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Postharvest Biology and Technology 44 (2007) 122–130
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Growth inhibition by gamma rays affects lipids and fatty acids in garlic sprouts during storage

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Received 5 October 2005; accepted 19 August 2006

Abstract

Bulbs of cv. Colorado garlic were irradiated at dormancy with a dose of 60 Gy of gamma rays and stored for 8 months, during which period the content and fatty acid composition of phospholipids (PL), glycolipids (GL) and neutral lipids were analyzed on three occasions. No significant changes were observed a few hours after irradiation, but the treatment resulted in a considerable reduction in lipid and fatty acid content 150 and 240 days post-harvest, with a concomitant reduction in the process of sprout growth. In total lipid, all fatty acids including the major linoleic acid (18:2) decreased, the largest decrease being in linolenic acid (18:3). The latter was a relatively minor component of PL (phosphatidylcholine and -ethanolamine) and a major acyl group of GL (monogalactosyl- and digalactosylglycerol). Radioinhibition had the opposite effect on polyunsaturated fatty acids of PL and GL, the 18:3/18:2 ratio decreasing in the former and increasing in the latter. Accretion of lipids and fatty acids is a normal biosynthetic process accompanying sprout growth, and the long-term effects of irradiation are interpreted to reflect a delay or slowing down of such process.

Keywords: *Allium sativum*; Garlic; Gamma irradiation; Sprouting inhibition; Lipids; Fatty acids; Linolenic acid

1. Introduction

^{60}Co gamma rays are widely used in food irradiation, and for several years now extensive research has been carried out on the structural and biochemical changes brought about by irradiation of plant cells and plant-derived foods (Kovács and Keresztes, 2002). When ionizing radiation is absorbed in biological materials, it acts directly on critical cell targets or indirectly through the generation of metabolites that can modify important cell components.

Low doses of gamma irradiation have been used to advantage in order to control the degree of ripeness and extend the shelf life of fruits and vegetables. In our laboratory, these low doses have been successfully used to inhibit sprouting and to extend the shelf life of cv. Colorado garlic bulbs (Croci and Curzio, 1983; Croci, 1988), as has also been achieved with onions and other alliums (Fenwick and Hanley, 1990; Croci et al., 1995). We have shown that gamma rays affect several chemical components of the sprout of cv. Colorado garlic, including growth regulators, total DNA, RNA, proteins, soluble carbohydrates and lipids (Croci et al., 1990, 1994; Pérez et al., 1998). More recently, we also reported histological and anatomical changes in garlic sprouts associated with gamma irradiation of the bulbs followed by storage under controlled conditions (Orioli et al., 2004).

A mature garlic bulb is made up of bulblets, commonly known as cloves, which develop from axillary buds of the younger foliage leaf (Rahim and Fordham, 1988). At harvest, each clove is considered a dormant bud whose edible part is composed of a differentiated fleshy leaf (the storage leaf), protecting the inner sprout. The latter is conformed by the sprout leaf containing an apical meristem encircled by three to four functional leaf primordia (Shah and Kothari, 1973).

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0925-5214/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.postharvbio.2006.08.018

During post-harvest storage, through a process regulated by hormonal factors (Argüello et al., 1991), the clove slowly comes out from dormancy and the sprout begins to grow, entering the sprouting stage. In the following stage the sprout begins to emerge from the clove. The duration of each of these stages differs according to the garlic cultivar (Burba, 1993). The developmental stage of tissue at the moment of irradiation is relevant in determining its radio-sensitivity (Gunkel and Sparrow, 1961), and since garlic bulbs are constituted by tissues at distinct physiological stages of differentiation, the possible effects of irradiation should also be studied later on, during the several months of post-harvest storage.

Rather than to radiolysis, the effects of low doses of radiation on living plant tissues have been ascribed to physiological and biochemical changes induced concomitantly with the phenomenon of growth arrest, changes which do not affect the safety of the product as a food (Diehl, 1990). The biochemical changes associated with growth inhibition processes in irradiated plant tissues have not yet been fully elucidated. In particular, the effects of ionizing radiation on the lipids of irradiated garlic have received relatively little attention in the literature. Kwon et al. (1988) concluded from their studies on a Korean garlic cultivar that immediately after gamma irradiation with 100 Gy there are no differences in the levels of linoleic, palmitic, oleic and linolenic acids, the predominant fatty acids of bulbs. However, a low, sprouting inhibitory dose of irradiation like this may have effects on lipids and fatty acids that become apparent several months afterwards (Pérez et al., 1998). The objective of the present work was to study the effects of an acute dose of 60 Gy gamma rays, applied to bulbs of cv. Colorado garlic at dormancy on the lipid and fatty acid composition of the sprout at different stages of the long period of post-harvest storage prior to commercialization.

2. Experimental

2.1. Plant material and irradiation process

Sound garlic (*Allium sativum* L.) cv. Colorado bulbs grown in the south-west of the Province of Buenos Aires, Argentina, were harvested manually in mid December and sun-cured in the field during 10 days. Bulbs were irradiated at facilities of the Comisión Nacional de Energía Atómica (CNEA), Argentina. Bulbs of uniform size (4–5 cm diameter) were irradiated at day 30 post-harvest, in air at 20 °C, with a dose of 60.0 Gy using ^{60}Co γ-rays. The dose-rate was 0.4 Gy/s as determined by Fricke dosimetry, and the dose uniformity ratio was 1.1. Bulbs were stored in a commercial warehouse at 19 ± 1 °C and 42 ± 2% relative humidity in dim light (23 μmol m^{-2} s^{-1}) for a total period of 8 months.

At the times specified in Section 3, cloves of uniform size (2.0 ± 0.2 cm) were obtained from the bulbs, and cut longitudinally using a scalpel to obtain the sprouts.

2.2. Sprout growth

Inner sprout growth was measured using the dormancy overcoming visual index (DVI) (Burba, 1993), which is the percentage ratio between the total length of the sprout and that of the storage leaf, measured in clove longitudinal cuts. These measurements were carried out every 30 days post-harvest on three samples each with 10 sprouts.

2.3. Lipid analysis

Three replicas of 1 g each were used for each time and treatment. Lipids were extracted from each replica using mixtures of $CHCl_3$–MeOH (2:1, v/v) (Folch et al., 1957). Fractions containing PL, GL and neutral lipids (NL), were separated by TLC on plates of silica gel G, running Me_2CO up to the middle of the plates twice, and Et_2O up to the top of the plates once. The position of the lipid fractions was determined with the help of standards and the lipids were then eluted from the silica support and separated into different classes.

The PL were resolved into classes on commercial TLC plates using $CHCl_3$–MeOH–HOAc–H_2O (50:37.5:3.5:2, v/v/v/v) (Holub and Skeaff, 1987). GL were resolved using $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (50:20:10:10:5, v/v/v/v/v) (Lepage, 1967) to separate MGDG and DGDG. The NL fraction was resolved using hexane–Et_2O–HOAc (80:20:1, v/v/v) to obtain triacylglycerols (TG) and free sterols (FS). All lipids under study were eluted from the silica support with $CHCl_3$–MeOH–HOAc–H_2O (50:39:1:10, v/v/v/v) (Arvidson, 1968).

PL and GL were quantified by measuring lipid phosphorus (Rouser et al., 1970), and galactose (Lepage, 1967), respectively. TG and FS were determined using commercial kits used for the determination of these lipids in clinical settings (Boehringer Mannheim Gmbh, Mannheim, Germany), by measuring the glycerol released after enzymatic hydrolysis from TG and cholesterol as standard, respectively.

Fatty acid methyl esters (FAME) were prepared from aliquots of the total lipid and from major lipid classes by heating the lipid samples in 14% BF_3 in MeOH (Morrison and Smith, 1964) at 45 °C overnight in screw cap tubes under N_2. The resulting FAME were recovered and purified by TLC using hexane–Et_2O (95:5, v/v), on plates of silica gel G that had been previously washed with MeOH–Et_2O (75:25, v/v). FAME were recovered from the silica support by vigorous agitation with H_2O–MeOH–hexane (1:1:1, v/v/v), followed by centrifugation, repeating the hexane extraction three times.

Fatty acid analysis was performed using a Varian 3700 gas chromatograph equipped with two (2 m × 2 mm) glass columns packed with 15% SP 2330 on Chromosorb WAW 100/120 (Supelco Inc., Bellefonte, PA) and two flame ionization detectors. The column oven temperature was programmed from 155 to 230 °C at a rate of 5 °C min^{-1}. Injector and detector temperatures were 220 and 230 °C, respectively,

and N_2 (30 mL/min) was the carrier gas. The fatty acids were identified with the aid of commercial standards.

2.4. Statistical analysis

Two-way analysis of variance (ANOVA) was carried out to determine the effects of treatment (irradiation) and storage time (Zar, 1999). The differences between the means were compared using least significant difference (LSD 5%) values.

3. Results

3.1. Length, shape and lipid content of sprouts

Two months after being harvested and stored under controlled conditions, the sprouts within the garlic bulbs started to grow. The rate of growth, expressed as the dormancy overcoming visual index (DVI), showed a significant increase ($p < 0.05$) between 60 and 180 days, exceeding the 100% value towards the end of this period, and then reaching a plateau around this maximum value for the duration of the next 2-month interval (Fig. 1). From the results based on sprout length it can be established that the dormancy, sprouting, and emergence periods corresponded to 30–60, 60–180, and above 180 days post-harvest, respectively. Most of the changes in length occurred at a significantly slower rate in sprouts from bulbs that had been previously subjected to irradiation ($p < 0.05$). The radioinhibited sprouts showed a marked growth arrest, their DVI values reaching at maximum 60% of that of non-irradiated sprouts at 180 days.

In addition to the differences in length, after being stored for 240 days the control and radioinhibited sprout samples showed obvious differences in their macroscopic characteristics (Fig. 2). Whereas the former had already acquired a clearly apparent greenish tint, the latter showed a brownish discoloration coinciding with a smaller number of leaflets and overall diminished turgor of the sprout.

The concentrations (g/kg fresh weight) of the main lipid classes of control and radioinhibited sprouts at the dormancy,

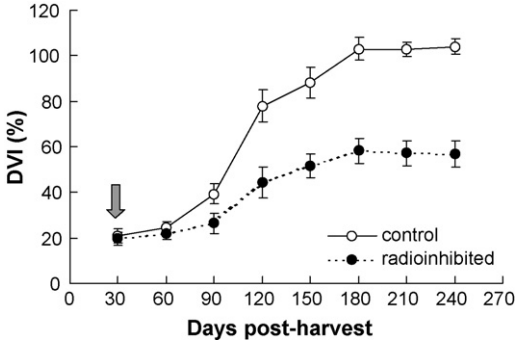

Fig. 1. Effect of garlic bulb irradiation and storage on the dormancy overcoming visual index (DVI). Each point represents mean value ± S.D. Treatment × days of storage $p < 0.05$. LSD: 3.77. The arrow points to the day on which the samples were irradiated.

Fig. 2. Long-term effects of irradiation. Comparison of sprouts obtained from a non-irradiated (A) and an irradiated (B) garlic bulb, 240 days after harvest.

sprouting and emergence stages of the post-harvest period under study are shown in Figs. 3 and 4. In control sprouts, at 30 days the major single lipid class was TG (Fig. 4), whereas at 150 and 240 days phosphatidylcholine (PC) predominated (Fig. 3). Furthermore, the period between 150 and 240 days was characterized by a clear enrichment in PC and TG as well as GL (Fig. 3).

The individual PL classes of control sprouts showed differential patterns of change during the storage of bulbs (Fig. 3). Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) content increased steadily during the 30–240 days of storage to a total of 2.6- and 2.1-fold for PC and PE, respectively. Diphosphatidylglycerol, phosphatidylserine (not shown) and phosphatidylinositol (PI) did not increase significantly from one stage to the next during the whole period ($p > 0.05$). Interestingly, sprout phosphatidylglycerol (PG) was the lipid class that increased most (2.6-fold) in the 30–150-day period and the only one to decrease in the 150–240-day period, to a value similar to the initial one (Fig. 3). GL content per gram of control sprout increased 2.7-fold during the 30–240-day period in the case of monogalactosyldiacylglycerol (MGDG) and 3.6-fold in the case of digalactosyldiacylglycerol (DGDG). These galactolipids also showed differential rates of accumulation with time of bulb storage, DGDG increased more in the 30–150-day period than in the 150–240-day period and MGDG behaving in the opposite manner. The incremental pattern of FS content (Fig. 4) resembled that of the major phospholipid, PC, whereas TG content (Fig. 4) remained similar during dormancy and sprouting, increasing significantly ($p < 0.05$) in the emergence phase.

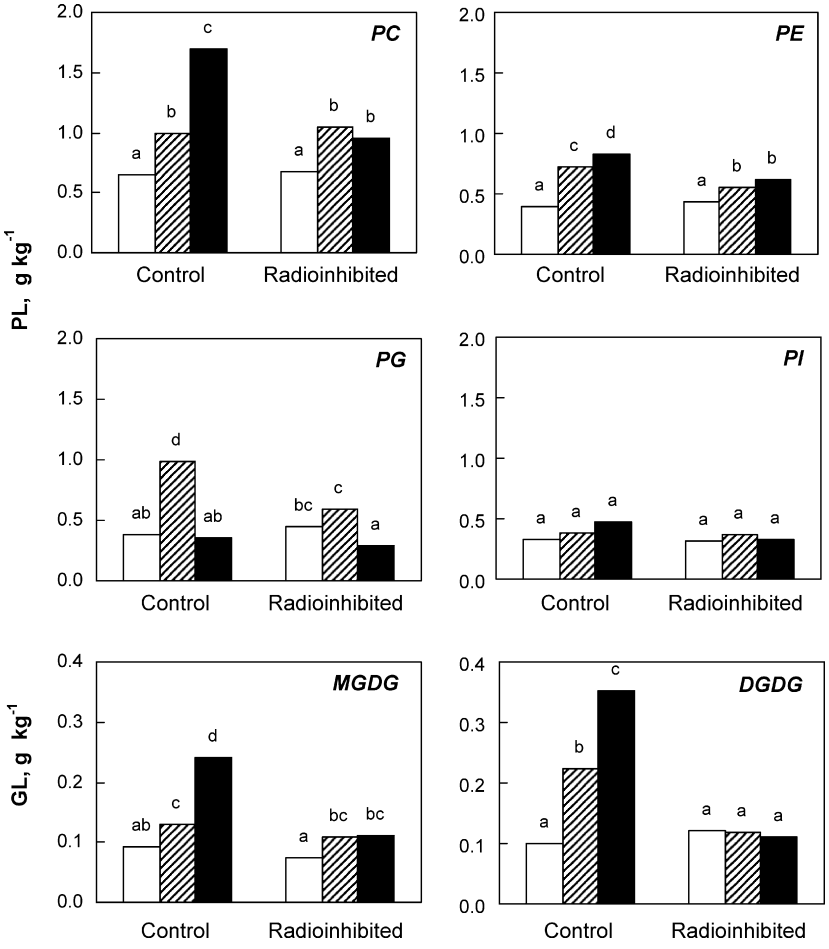

Fig. 3. Polar lipid content of garlic sprouts at different times during storage: 30, 150 and 240 days post-harvest (white bars, hatched bars and black bars, respectively). Results are expressed as mean values of three determinations. Different letters indicate significant differences at $p < 0.05$. For PC, PE, PG, MGDG and DGDG there was an interaction between treatment and days ($p < 0.05$).

In the sprouts of irradiated bulbs most of the lipid changes described above for control sprouts were significantly slower ($p < 0.05$). Thus, significantly less accumulation of most lipids occurred in the 30–150-day period in the radioinhibited sprouts, even though the pattern of accumulation was similar, with PC, PE, MGDG and FS content still increasing in this phase (Figs. 3 and 4). The most marked effect of irradiation on sprout lipids was during the 150–240-day period, when accumulation of PL, GL, TG and FS was almost completely blocked.

3.2. Total fatty acids

The major fatty acids of total lipid extracted from garlic sprouts are shown in Table 1. The sum of the fatty acids depicted in this and the following tables (Tables 2 and 3)

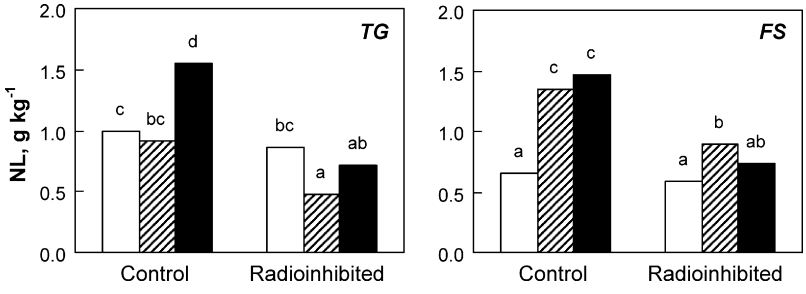

Fig. 4. Content of neutral lipids (NL) in garlic sprouts at different times during storage: 30, 150 and 240 days post-harvest (white bars, hatched bars and black bars, respectively). Results are expressed as mean values of three determinations. Different letters indicate significant differences at $p < 0.05$. There was an interaction between TG and FS between treatment and days ($p < 0.05$).

Table 1
Effects of irradiation and storage on major fatty acids of total lipid in garlic bulb sprouts

	Days post-harvest					
	Control			Radioinhibited		
	30	150	240	30	150	240
16:0*	24.24 ± 0.7 c	21.31 ± 0.3 b	19.86 ± 0.5 a	23.52 ± 0.5 c	23.48 ± 0.2 c	21.23 ± 0.1 b
16:1	1.29 ± 0.3 c	1.05 ± 0.1 ab	0.87 ± 0.1 a	1.21 ± 0.1 bc	1.03 ± 0.0 ab	0.90 ± 0.0 a
18:0	1.43 ± 0.1 d	0.49 ± 0.0 a	0.41 ± 0.0 a	1.63 ± 0.1 e	0.73 ± 0.0 c	0.62 ± 0.0 b
18:1	4.81 ± 0.2 a	6.34 ± 0.2 c	6.47 ± 0.5 c	4.42 ± 0.3 a	6.44 ± 0.2 c	5.62 ± 0.3 b
18:2*	63.73 ± 0.3 d	61.09 ± 0.2 b	59.70 ± 0.6 a	64.51 ± 0.4 e	60.21 ± 0.3 a	62.18 ± 0.4 c
18:3*	4.50 ± 0.3 a	9.73 ± 0.4 c	12.70 ± 0.5 d	4.81 ± 0.1 a	8.12 ± 0.1 b	9.44 ± 0.1 c

Untreated bulbs and bulbs irradiated at day 30 (60 Gy) were stored for the indicated periods after harvest. The sprouts were obtained from bulbs at the indicated times and the total lipid was extracted. Fatty acid methyl esters were prepared and analyzed by GC. Results are percentages (wt.%) and are expressed as mean values ± S.D. ($n = 3$). Different letters indicate significant differences at $p < 0.05$. An asterisk indicates the fatty acids for which there was a significant interaction between treatment and storage days ($p < 0.05$). Means followed by the same letter were not significantly different.

Table 2
Effects of irradiation and storage on major fatty acids of the two major glycerophospholipids in garlic bulb sprouts

	Days post-harvest					
	Control			Radioinhibited		
	30	150	240	30	150	240
PC						
16:0	28.50 ± 2.5 b	22.47 ± 1.2 a	21.73 ± 0.5 a	29.83 ± 2.0 b	21.9 ± 1.7 a	19.97 ± 1.6 a
16:1	0.90 ± 0.1 a	0.93 ± 0.1 a	0.77 ± 0.1 a	1.00 ± 0.2 a	1.00 ± 0.3 a	0.77 ± 0.1 a
18:0*	0.87 ± 0.1 cd	0.40 ± 0.1 a	0.47 ± 0.1 a	0.97 ± 0.1 d	0.77 ± 0.1 c	0.60 ± 0.1 b
18:1*	7.70 ± 0.9 bc	7.83 ± 0.1 c	7.67 ± 0.7 bc	6.33 ± 0.5 a	9.10 ± 0.3 d	6.77 ± 0.6 ab
18:2*	60.03 ± 1.5 ab	63.03 ± 0.9 c	62.40 ± 0.7 bc	59.60 ± 1.7 a	64.07 ± 1.9 c	68.00 ± 0.9 d
18:3*	2.10 ± 0.2 a	5.33 ± 0.1 d	6.97 ± 0.2 e	2.27 ± 0.1 a	3.17 ± 0.3 b	3.97 ± 0.2 c
PE						
16:0	32.97 ± 1.5 c	28.23 ± 1.3 b	25.77 ± 2.0 a	32.33 ± 0.5 c	27.20 ± 1.4 ab	28.40 ± 0.9 b
16:1	2.60 ± 0.9 b	1.73 ± 0.5 ab	1.47 ± 0.3 a	2.13 ± 0.6 ab	1.53 ± 0.5 a	1.17 ± 0.4 a
18:0	3.23 ± 0.7 c	1.63 ± 0.4 a	1.77 ± 0.6 ab	2.47 ± 0.4 bc	1.60 ± 0.1 a	1.53 ± 0.1 a
18:1	8.23 ± 1.5 c	6.03 ± 0.5 ab	7.10 ± 0.6 bc	6.53 ± 1.4 abc	5.97 ± 0.7 ab	5.03 ± 0.5 a
18:2	51.53 ± 2.8 a	57.60 ± 0.6 bc	58.43 ± 1.5 bc	54.70 ± 2.6 ab	60.50 ± 2.7 c	60.90 ± 1.6 c
18:3*	1.43 ± 0.2 a	4.73 ± 0.5 c	5.50 ± 0.5 d	1.83 ± 0.1 a	3.23 ± 0.1 b	2.93 ± 0.2 b

Details as in Table 1.

Table 3
Effects of irradiation and storage on major fatty acids of the two major glyceroglycolipids in garlic bulb sprouts

	Days post-harvest					
	Control			Radioinhibited		
	30	150	240	30	150	240
MGDG						
16:0*	21.75 ± 1.1 c	6.43 ± 1.9 ab	4.43 ± 0.9 a	19.41 ± 2.1 c	6.47 ± 1.8 ab	7.87 ± 2.5 b
16:1	9.50 ± 0.7 c	2.97 ± 1.0 b	1.60 ± 0.2 a	9.29 ± 1.0 c	2.63 ± 0.8 ab	2.27 ± 0.2 ab
18:0	8.11 ± 0.8 c	2.97 ± 0.8 b	1.30 ± 0.7 a	6.92 ± 0.9 c	1.43 ± 0.3 a	1.27 ± 0.3 a
18:1	11.12 ± 1.0 c	7.37 ± 0.9 b	5.07 ± 0.5 a	8.51 ± 1.4 b	5.47 ± 0.3 a	4.63 ± 0.5 a
18:2*	34.70 ± 2.1 c	28.57 ± 1.1 b	42.73 ± 1.3 d	32.79 ± 2.9 c	25.47 ± 0.5 a	29.13 ± 1.5 b
18:3	14.85 ± 0.2 a	51.67 ± 3.6 d	44.90 ± 0.9 c	23.14 ± 3.2 b	58.43 ± 3.3 e	54.83 ± 1.1 de
DGDG						
16:0	27.13 ± 5.1 b	17.90 ± 2.4 a	13.64 ± 3.4 a	24.90 ± 2.3 b	16.13 ± 1.9 a	13.18 ± 2.3 a
16:1	5.90 ± 1.3 bc	2.97 ± 0.7 ab	1.97 ± 0.6 a	8.50 ± 4.1 c	2.37 ± 0.6 a	2.10 ± 0.6 a
18:0	10.50 ± 3.1 b	3.20 ± 0.6 a	3.15 ± 3.2 a	5.70 ± 0.3 a	2.20 ± 0.2 a	3.63 ± 2.6 a
18:1	10.90 ± 1.6 c	9.23 ± 1.7 bc	7.95 ± 0.5 ab	8.75 ± 1.2 b	6.43 ± 0.5 a	6.32 ± 0.1 a
18:2	29.80 ± 2.9 bc	32.20 ± 3.4 cd	36.19 ± 1.6 d	25.80 ± 4.2 b	22.13 ± 1.7 a	24.92 ± 2.2 ab
18:3	17.40 ± 4.7 a	34.53 ± 1.9 c	37.09 ± 1.2 c	26.35 ± 3.0 b	50.70 ± 4.1 d	49.76 ± 2.8 d

Details as in Tables 1 and 2.

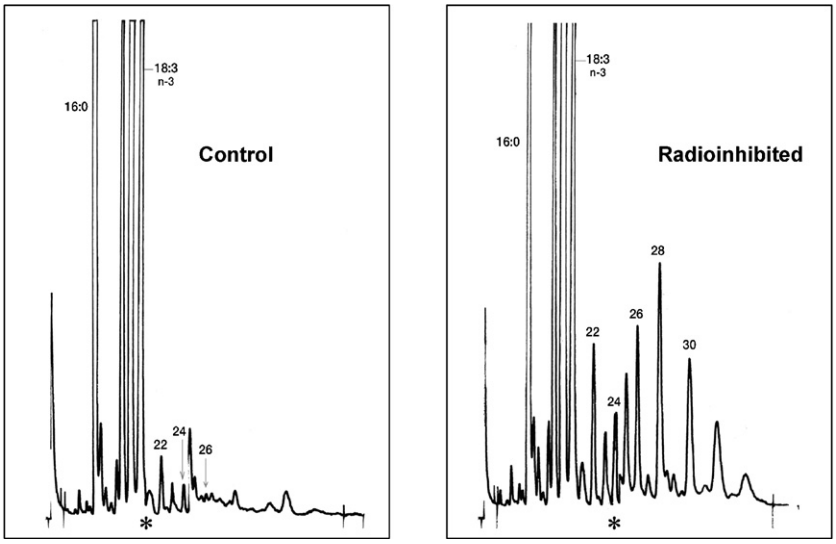

Fig. 5. Fatty acid profiles of total lipid in sprouts obtained after 240 days of storage from bulbs untreated and irradiated at day 30 post-harvest (60 Gy). The asterisks denote a 10-fold increase in detector sensitivity.

represent more than 90 ± 4% of the fatty acids detected, all other constituents presenting individual percentages of less than 0.5%. Palmitic acid (16:0) was the main saturated fatty acid in sprouts and linoleic acid (18:2) the main polyunsaturated fatty acid, the latter being almost three-fold more abundant than 16:0 and constituting the predominant fatty acid throughout the period analyzed. The analysis of variance carried out on the percentage of total lipid fatty acids showed a significant interaction between treatment and storage time ($p < 0.05$) only for 16:0, 18:2 and 18:3 (Table 1). A percentage decrease ($p < 0.05$) was observed for 16:0 and 18:2 in terms of storage time and an increase in terms of irradiation treatment ($p < 0.05$). Unlike the major fatty acid constituents (16:0 and 18:2), the percentage of 18:3 increased during storage ($p < 0.05$), whereas it decreased as a result of irradiation treatment ($p < 0.05$) (Table 1).

A second effect of irradiation on the fatty acids of total lipid in sprouts was an increase in the amount of a small group of long chain (C20–24) and very long chain (>C24) fatty acid constituents (Fig. 5) at days 150 and 240, with no significant differences between the values on these 2 days. The sums of long-chain and very long chain constituents represented 1.7 ± 0.1% and less than 0.5% of total fatty acids, respectively, in controls, and 3.5 ± 0.2% and 1.6 ± 0.2% of total fatty acids, respectively, in radioinhibited sprouts. Most of these fatty acids were saturated constituents, as ascertained by TLC on $AgNO_3$-impregnated plates, but the lipid class or classes to which they belong have not yet been identified.

3.3. Fatty acids of major lipid classes

Tables 2 and 3 show the fatty acid composition of two major PL and two major GL classes, respectively. Of the lipids studied, PL (Table 2) and TG (not shown) were the richest in 18:2 and GL the richest in 18:3 (Table 3). Since PL are the major lipid constituents (Figs. 3 and 4), the data in Tables 1 and 2 indicate the strong influence of 18:2-rich PL on the fatty acid composition of the total lipid.

The analysis of variance carried out on PC fatty acids showed significant interaction ($p < 0.05$) between treatment and storage time only for 18:0, 18:1, 18:2 and 18:3 (Table 2). The percentage of these 18-carbon fatty acids varied during post harvest ($p < 0.05$), 18:3 being the most affected. They were significantly but differently affected by irradiation, with the exception of 18:1, for which no significant effect was found as a result of treatment. Of the PE fatty acids analyzed, a significant interaction ($p < 0.05$) between treatment and storage time was only evident in for 18:3 (Table 2). As in the case of PC, this was the fatty acid most affected by storage time, increasing during post harvest ($p < 0.05$). 18:3 also reacted to treatment, showing a significant diminution.

The statistical analysis of the percentage values of MGDG and DGDG fatty acids showed significant interaction between treatment and number of storage days only for 16:0 and 18:2 in the case of the former ($p < 0.05$), no significant interaction being found for the fatty acids present in the latter ($p > 0.05$). In general, storage time affected ($p < 0.05$) the percentage of fatty acids in both GL, mainly 18:2 and 18:3. The 18-carbon fatty acids present in MGDG and DGDG were also affected by irradiation ($p < 0.05$).

The 18:3/18:2 ratios (Fig. 6) show that irradiation had opposite effects on the main two polyunsaturated fatty acids of the two major lipid constituents, GL and PL. In radioinhibited sprouts, the 18:3/18:2 ratio in PL increased continuously, but significantly less than in controls ($p < 0.05$), whereas in GL the ratio showed a bimodal pattern of change with time, but at all times it was significantly larger ($p < 0.05$) than in controls.

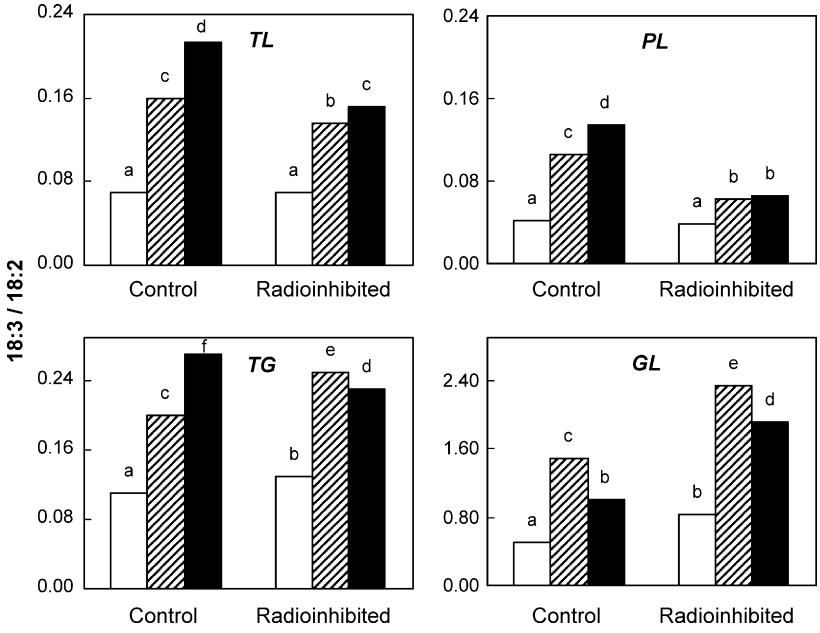

Fig. 6. Linolenic/linoleic acid ratio in phospholipids (PL) and galactolipids (GL) from control and irradiated sprouts (untreated and treated with 60 Gy at day 30, respectively) at different times during storage: 30, 150 and 240 days post-harvest (white bars, hatched bars and black bars, respectively). The ratio in total lipids (TL) and triacylglycerols (TG) is included for comparison. Results are expressed as mean values of three determinations. Different letters indicate significant differences at $p < 0.05$. In all cases (TL, PL, TG and GL) there was an interaction between treatment and storage days ($p < 0.05$).

4. Discussion

The present results show that a series of significant compositional changes takes place in the lipids of garlic sprouts over a storage period of several months, such changes being concomitant with the sprout growth within the cloves after dormancy. Previous studies have demonstrated that such growth is the result of two processes: (a) an increase in the number of cells as a result of normal mitotic activity and (b) an increase in the cell volume of the pre-formed tissues (Orioli et al., 2004). These processes eventually lead to sprouting and emergence, most likely supported by the significant energy reserves available within the clove (Argüello et al., 1991). An acute 60 Gy dose of gamma rays applied to garlic bulbs during dormancy resulted in a significant inhibition of sprout growth accompanied by considerable modifications in the concentration and fatty acid composition of the major lipid classes in the sprout. The main changes in lipids and fatty acids became manifest several months after the application of radiation, and were thus long-term effects. In the short-term (a few hours after irradiation), as seen here in the samples obtained 30 days post-harvest, most differences between control and radioinhibited sprouts were insignificant in the lipids under study. For this post-harvest stage, our results are in agreement with those of Kwon et al. (1988) in garlic and those of Hayashi et al. (1992) in potato, the cited authors observing a virtual lack of radioinhibition effects on main lipids and fatty acids after applying low doses of gamma rays. In the case of garlic, this may be explained not only by the low dose of radiation applied, but also by the considerable number of sulphur-containing molecules present in the bulbs (Lancaster and Boland, 1990), which could function as radioprotective compounds.

The low radiation dose used could have produced its long-term effects in part by means of the stimulation of lipid degradation, possibly mediated through the action of free radicals that are known to be generated after irradiation (Katsaras et al., 1986; Voisine et al., 1991). In plant tissues subject to different forms of stress, polar lipids are degraded to generate free fatty acids and diacylglycerols, resulting in an eventual accumulation of TG as a defense mechanism (Olsson, 1995; Navari-Izzo et al., 1990). In this connection, in sprouts from garlic samples 240 days after being irradiated at day 30, we have observed an 8.3-fold higher free fatty acid content than in controls (67.4 and 8.1 mg/kg fresh weight, respectively) and other lipid metabolites not yet fully identified (some of which could be diacylglycerols, unpublished work). Some of these substances could be the ones containing the long and very long saturated fatty acids shown in Fig. 5. The characterization of these lipid-related compounds may help discern the possible mechanisms involved in the long-term effects of gamma irradiation and their relative importance. However, most of the present results, rather than a stimulation of lipid degradation, seem to reflect a delay or a slowing down of the normal process of lipid synthesis that accompanies growth.

Sprout growth in non-irradiated samples was concomitant with an accretion of PL and GL rich in polyunsaturated fatty acids, 18:2 and 18:3, most specifically the latter, which behaved as a marker of growth. Radioinhibition of growth, manifested in a marked decrease in sprout length at day 150 and subsequent periods, was paralleled by a slower rate of formation of both these membrane-associated lipids as well

as of their constituent fatty acids. At day 240 post-harvest, the radioinhibited sprouts contained just two-thirds of the PL and one-half of the GL gained by their non-radioinhibited counterparts. On a weight basis, the most affected lipid class was the major PC, but on a relative basis, the lipids that decreased most were the GL. Since both lipids are membrane constituents, these results suggest that there were in general less membrane structures per gram in irradiated than in non-irradiated samples, in agreement with previous histological observations showing that sprouts from irradiated bulbs present cells of significantly larger size, and hence there is a lower number of cells in a given volume (Croci, 1988).

The different rates of PL and GL accretion during growth in non-irradiated samples, as well as the acquisition of a greenish color during the final stages of storage (Fig. 2, in agreement with Croci et al., 1987) suggest that, after dormancy, fully functional plastids may start to develop within sprout tissue (non-green plastids becoming chloroplasts). Though information on the plastidial and extraplastidial membranes of garlic parts and their lipids is very scanty, it is known that membranes of all plastid types in plants differ from other plant cell membranes (Joyard et al., 1998) in that they contain large amounts of GL (MGDG and DGDG), sulphogalactolipid, and a few PL, of which the major one is PG. In addition, a small amount of PC is present only in the cytosolic leaflet of the outer envelope and PE is negligible in purified plastids (Douce and Joyard, 1996). If this were the case in garlic sprouts, the present results suggest that extraplastidic (mostly PC and PE) as well as plastidic (MGDG, DGDG and PG) lipids were actively synthesized during storage in non-irradiated sprouts, and that the biosynthetic processes located in these structures were both significantly arrested, or their rate reduced, after irradiation.

The reduced contents we observed for all lipid-associated fatty acids in irradiated sprouts and the selectively larger inhibition of 18:3 formation suggest that a further radiation-induced effect occurred, namely reduced activity of (or expression of the genes encoding) the enzymes involved in the *de novo* synthesis as well as in the desaturation of fatty acids. Whether the reduction in fatty acids is a cause or a consequence of the reduction in lipid mass induced by irradiation is not known at present. Moreover, very little is still known on these two processes in non-irradiated garlic and their parts. In plants it is known that the plastidial stroma is the site of synthesis and the envelope is the site of desaturation of fatty acids, whereas the endoplasmic reticulum (ER) contains a different set of desaturases (Browse and Somerville, 1991). Thus, plastidial desaturases act on fatty acids bound to GL whereas ER desaturases act on fatty acids esterified to PC.

In conclusion, the changes observed in the content of sprout lipids and of their individual fatty acids in long-term post irradiation of garlic treated during dormancy with 60 Gy may be due to an alteration in the biosynthetic activity of the lipids induced by the gamma irradiation *per se*, or may be a consequence of the secondary effects associated with the radio-inhibition of sprouting.

Acknowledgements

The authors are grateful to Prof. R. Camina for carrying out the statistical analysis. This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas and in part by the Secretaría de Ciencia y Tecnología of the Universidad Nacional del Sur, Argentina.

References

Argüello, J.A., Ledesma, A., Bottini, R., 1991. Hormonal regulation of dormancy in garlic (*Allium sativum* L.) cv. Rosado Paraguayo. Agriscientia 8, 9–14.

Arvidson, G.A.E., 1968. Structural and metabolic heterogeneity of rat liver glycerophosphatides. Eur. J. Biochem. 4, 478–486.

Browse, J., Somerville, C., 1991. Glycerolipid synthesis: biochemistry and regulation. Annual review of plant physiology. Plant Mol. Biol. 42, 467–506.

Burba, J.L., 1993. Manual de producción de semillas hortícolas. In: Crnko, J. (Ed.), Producción de "semilla" de ajo. INTA—EE La Consulta, Mendoza.

Croci, C.A., 1988. Radioinhibición de la brotación en bulbos de ajo (*Allium sativum* L.). Algunos aspectos bioquímicos y fisiológicos. PhD thesis, Universidad Nacional del Sur, Bahía Blanca, Argentina.

Croci, C.A., Curzio, O.A., 1983. The influence of gamma irradiation on the storage life of "red" variety garlic. J. Food Process. Pres. 7, 179–183.

Croci, C.A., Argüello, J.A., Orioli, G.A., 1987. Effect of gamma rays on seed cloves of garlic (*Allium sativum* L.) at post-harvest: reversion by exogenous growth regulators. Environ. Exp. Bot. 27, 1–5.

Croci, C.A., Argüello, J.A., Orioli, G.A., 1990. Effect of gamma rays on sprouting of seed cloves of garlic (*Allium sativum* L.): levels of auxin-like substances and growth inhibitors. Environ. Exp. Bot. 30, 9–15.

Croci, C.A., Argüello, J.A., Orioli, G.A., 1994. Biochemical changes in garlic (*Allium sativum* L.) during storage following γ-irradiation. Int. J. Radiat. Biol. 65, 263–266.

Croci, C.A., Banek, S.A., Curzio, O.A., 1995. Effects of gamma irradiation and extended storage on chemical quality in onion (*Allium cepa* L.). Food Chem. 54, 151–154.

Diehl, J.F., 1990. Safety of Irradiated Foods. Marcel Dekker Inc., New York.

Douce, R., Joyard, J., 1996. Biosynthesis of thylakoid membrane lipids. In: Ort, D.R., Yocum, C.F. (Eds.), Advances in Photosynthesis. Vol. 4. Oxygenic Photosynthesis: The Light Reactions. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 69–101.

Fenwick, G.R., Hanley, A.B., 1990. Processing of alliums; use in food manufacture. In: Rabinowitch, H.D., Brewster, J.L. (Eds.), Onions and Allied Crops, vol. III. CRC Press Inc., Boca Ratón, Florida, pp. 73–91.

Folch, J., Lee, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Gunkel, J.E., Sparrow, A.H., 1961. Ionizing radiations: biochemical, physiological and morphological aspects of their effects on plants. Encyclopedia Plant Physiol. 16, 555–561.

Hayashi, T., Todoriki, S., Nagao, A., 1992. Effect of gamma-irradiation on the membrane permeability and lipid composition of potato tubers. Environ. Exp. Bot. 32, 265–271.

Holub, B.J., Skeaff, C.M., 1987. Nutritional regulation of cellular phosphatidylinositol. Methods Enzymol. 141, 234–422.

Joyard, J., Teyssier, E., Miège, C., Berny-Seigneurin, D., Maréchal, E., Block, M.A., Dorne, A., Rolland, N., Ajlani, G., Douce, R., 1998. The biochemical machinery of plastid envelope membranes. Plant Physiol. 118, 715–723.

Katsaras, J., Stinson, R.H., Kendal, E.J., McKersie, B.D., 1986. Structural simulation of free radical damage in a model membrane system: a small-angle X-ray diffraction study. Biochim. Biophys. Acta 861, 243–250.

Kovács, E., Keresztes, Á., 2002. Effect of gamma and UV-B/C radiation on plant cells. Micron 33, 199–210.

Kwon, J.H., Yoon, H.S., Byun, M.W., Cho, H.O., 1988. Chemical changes in garlic bulbs resulting from ionizing energy treatment at sprout-inhibition dose. J. Kor. Agric. Chem. Soc. 31, 147–153.

Lancaster, J.E., Boland, M.J., 1990. Flavor chemistry. In: Rabinowitch, H.D., Brewster, J.L. (Eds.), Onions and Allied Crops, vol. III. CRC Press Inc., Boca Ratón, Florida, pp. 33–72.

Lepage, M., 1967. Identification and composition of turnip root lipids. Lipids 2, 244–250.

Morrison, W.R., Smith, L.M., 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride. J. Lipid Res. 5, 600–608.

Navari-Izzo, F., Vangioni, N., Quartacci, M.F., 1990. Lipids of soybean and sunflowers seedlings grown under drought conditions. Phytochemistry 29, 2119–2123.

Olsson, M., 1995. Alterations in lipid composition, lipid peroxidation and anti-oxidative protection during senescence in drought stressed plants and non-drought stressed plants of *Pisum sativum*. Plant Physiol. Biochem. 33, 547–553.

Orioli, G.A., Croci, C.A., Pellegrini, C.N., 2004. Sprouting radioinhibition: a method to extend the storage of edible garlic bulbs. In: Dris, R., Jaim, S.M. (Eds.), Production Practices and Quality Assessment of Food Crops. Vol. 4. Postharvest Treatment and Technology. Kluwer Academic Publishers, Dordrescht, The Netherlands, pp. 229–259.

Pérez, M.B., Curzio, O.A., Aveldaño, M.I., Croci, C.A., 1998. Effects of γ-irradiation on the lipid composition of inner sprout of garlic. Radiat. Phys. Chem. 52, 113–117.

Rahim, M.A., Fordham, R., 1988. Effect of storage temperature on the initiation and development of garlic cloves (*Allium sativum* L.). Sci. Hortic. 37, 25–38.

Rouser, G., Fleischer, S., Yamamoto, A., 1970. Two-dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorous analysis of spots. Lipids 5, 494–496.

Shah, J.J., Kothari, I.L., 1973. Histogenesis of garlic clove. Phytomorphology 23, 162–170.

Voisine, R., Vézina, L.P., Willemont, C., 1991. Induction of senescence-like deterioration of microsomal membranes from cauliflower by free radicals generated during gamma irradiation. Plant Physiol. 97, 545–550.

Zar, J.H., 1999. Biostatistical Analysis, 4th ed. Pretice-Hall, New Jersey.