas for Arnon: $\rho_a = 12.72\ A_{663} - 2.59\ A_{645}$ ; $\rho_b = 22.88\ A_{645} - 4.67\ A_{663}$ ; $\rho_T = \rho_a + \rho_b = 20.29\ A_{645} + 8.05\ A_{663}$

#### 2.3.2. Determination of SOD activity, CAT activity, POD activity

SOD activity was determined using the method reported by Crosti et al. (1987) based on the photoreduction of nitro-blue tetrazolium (NBT) as an indicator. One unit (U) of enzyme activity was the amount of extract that gave 50% inhibition of the rate of reduction NBT.