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A PHYSIOLOGICAL INDICATOR TO ESTIMATE ALLICIN CONTENT IN GARLIC DURING STORAGE

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ABSTRACT

The aim of the present work was to evaluate the application of a physiological indicator as marker of allicin content during postharvest storage at different temperatures. Sureño INTA garlic cultivar was stored at room temperature (20C ± 3) and cold storage (0C ± 0.5). Samples were taken periodically and allicin, pyruvate content were analyzed and the sprout growth was measured through of the visual index of dormancy (VID). The results showed that regardless of storage temperature, allicin, pyruvate content and VID changed significantly during the period of the assay. For the conditions evaluated, the highest allicin content was observed at 50% and 100% VID. These facts suggest that VID may be a suitable tool to estimate allicin level during storage.

PRACTICAL APLICATIONS

This work was addressed to resolve the problem of standardization of garlic subproducts according to their bioactive compounds levels. The information acquired is important for farmers and the food and phytotherapic industry in order to guarantee the adequate supply of garlic subproducts to the consumer. Therefore, we found that it is important to consider a physiological indicator as a tool to estimate the bioactive compound levels during postharvest storage.

INTRODUCTION

Garlic (*Allium sativum* L.) has been cultivated for many centuries due to their characteristic flavor and medicinal properties. Beneficial clinical effects related to the consumption of garlic preparations have been reported (Amagase *et al.* 2001), including reduced risk for developing cardiovascular diseases, cancer, obesity, hypercholesterolemia, diabetes type II, hypertension, cataract and disturbances of the gastrointestinal tract (Lanzotti 2006). There is evidence that many organosulphur compounds (OSCs) found in *Allium* preparations are responsible for several of the biological activities mentioned above (Tapiero *et al.* 2004).

The major OSCs in intact *Allium* plants are S-alk(en)yl-L-cysteine sulfoxides (ACSOs) (Block 2010). In garlic bulbs, the main flavor precursor is alliin, with fewer amounts of isoalliin and methiin (Lancaster and Boland 1990). Others OSCs are present as γ-glutamyl peptides such as γ-glutamyl allyl cysteine sulphoxide (γ-GLUACSO), and γ-glutamyl isoallyl cysteine sulphoxide (γ- GLUisoACSO) (Hughes *et al.* 2006). When garlic tissue is damaged, the vacuolar enzyme alliinase rapidly hydrolyses the cytosolic ACSOs, including alliin, to form the thiosulfinates (which provide the characteristic flavor and odor), pyruvate and ammonia. Allicin (diallyl thiosulfinate) represents about 70% of the overall thiosulfinates formed, and it is considered the principal bioactive compound found in fresh garlic.

The OSCs exhibit variability due to genotype, environmental and postharvest storage conditions. The environmental factors include sulfate, nitrogen and selenium availability in the soil; growing temperature; and water supply (Randle and Lancaster 2002). Regarding storage conditions after harvest, temperature is considered the most important one. In particular, storage temperature affects the break of dormancy and sprouting of garlic cloves and root development (Ichikawa *et al.* 2006). On the other hand, the profile of bioactive compounds in commercial garlic products varies according to the methods used to elaborate these preparations (Lawson *et al.* 1991a). All these aspects make difficult to standardize the levels of bioactive compounds in commercial garlic products. In addition, there is evidence about numerous cases of patients that experienced negative health consequences linked to the poor quality of phytotherapics (World Health Organization 2003a). This situation is evidenced by the WHO global survey on the national policy and regulation of traditional medicine who has reported that there are three common difficulties and challenges: lack of information sharing; lack of safety monitoring for herbal medicines; and lack of methods to evaluate their safety and efficacy (World Health Organization 2003b).

We have characterized Argentinean garlic cultivars by organosulfur (allicin, S-alk(en)yl-L-cysteine sulfoxides, thiosulfinates), pyruvate levels, solids and mineral content; and biological activities like platelet aggregation, antioxidant activity and 15-lipooxigenase inhibition (Camargo *et al.* 2005; Sance *et al.* 2006; Camargo *et al.* 2007; Cavagnaro *et al.* 2007; González *et al.* 2009; Camargo *et al.* 2010; Soto Vargas *et al.* 2010; Vazquez-Prieto *et al.* 2010). In the present work, we have focused on the study of OSCs content changes during postharvest. Several studies have been carried out on this area (Lawson *et al.* 1991b; Hughes *et al.* 2006; Ichikawa *et al.* 2006). In addition, it is well known that garlic bulb storage has problems, such as sprouting and weight loss. Therefore, different treatments have been aimed at minimizing them. Ledesma *et al.* 1980, have reported that garlic cloves did not grow immediately after harvest due to dormancy which gradually diminished during storage. The dormancy rupture is triggered by biochemical changes (Ceci and Curzio 1992). Some of them are the increase of catabolic processes and the transport of nutrition substances to the sprout (Pellegrini *et al.* 2000). Furthermore, it has been reported that sprouting garlic and onion show higher γ-glutamyl transpeptidase activity than dormant bulbs (Ichikawa *et al.* 2006). This enzyme is responsible for converting γ-glutamyl peptides into flavors precursors, which in turn are hydrolyzed by alliinase to form bioactive compounds (Debaene *et al.* 1999). All these physiological changes demonstrate that the biosynthetic flavor pathway is active during bulb storage (Kopsell and Randle 1999).

A tool used to measure the break of dormancy in garlic bulbs is the visual index of dormancy (VID), which represents the percent relationship between length of the sprouting and the storage leaf (Burba *et al.* 1989). This measurement is often applied to decide when garlic cloves can be used for seed, and also as an indicator of shelf life (Portela *et al.* 2005).

We hypothesize that VID could be used as a tool to estimate when allicin would reach the maximum level in order to obtain garlic subproducts with the highest yield of OSCs. Finding the relationship between these garlic bulb features could be useful for food and phytotherapy industry. Thus, the main point of this work was to investigate the relationship between analytical data (allicin and pyruvate levels) and VID in order to obtain more detailed information about the dynamics at different storage temperatures. This is the first time that VID was applied to relate physiological state with OSCs levels.

MATERIALS AND METHODS

Plant Material

The cultivar selected for this study was Sureño INTA belonging to the garlic germplasm bank of INTA La Consulta, Argentina. This cultivar was chosen because it showed the highest level of allicin in our previous work (Camargo *et al.* 2005). Sureño INTA cultivar was grown in a loam soil in four-row plots arranged in a complete randomized block design in La Consulta, Mendoza, Argentina (33°44′ S, 69°07′ W).

Garlic Samples

Bulbs were harvested in December, fully cured under environmental conditions during a period of 102 days. After that, uniform bulbs were selected and placed in boxes for storage under two experimental conditions: room temperature (20C ± 3) and cold storage (0C ± 0.5). The assay was completed once garlic bulbs reached 100% VID, under both conditions. Ten whole bulbs were taken at random per triplicate at intervals of 15 days. These 10 chosen bulbs were peeled to remove the dry outer scales and then, in each sample VID, allicin and pyruvate levels were measured.

Allicin Determination

Allicin levels were analyzed according to González *et al.* 2007. The samples were conditioned through garlic powder preparation as previously described (Lawson *et al.* 1991b). Distilled water was added to 1 g of garlic powder (30 mL per g), mixed, kept 10 min at room temperature and then centrifuged at 14.000 rpm for 5 min. Methanol was added to 600 μL of the supernatant (1:1 v/v) and centrifuged at 14,000 rpm for

5 min. The resulting supernatant was filtered through a 0.45 µm membrane before injection. A 20 µL aliquot was injected into an HPLC. The assay was performed on HPLC Konik A 500 system, coupled to a UV detector of variable wavelength. The reversed-phase column (250 × 4.6 mm; Waters Spherisorb ODS2, 5 µm) was fitted with a guard column of the same characteristics. Samples (10 µL) were injected into the column, which was maintained at room temperature (25 ± 3C). Aqueous methanol (50% v/v) was used with isocratic elution and a flow rate of 0.8 mL/min. Eluted components were detected at 254 nm. Using this system, allicin eluted at 9 min. The quantification was done as previously described (González *et al*. 2007). Values were expressed as mg/g of fresh weight (fw).

Basal and Total Pyruvate Acid Levels Determination

Basal and total pyruvate concentrations were analyzed according to Natale and Camargo 2005. For total pyruvate determination, garlic cloves were blended for 1 min in distilled water (1:10 w/v). The juice was collected, filtered and kept at room temperature for 15 min to allow enzymatic hydrolysis of the flavor precursors. Garlic cloves were microwaved at 1,000 w per 100 g for 3 min to inactive allinase for basal pyruvate determination. After that, they were blended for 1 min in distilled water (1:10 w/v). Aliquots of both juices were added to an equal volume of 5% trichloroacetic acid (v/v) and centrifuged for 10 min at 10,000 rpm. One milliliter of 0.0125% 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl was added to 2 mL of juice/trichloroacetic acid (TCA) (1:20 v/v). The tubes were incubated at 37C for 10 min in a temperature controlled bath, and then 5 mL of OHNa 0.6 N was added. Absorbances were measured at 420 nm. The pyruvate concentration of each garlic juice was determined using a reference by a standard curve developed with known concentrations of pyruvate. Values were expressed as µmol/g fw.

Visual Index of Dormancy

VID was calculated according to the following formula: (length of sprouting leaf/length of storage leaf) × 100 (Burba *et al*. 1989).

Data Analysis

Allicin content, pyruvate levels and VID were expressed as means and standard deviations (SD) of three replicates per sample. The statistical analysis was done applying one-way analysis of variance followed by Scheffe's Multiple Comparison Test using STATGRAPHICS Centurion XV for Windows.

RESULTS AND DISCUSSION

VID Evolution during Storage at Different Temperatures

As previously mentioned, the observations were made until garlic bulbs reached the 100% VID. Under room temperature storage conditions (20 ± 3C), the experiment lasted 90 days, while at cold storage conditions (0 ± 0.5C) the experiment ended after 120 days. As Fig. 1 shows, VID value at the beginning of the assays was 39% ± 7.2. Comparing the same period of treatment, VID values of bulbs stored at 20C were always higher than bulbs stored at 0C.

In addition, Fig. 1 shows that the increase from 40 to 50% VID occurred in 15 days at 20C and 60 days at 0C. After that, the increase from 50 to 60% VID took in 15 days at 20C and 30 days at 0C. However, the increases from 60 to 100% VID were observed in 30 days under both conditions. Our data indicated that an increase from 40 to 60% VID under cold storage condition was remarkably extended. The rate of sprout development beyond 60% VID was similar under both storage conditions.

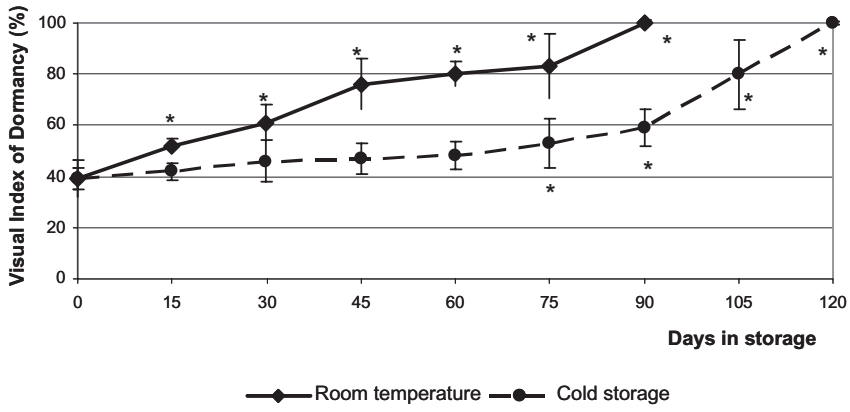

FIG. 1. CHANGES IN VID VALUES DURING STORAGE AT 20C AND 0C
Each value is the mean of three replicate samples ± SD ($N = 3$). Symbols indicate of differences as compared with day 0 (Scheffe's test); *$P < 0.05$.

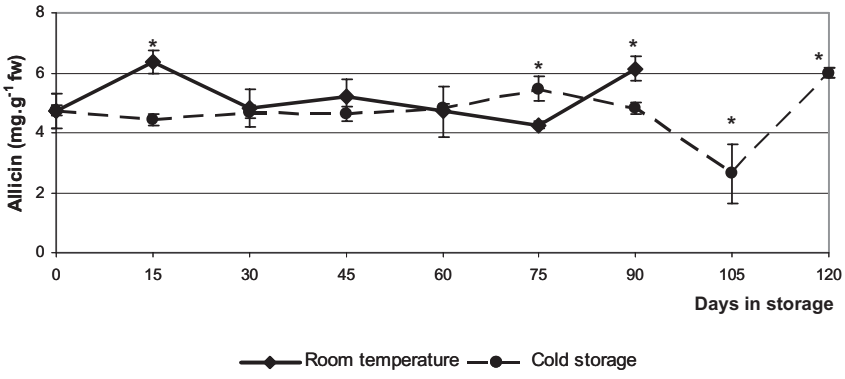

FIG. 2. CHANGES IN ALLICIN LEVELS DURING STORAGE AT 20C AND 0C
Each value is the mean of three replicate samples ± SD ($N = 3$). Symbols indicate of differences as compared with day 0 (Scheffe's test); $*P < 0.05$.

In general, a value of 70% VID is considered the limit for fresh garlic consumption because at this value the bulb quality degradation starts (Portela *et al.* 2005). Garlic bulbs at 0C reached 70% VID 60 days later than garlic at 20C.

Allicin Content during Storage at Different Temperatures

The changes of allicin content in garlic bulbs during storage at 20 ± 3C and 0 ± 0.5C are shown in Fig. 2. Allicin content on day 0 was 4.74 ± 0.58 mg/g fw. During the storage of garlic bulbs at both temperatures and for the period of observation, the allicin content showed significant differences (Scheffe's test, $P < 0.05$). At 20C between 15 and 75 days allicin content decreased 33% (from 6.36 ± 0.38 to 4.26 ± 0.13 mg/g fw), and then increased reaching 6.13 ± 0.4 mg/g fw at a storage of 90 days. At 0C, allicin content decreased during the first 15 days of storage, and thereafter showed a slight and steady increase for 75 days. After that, they fell sharply to 52% (from 5.47 ± 0.39 to 2.64 ± 0.99 mg/g fw) at 105 days.

From the beginning to the end of the assay, an increase of 29% at 20C and 26% at 0C were observed in allicin content, respectively. The lowest allicin content under both conditions was 2.64 ± 0.99 mg/g and the highest 6.36 ± 0.38 mg/g fw, with mean values of 4.69 ± 0.91 mg/g fw at 0C and 5.18 ± 0.78 mg/g fw at 20C, respectively. According to Baghalian *et al.* 2005, the minimum allicin content to ensure pharmaceutical and economic viability of garlic should be 4.5 mg/g fw. This value was observed during all the experiments except when garlic cloves reached 80% VID approximately at both storage conditions.

Allicin and Pyruvate Content versus VID during Storage at Different Temperatures

Regarding VID versus allicin content, Fig. 3 shows that the maximum and minimum allicin levels were reached at similar VID values (50%, 100% VID and 80% VID, respectively). As mentioned previously, garlic and onion bulbs metabolism are active during storage. This fact has been demonstrated by previous works that show decreases in peptides concentration accompanied by increases in sulfoxides levels as result of activity of γ-glutamyl transpeptidase during storage at different temperatures (Kopsell and Randle 1999; Ichikawa *et al.* 2006). Also, significant correlations have been found between ACSOs levels and thiosulfinates content (Sance *et al.* 2008; González *et al.* 2009). These behaviors may explain the increases of allicin levels observed during storage. On the other hand, the lowest allicin levels were remarkably exhibited at 80% VID in both treatments (Fig. 3). This could be

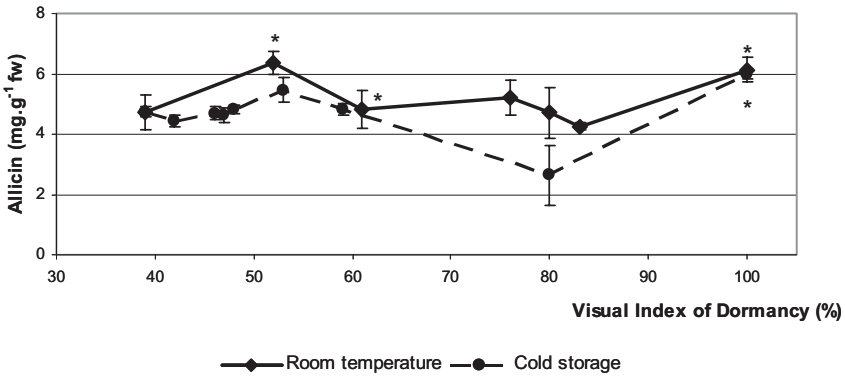

FIG. 3. RELATIONSHIP BETWEEN ALLICIN LEVELS AND VID VALUES DURING STORAGE AT 20C AND 0C
Each value is the mean of three replicate samples ± SD ($N = 3$). Symbols indicate of differences as compared with day 0 (Scheffe's test); $*P < 0.05$.

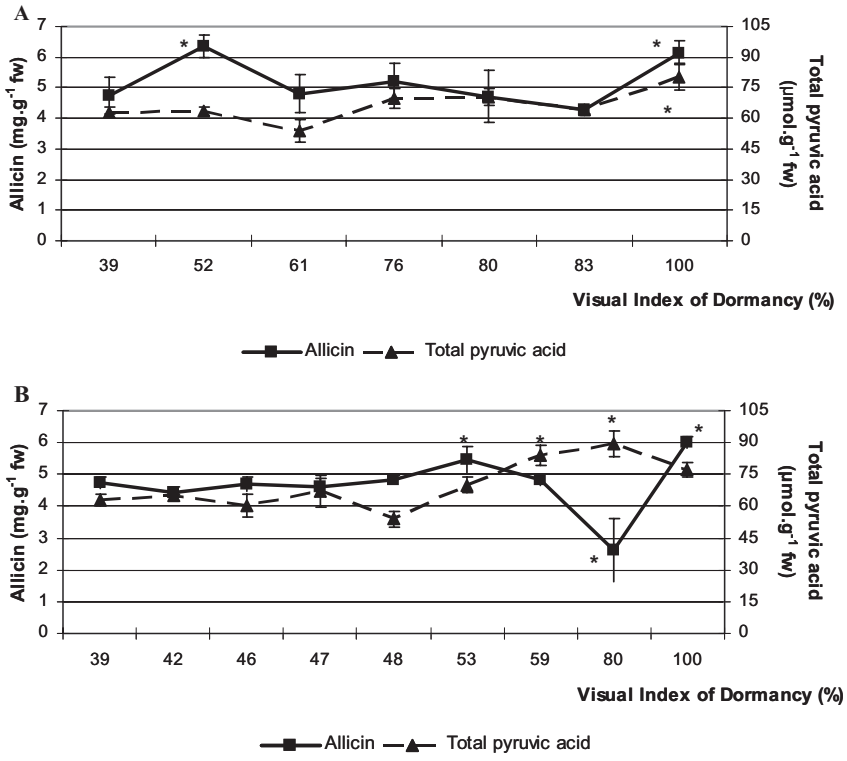

FIG. 4. RELATIONSHIP BETWEEN ALLICIN AND TOTAL PYRUVIC ACID LEVELS, AND VID VALUES DURING POSTHARVEST: (A) STORAGE AT 20C, (B) STORAGE AT 0C Each value is the mean of three replicate samples ± SD ($N = 3$). Symbols indicate of differences as compared with day 0 (Scheffe's test); $^*P < 0.05$.

related to breaking of dormancy. At this point, the sulfoxides might be served as reserve compounds due to an increasing the metabolic-respiratory because of sprout growing. This is consistent with studies reported by Ceci and Curzio (1992) and Ichikawa *et al.* (2006).

Figure 4 shows allicin and pyruvate levels at both temperature treatments. We did not find a significant correlation between them $P \leq 0.05$ ($r = 0.33$). On the other hand, the data of nonenzymatic pyruvate levels during both treatments did not show significant differences at $P \leq 0.05$ (data not shown). Several reports about relationships between pyruvate levels and OSCs content are contradictories. Some authors reported that pyruvate levels have been used as indicator of onion and garlic pungency due to a good correlation between it and organosulfur compounds levels (Goldman *et al.* 1996; Sance *et al.* 2008; González *et al.* 2009). Nevertheless, others authors have mentioned that changes in pyruvate levels during postharvest would be not related with changes in organosulfur compounds levels (Debaene *et al.* 1999; Kopsell and Randle 1999). Our results are in agreement with the last observations, then we considered that pyruvate levels would be not a good indicator for organosulfur content during postharvest storage.

Burba *et al.* (1989), have proposed a classification of Argentinean garlic cultivars based on their requirements for bulbing (low-temperature and day-length) into four ecophysiological groups. Besides, it has been designed a table of equivalence of groups and types of garlic cultivars, which includes the classification used in France, Japan and China (Burba 1997). On the other hand, the VID evolution has been evaluated for Argentinean cultivars. The results showed a response cultivar dependent; however cultivars belonging to the same ecophysiological group have shown a similar behavior. Based on the above mentioned, we could expect that the data herein obtained would be extrapolated to other garlic cultivars. However, future investigations should be done in order to confirm these aspects.

In summary, the data demonstrated that IVD should be taking into consideration as useful tool to estimate allicin level during storage. Respect pungency, the changes in pyruvate levels did not show a relationship with changes in allicin levels during postharvest. Consequently, pyruvate levels were rejected as indicator of bioactive compounds levels. For our experimental conditions, when Sureño INTA reaches 50% and 100% VID, it would be the suitable moment to elaborate pharmacological products with high content of bioactive compounds.

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