RESPONSES OF GARLIC BULBS TO GAMMA IRRADIATION. CHANGES IN MAJOR AMINO ACIDS

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ABSTRACT

Studies were conducted to provide information about the amino acids composition of garlic bulbs cv Colorado and to determinate the effect of a dose of 60 Gy of gamma rays on the behavior of the major free amino acids in relation to sprout growth radioinhibition. TLC and HPLC were used for identification and quantification of free amino acids. Eighteen free amino acids were identified in both parts of garlic bulbs: alanine, glycine, proline, methionine, serine, phenylalanine, aspartic acid, glutamic acid, lysine, arginine, tyrosine, threonine, cystine, cysteine, leucine + isoleucine and asparagine. In the inner sprout the major amino acids founded were: glutamine, glutamic acid, threonine, asparagine, cystine, cysteine and methionine; in the storage leaf also arginine was also predominant. In general, concentration of amino acids appeared to be less affected by irradiation in the storage leaf that in the inner sprout. An increase in the short time post-irradiation in glutamine, glutamic acid, asparagine, threonine and methionine was observed. Sprout growth radioinhibition was evident about 70 days after treatment and was preceded by a decrease in the major amino acids except methionine. It appears that concentration of same major amino acids can be used as monitors of radioinhibition process in inner sprout of garlic.

I. INTRODUCTION

The sprout inhibition of garlic by irradiation shows to be an economically feasible method and can replace chemical sprout inhibitors [1]. This technology has been accepted in several countries as well as in Argentina [2], where irradiation of garlic bulbs is important due to the MERCOSUR since it enables producers and handlers to spread supply, meet peak demands, and transport bulbs over long distances. Methodologies are required to control the processing and trading of irradiated bulbs by governmental organizations and for enhancing consumer confidence [3].

We have reported earlier on chemical changes that take place on cv Colorado garlic induced by gamma irradiation [4]. These include dry matter, acidity, ascorbic acid, soluble carbohydrates, proteins and piruvic acid. Information on amino acids in irradiated garlic is very scanty. It was found in garlic grown in Korea that significant reduction in the level of glutamic and aspartic acids take place immediately after irradiation with 100 Gy of gamma rays while little affected were sulfur containing amino acids [5]. However, the pattern of changes of amino acids in irradiated garlic during long-term storage are lacking. This information is important in order to find out chemical parameters that can be used in quality control, allowing the differentiation between irradiated and non irradiated bulbs during storage.

The aims of the present work were: i.- to provide information about the amino acids composition, both for the inner sprout and the storage leaf from cloves of cv Colorado garlic bulbs. ii.- to follow up the changes that take place in the major free amino acids concentration in garlic clove under the influence of irradiation and long-term storage.

The data indicate that the sprout and storage leaf of garlic present the same amino acids composition but a different behavior during storage. Furthermore, the determination of the effect of gamma irradiation on the concentration of the seven predominant amino acids in
the inner sprout, during different moments in the storage, is likely to contribute to methods of detection of irradiated garlic bulbs.

II. MATERIALS AND METHODS

Plant Material and Treatment. Sound garlic bulbs cv Colorado, harvested in the southwest of the Buenos Aires province, were used for these studies.

Garlic bulbs were treated in the irradiation facilities of IONICS Corp. at 30 days after harvest, in air and 20 ºC with an average dose of 60 Gy using 60 Co gamma rays. The dose rate was 0.4 Gy/s as determined by Fricke dosimetry, and the dose uniformity ratio was 1.1 [6]. Bulbs were stored at 19 ± 1°C and 42 ± 2 % relative humidity. Determinations were made on selected clove samples of uniform size.

Growth Measurement. At selected intervals after irradiation (20, 40, 70, 100 and 130 days), 30 inner sprouts were removed from cloves and weighted individually. The mean fresh weight was used as the criterion for inner sprout growth.

Amino Acids Assay. Analysis of free amino acids were conducted on the inner sprout and the storage leaf separately. One gram of tissue was dropped into 2 mL methanol/chloroform/water 12/5/3 v/v (MCW) in refrigerated bath [7]. The tissue was homogenized in a Ultra-Turrax homogenizer, the homogenate was centrifuged and the supernatant was collected. The residue was reextracted with a further 2 mL MCW. Then the tissue residue was extracted a further 4 times with 2 mL portions of 80 % ethanol. To the MCW extract was added 1 mL chloroform and, then, 1.5 mL water: the resulting two phase mixture was centrifuged and the chloroform layer was descarted while the aqueous layer was added to the ethanol extract. The combined extract was dried under vacuum at 35 º and the dried extract was taken up in water for directly amino acids analysis.

To 0.5 mL of extract, 5% NaHCO3 were added up to alkalinity, 100 µL of 2,4 dinitrofluorobenzene (DNFB) was added and shaken at 40 º for 3h [8]. Excess DNFB was removed by extracting it with 5 x 1 mL of diethyl ether. The remaining aqueous fraction was acidified by 100 µL of 6N HCL and the DNP - amino acids were extracted with diethyl ether until the ether no longer became colored. The ether was evaporated and the residue was taken up in 0.5 mL HPLC grade methanol. Amino acid standards were derivated in the same way.

The chromatograph used was a KONIK KNK 500 equipped with a UV detector. The column was a ODS-Hypersyl (5µm), 200 x 4.6 mm and the DNP- amino acids were eluted in a linear gradient from 20 % to 75 % of acetonitrile in 1% glacial acetic in water at flow rate of 1.2 mL/min. DNP amino acids were detected at 254 nm.

The quantitative determinations were made by external standard method, using peak heights.

III. RESULTS AND DISCUSSION

Free Amino Acids of Garlic Clove. The amino acids identified in garlic bulbs are those common in higher plants. The inner sprout and the storage leaf of garlic clove showed to have identical aminoacids composition during storage. Eighteen free aminocids were identified in both parts: alanine, glycine, proline, methionine, serine, phenylalanine, aspartic acid, glutamic acid, lysine, glutamine, arginine, tyrosine, threonine, cystine, cystéine, leucine + isoleucine, asparagine. Although the presence of histidine, valine and tryptophan in storage bulbs has been reported [5], it was not observed by our method. Gutamine, glutamic acid, threonine, asparagine, cystine, cystéine and methionine were the seven major free amino acids detected in inner sprout tissues. Considerable amounts of arginine were detected in storage leaf tissues as well. Glutamic acid and its amide glutamine concerning the glutamato family are the primary products of inorganic nitrogen assimilation, as such, they occupy central positions in the intermediary nitrogen metabolism [9]. Threonine and asparagine belong to aspartate family. The latter has long been recognized as an important compound in nitrogen transport [10]. It is known that arginine is accumulated in storage tissues [9], probably as a consequence of their comparative advantages as carrier of organic nitrogens. On this basis the abundance of arginine in storage leaf of garlic can be explained.

The fact that among predominant garlic amino acids there are organosulfur compounds is not surprising since the distinctive flavor characteristics of tissues of plants from the genus Allium are conferred by an array of organosulfur compounds generated from non-protein sulfur amino acids precursors [11].

Effect of Gamma Rays on Growth to the Inner Sprout. The growth pattern of the inner sprout of garlic clove after treatment with 60 Gy dose of gamma rays in dormancy is shown in Fig. 1. On the fresh weight basis the growth pattern of irradiated and control sprouts was identical throughout the first 40 days, but on the 70 days the pattern changed: in the control sprout the fresh weight increased notably whereas in the irradiated sprout a slight increase was detected. The inhibition of growth observed after 40 th day agrees with the fact that under sown conditions the irradiated garlic seed cloves show lack of capacity to reach the seedling stage [12] and that in apical meristems and leaf primordia gamma radiation inhibited cell division [4].
Effects of gamma irradiation on behavior of major amino acids of inner sprout. The effects of radiation on the major amino acids differ in the two parts of garlic cloves studied and also vary with the post-irradiation time considered. In general, the levels of amino acids appeared to be less affected by treatment in the storage leaf (data not shown) that in the inner sprout. The results obtained with sprout tissue are given in Fig. 2 to 8.
Data showed that in a short time (20 days) after a 60 Gy dose of gamma irradiation there was a sharp increase in the concentration of glutamine, glutamic acid, asparagine and threonine; methionine showed only a slight increase. Furthermore, a decrease was observed in cystine concentration. When sprout tissues were studied 40 days after irradiation it was found that glutamine, glutamic acid, asparagine, threonine and cystine definitely decreased when compared with control. The concentration of cysteine remained unchanged, while a remarkable increase was detected in methionine.

70 days after irradiation no remarkable changes were observed in the concentration of glutamic acid, asparagine, threonine and methionine. A moderate decrease was detected in glutamine, cystine and cysteine.

After 100 days of gamma irradiation the concentration of glutamic acid showed a remarkable increase while the concentration of cystine was lower when compared with control. Levels of glutamine, asparagine, threonine, methionine and cystine remained practically unchanged.

In relation to the long term effects variations in the level of amino acids subsequent to irradiation have been reported for different tissues of several species [14]. These observations have not been pursued within the context of growth parameters. Our experimental evidence clearly shows that growth inhibition in the sprout of garlic is reflected in decreased fresh weight as of 70 days later, which is preceded by a decrease in major amino acids except methionine. The specific mechanism of these effects has not clearly defined. It would seem that interrelated non specific factors are involved in increased amino acid uptake [14] and promotion of the scavenger production [1].

It has been reported that tissues from potatoes irradiated at sprout inhibition dose are at a transient, higher level of metabolism, which is required for the recovery of the tissue from radiation damage [13]. The fact that gamma ray treatment on inner sprout tissue produced an increase in the short time in aminoacids belonging to glutamate and aspartate families supports this statement.

The observed changes in the major amino acid concentration in sprout of garlic cloves can be used for detection of irradiated garlic bulbs as a complement of existing methods based on chemical changes in nucleic acids [15]. On the other hand, it would be appropriate to examine the applicability of this technique to distinguish between irradiated and chemical sprout inhibited garlic.

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REFERENCES


