Postharvest Biology and Technology 131 (2017) 39–45

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Total polyphenols, total flavonoids, allicin and antioxidant capacities in garlic scape cultivars during controlled atmosphere storage

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ARTICLE INFO

Keywords:
Garlic scape
Quality
Polyphenol
Flavonoids
Allicin
Antioxidant

ABSTRACT

Five commercial cultivars of garlic scapes were subjected to a controlled atmosphere (O_2 = 2-5%, CO_2 = 3–6%) at temperature = 0 ± 0.5 °C, RH = 85-95%, for 140–224 d to document the quality and related changes in components during storage in two consecutive years. Polyphenols, flavonoids, allicin, and 2,2-diphenyl-1-picryl-hydrazl (DPPH) as well as the ferric ion reducing antioxidant power (FRAP), metal chelating capacity (MCC), and hydroxyl radical scavenging activity (HRSC) were analyzed to study overall antioxidant properties of garlic scapes in storage. The storage life was 224, 196, 196, 168, and 140 days for G025, G107, G2011-04, G110, and G064. G025 had the highest total polyphenol concentrations at 140 and 168 days in 2014 and 2015, respectively, whereas G2011-4 had the lowest concentrations of total polyphenols. The highest total polyphenols, total flavonoids and allicin concentrations were observed in G025, whereas G2011-04 displayed the lowest concentrations of total polyphenols and allicin in both years. For all cultivars, total flavonoid concentrations decreased with time. The highest weight loss was observed in G064 both in 2014 and 2015. The antioxidant capacity of G025 and G110 was higher than that of the other cultivars. DPPH and HRSC were highest in G025, and MCC and FRAP were high in G110 and G107 in both years. These results demonstrate that cultivar influences the rate of garlic scape deterioration, chemical composition properties and antioxidant activities.

1. Introduction

Garlic scape is the flower stalk of garlic plant. The hardneck type of garlic scape is thin and has a slight garlicky scent, a moderately spicy flavor and an especially fresh and pleasant taste. Garlic flower stalk is widely consumed in various parts of Asia, especially in China, as it is a common ingredient in Chinese cuisine (Simon and Jenderek, 2003). Garlic is a rich source of health-promoting phytochemicals including antioxidants such as phenolics, flavonoids, and allicin (Lanzotti, 2006).

Garlic bulbs are good source of natural antioxidants and possess potential health-promoting effects due to their high phenolic phytochemical concentrations (Nuutila et al., 2003). Phenolic concentrations in garlic are affected by agronomic and environmental factors (Waterer and Schmitz, 1994), but cultivar is the primary factor that determine this variation. Bulb firmness, pH, total soluble solids, moisture concentration, and sugar concentration have been reported to differ across 14 garlic cultivars (Volk and Stern, 2009). Most research on garlic has focused on the bulbs, while scapes of garlic receive very little attention, which have gained popularity in recent years. Garlic scapes are used for strong desirable fresh flavor in various recipes and stir-fry preparations as well as an additive for meat products (Rekowska and Skupien, 2009). Despite its popularity as a high-value vegetable and as a source of flavorful ingredient, very limited research on the antioxidant components of garlic scapes has been carried out (González et al., 2012). The quality of garlic scapes after long-term storage affects consumer acceptance. Many factors have to be considered for optimum quality, such as genotype and pre- and post-harvest conditions. Curing methods, environmental conditions and genotype can affect maximum quality, and post-harvest handling (storage temperature and relative humidity) is essential for maintaining high quality of *Allium* crops (Brewster, 2008; Gubb and MacTavish, 2002).

Variation in allicin, allyl methyl thiosulfinate, and allyl *trans*-1-propenyl thiosulfinate concentrations was observed in 93 garlic cultivars (Kamenetsky, 2007). Allicin (diallylthiosulfinate), an active compound in garlic, represents approximately 70% of the overall thiosulfinate concentrations formed upon crushing of cloves (Kim et al., 2013; Lanzotti, 2006). Garlic cloves contain a large amount of alliinase in sheath cells and alliin in storage cells; these compounds interact once garlic is damaged and alliin is chemically converted to alkenyl sulfinyl compounds (Ellmore and Feldberg, 1994). Allicin has antimicrobial,

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http://dx.doi.org/10.1016/j.postharvbio.2017.05.002
Received 16 February 2017; Received in revised form 2 May 2017; Accepted 2 May 2017

anti-inflammatory, antithrombotic, and anticancer properties (Beato et al., 2011; Bommareddy et al., 2016; Kim et al., 2013; Lanzotti, 2006).

Garlic scape can be stored in controlled atmosphere for 10 months at a temperature of 0 ± 0.5 °C and a relative humidity of 85–95% to fill supply gaps in the market, but the consumers complain about the deterioration of quality compared with freshly harvested scapes (Pers.com.). With respect to the quality of green onion cultivars in a modified atmosphere, the total polyphenol concentration was reduced by 6.6% after 20 days of storage (Viškelis et al., 2012).

The objective of this study was to investigate the deterioration of quality in different garlic scape cultivars during long-term storage.

2. Materials and methods

2.1. Samples and storage conditions

Garlic scape cultivars G110, G107, G2011-4, G025, and G064 were obtained from the germplasm program of the Horticultural experimental station of Northwest A & F University, Yangling, China. Garlic scapes were harvested during March and April in 2014 and 2015 respectively, at the commercial ripening stage with no disease and pest damage, without aging of the base and bracts. The scapes had fresh green color and crisp texture, and were harvested at the proper maturity stage (floral axis with bending hooks), without deformities. The garlic scapes were harvested on sunny days, transported to lab immediately in well-ventilated conditions and precooled at 5 °C before being kept in cold storage. The base of the scape was trimmed, cleaned and cured in pre-cooling room for 24 h. In 2014, data were recorded at 28 d intervals, whereas in 2015, data were recorded at 56-d intervals. The cultivars were stored at 0 ± 0.5 °C (RH = 85-95%, O_2 = 2-5%, CO_2 = 3-6%) for a maximum of 224 d, following the commercial storage recommendations (SBT 10887/2012). The fresh garlic scapes were stored at the eating-quality stage. Three boxes each of 360 L had two nozzles, one for injecting O_2 and the other for CO_2. After taking samples, the lid was closed, and nitrogen gas was slowly injected until the O_2 reached the desired level through one nozzle. Then, the nozzle was quickly closed, and CO_2 was slowly injected through the second nozzle, after which it was closed upon reaching the desired limit. A general analyzer CYCK-401 (Yantai Venture of Measurement and Control Engineering limited, Xian, China) was used to measure temperature, relative humidity, O_2 and CO_2 concentrations. The same procedure was repeated every time in sampling. Commercial garlic scapes are usually stored in the cold storage room, and the boxes were used only for experiment. Three replicate samples of 1 kg each, were placed on the shelves in 3–4 layers, 300 mm apart. The boxes were kept open for ventilation 2–3 times per month for 15–20 mins each time. O_2 and CO_2 were maintained at 2–5% and 3–6%, respectively, to avoid sulfur-containing volatiles. Refrigeration time varied for different cultivars. The refrigeration time was 150–300 days. 150 days, the CO_2 was kept at the high limit index and O_2 at the low limit index; after 150 days, the CO_2 was lowered to the low limit, and O_2 was raised to the high limit index. Quality evaluations were performed on each sampling day. A panel of five experts subjectively assessed the samples for each quality characteristic (appearance, taste and texture) using a 1–5 scale. In the case of appearance, a scale composed of pictures and a brief description for each score value was used as follows: 5 = excellent, no defects; 4 = very good, minor defects; 3 = fair, moderate defects; 2 = poor; major defects; and 1 = inedible. A score of 3 was considered the limit of marketability and a score of 2 as the limit of edibility. Due to the small size of our taste test panel and the difficulty in tasting several garlic scape samples at one time, only generalizations from their records are presented.

2.2. Determination of total polyphenols, total flavonoids, antioxidant capacity and weight loss

50 g of scapes were subsampled from each replicate, homogenized and extracted in 200 mL of ethanol: acetone (7:3, v/v) for 1 h at 37 °C (Lee and Wicker, 1991). The extract was filtered through Whatman No. 41 paper and rinsed with 50 mL of ethanol: acetone (7:3, v/v). Extraction of the residue was repeated using the same procedure. The two combined filtrates were stored at −20 °C until total polyphenol, total flavonoid and antioxidant capacity analyses were carried out.

Total polyphenols (TP) were determined using the Folin-Ciocalteu method (Jayaprakasha et al., 2001) with minor modifications. In a 10-mL Eppendorf tube, 7.9 mL of distilled water, 0.1 mL of garlic scape extract and 0.5 mL of Folin-Ciocalteu reagent (1:1 with water) were added and mixed. After 1 min, 1.5 mL of sodium carbonate (1.8 mol L^{-1}) was added. The prepared mixture sat at room temperature in the dark for 2 h. The absorbance was measured at 765 nm; the total polyphenol concentration was calculated from the calibration curve using gallic as the standard. All concentrations were expressed as grams per kilogram on a fresh weight basis.

Total flavonoids (TF) were determined according to the methods of Yong et al. (2008). In a 10-mL Eppendorf tube, 0.3 mL of garlic scape extract, 3.4 mL of 30% ethanol, 0.15 mL of 0.5 mol L^{-1} $NaNO_2$ and 0.15 mL of 0.3 mol L^{-1} $AlCl_3$ were added and gently mixed. After 5 min, 1 mL of 1 mol L^{-1} NaOH was added. The absorbance was measured at 506 nm. The total flavonoid concentration was calculated from a calibration curve using rutin as the standard. All concentrations were expressed as grams per kilogram on a fresh weight basis.

Weight loss of each replicate was measured every 28 d in 2014 and 56 d in 2015. Weight loss was calculated on the basis of differences between the initial and final weights recorded for each sample. Cumulative weight loss was expressed as the percentage loss of the initial total weight.

2.3. Determination of allicin using HPLC

For allicin analysis, we followed the procedure of Tan (2008). 10 g of scapes were subsampled from each replicate and crushed in 50 mL of methanol. The samples were then centrifuged at 4 °C at 12,000*g* for 20 min, after which 1 mL of supernatant was filtered through 0.45-μm filter paper.

An allicin standard was obtained from Sigma International Co., China. For HPLC, we followed the procedure of Fujisawa et al. (2008), with some modifications. Quantitative analyses were carried out on an HPLC machine (Waters 600E, Milford, USA). The column dimensions were 150 × 4.6 mm, with a 5-μm thickness (C18 Aeris Peptide, Dikma Technologies, China) operating at a constant flow rate of 1.0 mL min^{-1} at 25 °C. An injection of 10 μL was used for the standard and for all samples. Detection was carried out using a UV detector at 220 nm. The mobile phase was as follows: methanol (9); water (41); acetonitrile (50). Quantification of allicin was performed by comparing the peak area produced by crushed garlic scape extracts with that of authentic allicin. The results of allicin were expressed as milligrams per kilogram on a fresh weight basis.

2.4. Measurements of antioxidant enzyme activity

The ability to scavenge 2,2-diphenyl-1-picryl-hydrazl (DPPH) was determined using the method of Hatano et al. (1988). One milliliter of each extract was added to 1 mL of the DPPH radical solution in methanol (the final concentration of DPPH was 0.1318 mmol L^{-1}). The mixture was then stirred vigorously and sat for 30 min; the absorbance of the resultant solution was measured at 517 nm with a spectrophotometer. DPPH was calculated as follows:

$$\text{Scavenging\%} = (1 - A_{sample})/A_{control}) \times 100$$