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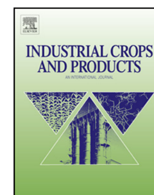

Changes in antioxidant capacity, levels of soluble sugar, total polyphenol, organosulfur compound and constituents in garlic clove during storage

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A B S T R A C T

The present study was designed to study the effect of storage duration on changes of antioxidant capacities and bioactive compounds in garlic clove by DPPH and FRAP assays and GC–MS analysis. Both DPPH and FRAP assays displayed that the antioxidant capacities in garlic clove extracts reached maximum values at 8 weeks. The levels of soluble sugar decreased during storage, and the contents of total polyphenols and organosulfur compounds reached maximum value at 6 and 8 weeks, respectively, and then decreased significantly. The GC–MS analysis results of ethyl acetate extract showed that among of the eighteen typical organosulfur compounds, ten compounds reached maximum levels at 8 weeks. The present study indicated that organosulfur compounds in garlic cloves might play a key role in antioxidant capacities due to their positive relationship during storage. The results in this study would provide beneficial information for garlic processing industries and consumers to make full use of garlic during storage.

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1. Introduction

Garlic (*Allium sativum* L.) belongs to the vegetables of the *Allium* genus, it has a long history as being a food having a unique taste and odor (Ariga and Seki, 2006; Ngo et al., 2007). Nowadays, Garlic has been not only widely used in antibacterial, antiviral, antifungal and antiprotozoal, but also beneficial effects on the cardiovascular and immune systems (Harris et al., 2001). Researchers have proved that there are more than 200 components identified from garlic, such as vitamins, proteins, lipids, trace elements Se, flavonoids and at least 33 different organosulfur compounds (Stajner et al., 2006; Ngo et al., 2007).

Garlic clove contains a surprisingly large amount of alliinase (10 mg/g fresh weight), alliinase in the bundle sheath cells and alliin in the storage cells are separated, and only they encounter each other once garlic is damaged, then generate chemical reaction and convert alliin immediately to the alk(en)yl sulfinyl compounds (Ellmore and Feldberg, 1994). Alk(en)yl sulfides are characteristic flavor components of garlic. Modern scientific research has revealed that the wide variety of dietary and medicinal functions of garlic can be attributed to the sulfur compounds from garlic (Ariga and Seki, 2006). Studies suggested that diallyl trisulfide (DATS) was responsible for the anticancer effect for garlic eaters (Seki et al., 2008). Moreover, garlic contained stable organosulfur compounds, flavonoids and polyphenols, which had potent antioxidant properties (Nencini et al., 2011; Beato et al., 2011). In addition, garlic polysaccharide, mainly consisted of water-soluble fructose polymers, displayed bioactivity in many aspects, such as controlling blood lipids, lowering blood sugar and promoting the absorbability of minerals (Huang et al., 2012).

The flavor compounds that are important quality parameters will be affected by many factors, such as storage temperature, cultivar, storage duration, etc (Randle and Lancaster, 2002; Bloem et al., 2011). Bloem et al. (2011) demonstrated that sulfur fertilization was shown to significantly increase the alliin concentration in garlic cloves, while high nitrogen levels had an adverse effect, and best quality in terms of high alliin contents was obtained during the entire storage time at an sulfur level of at minimum 30 kg/ha if no nitrogen was applied. Montaño et al. (2011) presented that there was a significant effect of the location, cultivar, and garlic ecotype on individual organosulfur compound contents.

Generally, garlic cloves are stored for varying lengths of time before being consumed, so it is important for garlic processing industries and consumers to know the changes of chemical constituents and antioxidant capacities during storage, but so far these have not yet been investigated. In the present study, the changes of antioxidant capacities, soluble sugar, total polyphenols and organosulfur compounds were determined and identified by

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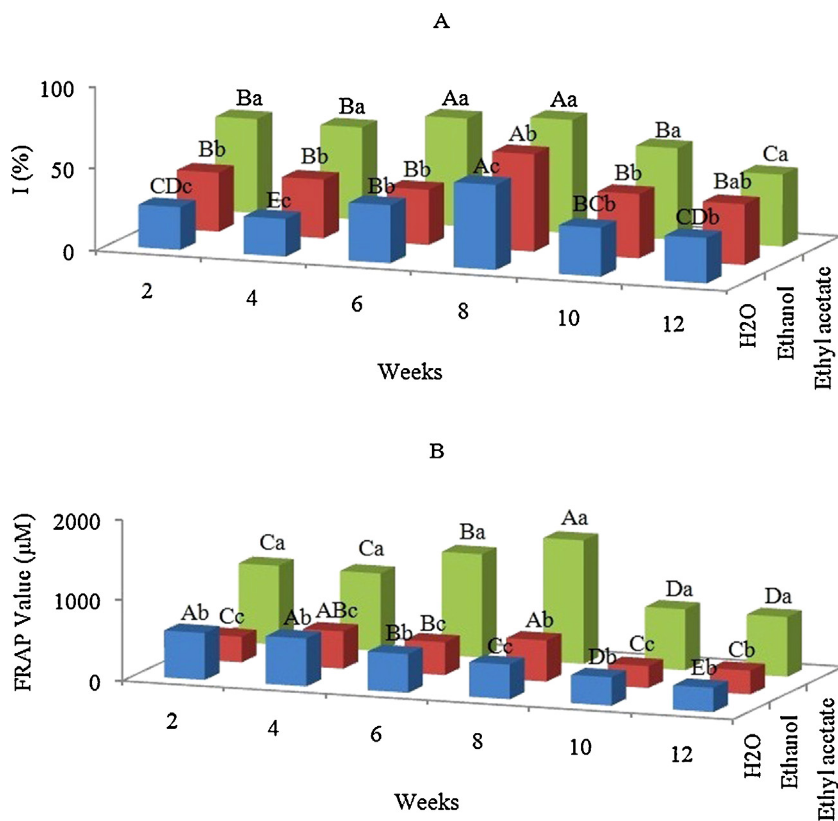

Fig. 1. Changes in antioxidant capacities of different solvent extracts in garlic by the assays of DPPH (A) and FRAP (B) during storage. The data are the means of triplicate measurements. Different lowercase and uppercase letters on top of the column were considered significant at $P < 0.05$ level among different solvent extracts and storage times, respectively.

DPPH and FRAP assays and GC–MS (gas chromatography–mass spectrometry), with the aim of gaining insight into metabolism and evaluating the effect of storage duration on antioxidant capacity in garlic cloves.

2. Experimental

2.1. Plant material

Fresh garlic cloves, harvested from the Gansu Provincial Key Lab Foundation of Aridland Crop Science, Lanzhou, China, in June 2012, were used for the present study. Garlic cloves were stored in an incubator at 20 ± 2 °C with 45% relative humidity on a 1000 lx light/dark (12 h/12 h) cycle, for detecting the changes of antioxidant capacities, soluble sugar, total polyphenols and organosulfur compounds and chemical constituents during storage duration.

2.2. Extracts preparation

The extracts preparation was performed according to the literature (Brankovic et al., 2011) with slightly modification, and the specific steps as follows. Garlic cloves were peeled, weighed (50.0 g), and pulverized into a fine juice by a laboratory blender, extracted separately with 200 mL of H_2O, 80% methanol, ethanol and ethyl acetate, respectively, for 4 h at room temperature in a shaker, carried out in an ultrasonic vibration for 30 min, and then filtered three times through cheesecloth. The filtrate was added 10 mL of cold 1.0% saline solution and stirred at high speed for 20 min. The mixture was centrifuged at 12000 rpm for 15 min, and then the supernatant was evaporated and condensed in a rotary evaporator at 35 °C. The obtained extracts were stored at −20 °C.

2.3. Antioxidant assays

2.3.1. DPPH assay

The measurement was performed according to the literature (Li et al., 2012, 2013), and the specific steps as follows. The extracts of H_2O, ethanol and ethyl acetate in Section 2.2 were diluted with 80% methanol with concentration of 10 mg/mL, and then 50 μl of sample solution was added into 950 μl of 10^{-4} M DPPH methanol solution. After shaking and incubating in dark at room temperature for 30 min. The absorbance was evaluated at 515 nm, and 500 μM 90% methanol ASA was tested as a positive control. The assay was carried out in triplicate, and the capability to scavenge the DPPH radicals was calculated as:

$$\text{Scavenging effect}(I\%, \text{Percentage of inhibition}) = \left[\frac{(A_0 - A)}{A_0}\right] \times 100$$

where A_0 and A were the absorbance of DPPH without and with sample, respectively.

2.3.2. FRAP assay

The measurement was described by the literature (Benzie and Strain, 1996; Li et al., 2012), and the specific steps as follows. The 300 μL FRAP reagent [prepared ex tempore by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6, with 10 mmol/L TPTZ in 40 mmol/L HCl, and 20 mmol/L $FeCl_3 \cdot 6H_2O$ at 10:1:1 (v/v/v)] and the 10 μl standard samples ($FeSO_4 \cdot 7H_2O$, 500 μM) or test samples of H_2O, ethanol and ethyl acetate in section 2.2 (10 mg/mL 80% methanol) were mixed. The mixture reacted at 37 °C and the absorbance was taken at 593 nm immediately and 4 min later. And 500 μM 90% methanol ASA was tested as a positive control. The assay was carried out in triplicate. The FRAP value of the test

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