ANTIOXIDANT ENZYME ACTIVITIES AND LIPID PEROXIDATION AS BIOMARKER COMPOUNDS FOR POTATO TUBER STORED BY IODINE VAPOR

Abd El-Moneim M.R. Afify[1], Hossam S. El-Beltagi[1], Amina A. Aly[2] and Abeer E. El-Ansary[1]

[1] Department of Biochemistry, Faculty of Agriculture, Cairo University, P. Box 12613, Gamma st, Giza, Cairo, Egypt
[2] Natural Product Dept., National Center for Irradiation Research and Technology. P. Box 29, Nasr City, Cairo- Egypt

ABSTRACT

The present study was conducted to evaluate effects of four iodine vapor concentrations (9.375, 18.75, 37.5 and 75 g/m^3) as sprout inhibitors of potato tubers. The activities of the biochemical enzymes catalase, glutathione-S-transferase, peroxidase, polyphenol oxidase and superoxide dismutase, in addition to lipid peroxidation level (MDA), were tested in potato tubers stored for 3, 6 and 9 weeks. The results of enzyme activities varied depending on the function of enzymes. As general trend, the activity of the enzymes recorded were significantly found to be in the range of control or less, preventing potato tubers from sprouting. Glutathione-S-transferase activity was significantly decreased and reached minimum activity (2.8±2.41) when treated with 75 g/m^3 iodine vapor. Polyphenol oxidase and peroxidase activities increased to the level of control at which potato tubers were maintained for 6 weeks. The results proved that inhibition of catalase enzyme inhibited germination but increasing catalase activity broke potato dormancy initiating germination.

KEYWORDS:
Antioxidant enzymes, iodine, potato tubers, storage

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth-most important food crop in the world mainly due to its starch content and high quality protein, substantial amounts of essential vitamins, minerals, and very low fat content [1]. Potato suffers from undesirable sprouting during storage for the fresh market; prior to industrial processing, this serious problem occurs when dormancy is broken and sprouting is activated Sprouting reduces the weight, the nutritional and processing quality of tubers, and the number of marketable potatoes, being responsible for important economic losses during potato storage. In particular, potato tuber dormancy was accompanied by a transient but remarkable increase in H_2O_2 content. The effect of a catalase inhibitor (thiourea), or of exogenous H_2O_2 application, on tuber sprouting behavior was assessed. Both treatments resulted in a reduction of the dormancy period, as well as rapid and synchronized sprouting of the treated tubers when compared to control as well as in increased sprout number per tuber [2].

As potatoes are grown better in moderate climates, where continuous production is impossible, storage of the tubers is necessary to allow availability throughout the year. However, during storage, tubers sprout after a dormancy period that is under genetic and environmental control. Pressure treatments of 100 MPa applied for 5 and 10 min inhibited the sprouting of potato tubers which have been stored for 3 months before the pressure treatments, for, at least, 6 weeks at 18 °C. Less intense pressure treatments of 30 and 50 MPa for 5 and 10 min already showed some inhibitory effects on sprouting and sprout development. Pressure treatments show a great potential to be used as non-thermal and chemical-free method, to control sprouting of potato tubers [3]. Pressure can also inactivate enzymes present in foods [4, 16], and induce gene expression modification [5, 6]

Commercial methods used to control potato sprouting are storage at low temperature, the use of chemical sprouting inhibitors, and irradiation. However, storage at low temperatures promotes the conversion of starch to sugars, increasing tubers' sweetness, with the consequent change in taste and undesirable browning because of Maillard reactions, when tubers are processed at high temperature. Chemical sprouting inhibitors like chloroprofram and maleic hydrazide are increasingly facing restrictions in their use in many countries, while essential oils were used [7-8] to control *Tetranychus urticae Koch*.

Iodine-saturated atmosphere was found to inhibit the sprouting of potato (*Solanum tuberosum* L.) tubers. The iodine concentration in tuber tissues increased as a function of exposure length, and the onset of inhibition of sprouting was found to depend on tubers genotype. During the time course of the treatment, the transcription of polyphenols was undetectable in tuber peels, whereas in bud tissues featured an increase, followed by a decrease

occurring simultaneously with the suppression of sprouting. The treatment of tubers with iodine strongly affected the expression of polyphenol oxidases at the transcriptional level. Polyphenol oxidase activity in buds poorly reflected the corresponding level of transcription; similarly, little differences were found among the enzyme isoforms expressed in buds as a function of length of exposure to iodine. These findings suggest that the induction of polyphenol oxidases mRNAs transcription could probe the inhibition of sprouting by iodine [9].

Essential oils of fennel, peppermint and caraway were formulated in the form of emulsifiable concentrates and used for controlling germination of potato tubers. The results showed that different concentrations of these formulated fennel, peppermint and caraway oils exerted high toxicity against the inoculated fungus. In addition, all formulated oils with different forms showed high activity for controlling the decay when applied as protective and therapeutic agents [10].

Lipid peroxidation is a major cause for the deterioration of fat-containing food. It initiates other undesirable changes in food, affecting nutritional quality, color, flavor and texture. Auto-oxidation of polyunsaturated lipids involves a free radical chain reaction, generally initiated by exposure of the lipids to light, heat, ionizing radiation, metal ions, or metaloprotein catalysts. Therefore, the inhibition of free radical oxidation by antioxidants is of great practical importance in preserving polyunsaturated lipids from deterioration [11].

The aim of the present investigation is to study the capability of using different concentration of iodine vapor for inhibiting sprouting of potato tubers (*Solanum tuberosum* L.) variety Diamont during storage, and extending its shelf-life instead of using pesticide chemicals with bad impact to humans [12]. Contextually, antioxidant enzyme activities of peroxidase, polyphenol oxidase, glutathione-S-transferase, superoxide dismutase and catalase were studied, in addition to MDA determination of lipid peroxidation.

2. MATERIALS AND METHODS

Potato tubers, *Solanum tuberosum* L. ssp. *tuberosum* cv. Diamont, of a uniform size (60–65 mm) were obtained at harvest time from the farm of the Faculty of Agriculture, Cairo University. They were washed and allowed to dry at room temperature, and then divided to groups according to the different treatments. Using 4 different concentrations of iodine vapor showed that the activity of peroxidase enzyme increased and exceeded the normal limits, but protecting potato tubers from sprouting for only 6 weeks.

2.1. Experimental design

Five storing boxes with small entrance door (32.5x 32.5x39.5 cm) were manufactured, 4 for samples treated by iodine, and 1 for control.

2.2. Sprout inhibitor treatments

Iodine treatments: 20 potato tubers were put into air-tight boxes (32.5x32.5x39.5 cm) and kept in dark conditions. The iodine to be tested was placed in a beaker, inside the boxes, in quantities of saturation 0.39, 0.78, 1.56 and 3.129 g corresponding to 9.375, 18.75, 37.5 and 75 g/m^2, respectively. Experiments lasted for 6 weeks. Throughout the experimental period, the temperature in the dark boxes varied from 20 to 25 °C.

2.3. Biochemical analysis

2.3.1. Preparation of enzyme extracts

Ground samples (10.0 g each) were homogenized in 10 ml of 50 mM phosphate buffer pH 7.0, 1% PVP (Sigma), 1 mM ascorbate (Sigma) at 4 ºC. After centrifugation at 15,000×g for 15 min, the supernatant was collected according to [13].

2.3.1.1. Determination of soluble proteins

Soluble proteins were measured by the Bio-Rad micro assay modification of the procedure using crystalline bovine serum albumin as a standard [14].

2.3.1.2. Peroxidase activity (POD; EC 1.11.1.7):

Peroxidase activity was assayed by monitoring the increase in absorbance at 430 nm due to the oxidation of pyrogallol (2.6 mM^{-1} cm^{-1}), as described by [15]. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 20 mM pyrogallol, 5 ml H_2O_2 and 20 µl of enzyme extract. POD activity was expressed as U/100g tuber fresh weight (fw). One unit of enzyme was the amount necessary to decompose 1 µmol of substrate per min at 25 °C.

2.3.1.3. Polyphenol oxidases (PPOs; EC 1.10.3.1 and EC 14.18.1)

PPO activities were determined by spectrophotometry at 20 °C, in triplicate. The reaction mixture consisted of 0.1 M sodium phosphate buffer pH 6.8, 20 mM, 4-dihydroxy L-phenylalanine (L-DOPA, Merck) and 50 µl of the sample. The increase in absorbance was measured in a 1-cm light path cuvette at 475 nm, in a final volume of 1 ml. PPO activity was calculated considering molar extinction coefficient for dopaquinone (3600 M^{-1} cm^{-1}) [16]. PPO activity was expressed as U/g tuber fw.).

2.3.1.4. Glutathione-S-transferase (GST; EC 2.5.1.18)

Glutathione-S-transferase activity was measured according to the method of [17] by following the changes in the absorbance at 340 nm in a mixture containing 0.17 mM sodium phosphate buffer, pH 6.5, 1 mM GSH, 1 mM 1-chloro-2,4 dinitrobenzene (CDNB) in ethanol, and enzyme extract. EU = the amount of enzyme that catalyses the formation of 1 µmol of S-2,4-dinitrophenylglutathione min^{-1}. Glutathione-S-transferase was expressed as U/100 g tuber fw.

2.3.1.5. Superoxide dismutase (SOD; EC 1.15.1.1):

Superoxide dismutase activity was measured by the photochemical method as described by [18]. One unit of

SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm, in the presence of riboflavin and light. The reaction mixture contained 45 mM potassium phosphate buffer, pH 7.0, 0.1 mM EDTA and 13 mM methionine, 0.17 mM NBT in ethanol, 0.007 mM riboflavin, and enzyme aliquot. Blanks were kept in the dark, and the others were illuminated for 15 min. One unit of SOD is the amount of extract that gives 50% inhibition to the rate of NBT reduction. Superoxide dismutase was expressed as U/100 g tuber fw..

2.3.1.6. Catalase (CAT; EC 1.11.1.6)

Catalase activity was determined as H_2O_2 consumption measured as the decrease in absorbance at 240 nm according to [19]. The assay contained 50 mM KH_2PO_4/K_2HPO_4 (pH 7.0), and 10 mM H_2O_2 in phosphate buffer. Extinction coefficient of 39.4 mM^{-1} cm^{-1} was used to calculate activity. Enzyme activity was ex pressed in µM H_2O_2 min^{-1}. Catalase activity was expressed as U/100 g tuber fw.)

2.3.1.7. Lipid peroxidation (MDA)

The lipid peroxidation was measured in terms of malonyldialdehyde (MDA) content by thiobarbituric acid (TBA) reaction. The level of lipid peroxidation is expressed as mmol of MDA formed using an extinction coefficient of 155 mM^{-1} cm^{-1} [20]. MDA was expressed as nmol. g^{-1} tuber fw.

2.4. Statistical analysis

All analyses were performed in triplicate (n=3). Statistical analysis was done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered to be significant.

3. RESULTS

Sprout control is essential for successful management of stored potatoes. Sprouting is responsible for remarkable economic losses during potato storage: sprouted potatoes are subjected to high water evaporation and quality deterioration, which reduce both their weight and marketability. Accordingly, the inhibition of sprouting is crucial in potato industry, as it provides flexibility in storage or transport, and extension of the marketing period. There are 5 stages of tuber physiological ageing: dormancy (no sprout growth), apical dominance (one sprout growth), multiple sprouting, branching (branched sprout growth), and senility (« little potato » growth). Since the reactive oxygen species (ROS) removal rate is controlled by antioxidant enzymes and a variety of low-molecular weight antioxidants, it is of interest to determine the global change of antioxidant activity present in potato tubers during different times of storing after treatments with different concentrations of iodine vapors. POX**,** PPOs , GST, SOD, CAT, and also MDA were studied in potato tubers treated with iodine vapor and stored for 9 weeks, as indication for potato tuber validity during storage

3.1. Peroxidase enzyme activity

Data presented in Table 1 reveal that treatment of potato tubers with iodine vapor maintained them from germination for 6 weeks. The data showed that the best treatment is 37.5 g/m^3 of iodine, with POX activity 72.87±1.21 after 6 weeks, which may exceeds the normal limit of potato cells recovery. The high concentration of iodine showed the same trend which encouraged the next step of sprouting after 6 weeks (Table 1). POX activity was inhibited and reached its maximum (13.26±3.9) after 3 weeks with 37.5 g/m^3 iodine but a minimum (9.31±1.91) with 75 g/m^3 iodine. After 6 weeks, the activity of peroxidase was increased to reach 92.31±3.21 with 18.75g/m^3 iodine. Therefore, the results encouraged us to use lower concentration of iodine (9.375g/m^3) during potato storage. These results proved that inhibition of POX activity keeps potato tuber dormancy as well as storage for a longer time whereas increasing the activity of POX breaks down potato tuber dormancy.

3.2. Polyphenol oxidase enzyme activity

Iodine treatments showed that potato tubers could be stored for 6 weeks only, and after that, the potato tubers were sprouted. The data showed that the best treatment of iodine vapor is 9.375 g/m^3 with PPO activity of 42±4.00 after 6 week which exceeds the normal limit of potato cells recovery. The high concentration of iodine showed the same trends encouraging the next step of breaking potato tuber

TABLE 1 - Influence of different treatments on sprout suppression effects during storage period (weeks) and on peroxidase (U/100 g tuber f.w.) activity.

Treatments	Storage time			
	Zero week	3 weeks	6 weeks	9 weeks
Control	19.03±1.9			
Iodine (g/m^3)				
9.375 g/m^3		10.60±4.60^{d}	84.08±2.93^{b}	-
18.75 g/m^3		12.38±4.65^{c}	92.31±3.21^{a}	-
37.5 g/m^3		13.26±3.93^{b}	72.87±1.21^{c}	-
75 g/m^3		9.31±1.91^{e}	74.09±3.21^{c}	-
LSD at 0.05		5.00	4.00	-

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different ($P \leq 0.05$).

TABLE 2 - Influence of different treatments on sprout suppression effects during storage period (weeks) and on polyphenol oxidase (U/g tuber f.w.) activity

Treatments	Storage time			
	Zero weeks	3 weeks	6 weeks	9 weeks
Control	39.6±7.56			
Iodine (g/m^3)				
9.375 g/m^3		70.0±1.73^{defg}	42±4^{gh}	-
18.75 g/m^3		58.3±10.41^{ghi}	76±9.87^{cd}	-
37.5 g/m^3		130.0±10^{a}	58±6^{e}	-
75 g/m^3		48.0±5.68^{hij}	64±2.31^{d}	-
LSD at 0.05		15.03	11.8	-

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different (P ≤ 0.05).

dormancy and stating sprouting. Therefore, using low iodine vapor concentration was recommended (Table 2). These results approved that PPO activity was increased from 1.5-fold (iodine 9.375g/m^3, PPO 70.±1.73) to 3-fold (iodine 37.5 g/m^3, PPO 130±10) values compared to PPO activity in control (39.6±7.56) .

3.3. Glutathione-S-transferase enzyme activity

Data in Table 3 showed that treatment of potato tuber with iodine inhibits sprouting only for 6 weeks because activity of this enzyme was reduced below the control levels. GST activity was significantly decreased and reached a minimum (2.8±2.41) when treated with high concentration of iodine vapor (75 g/m^3), after 6 weeks. Maximum reduction of GST activity (4.4±0.33) was found in iodine vapor-treated potato tubers when using low levels of iodine vapor, and keeping the enzyme activity (8.4±0.16) in the range of control (7.86±3.26), after 3 weeks.

3.4. Superoxide dismutase enzyme activity

It is very important to note that the activity of SOD in potato tubers after treatment with iodine vapor was slightly decreased in all treatments and maintained potatoes for 6 weeks. The maximum decrease in enzyme activity (150±10^{i}) was observed after treatment with iodine vapor concentration of 37.5 g/m^3. These results proved that activity of SOD is not a major factor to inhibit potato sprouting (Table 4) but is involved in steps needed to maintain potato tubers during storage. It is known that SOD activity gradually increased throughout storage time while after treat ment with iodine vapor, SOD activity was inhibited.

TABLE 3 - Influence of different treatments on sprout suppression effects during storage period (weeks) and on glutathione-S-transferase (U/100 g f.w.) activity.

Treatments	Storage time			
	Zero weeks	3 weeks	6 weeks	9 weeks
Control	7.86±3.2614			
Iodine (g/m^3)				
9.375 g/m^3		8.4±0.16^{ijkl}	4.2±5.92^{e}	-
18.75 g/m^3		5.0±0.94^{kl}	7.1±7.39^{e}	-
37.5 g/m^3		5.6±0.48^{ijkl}	5.6±2.796^{e}	-
75 g/m^3		4.4±0.33^{jkl}	2.8±2.41^{e}	-
LSD at 0.05		6.7	7.5	-

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different (P ≤ 0.05).

TABLE 4 - Influence of different treatments on sprout suppression effects during storage period (weeks) and on superoxide dismutase (U/100 g tuber f.w.) activity.

Treatments	Storage time			
		3 weeks	6 weeks	9 weeks
Control	920.±88.4			
Iodine (g/m^3)				-
9.375 g/m^3		833.3±10^{abcd}	683.3±47.14^{def}	-
18.75 g/m^3		825±82.49^{abcd}	637.5±100.17^{efg}	-
37.5 g/m^3		908.3±76.60^{abc}	150±10^{i}	-
75 g/m^3		925±0.0100^{a}	245.8±88.39^{i}	-
LSD at 0.05		180.00	99.00	

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different (P ≤ 0.05).

TABLE 5 - Influence of different treatments on sprout suppression effects during storage period (weeks) and on catalase (U/100 g tuber f.w.) activity.

Treatments	Storage time			
	Zero week	3 weeks	6 weeks	9 weeks
Control	191.4±18.64			
Iodine (g/m^3)				-
9.375 g/m^3		118.52±8.19^{efgh}	266.7±55.9247^{d}	-
18.75 g/m^3		522.2±21.11^{b}	588.9±10.791^{c}	-
37.5 g/m^3		118.89±12.51^{efgh}	300±33.68^{c}	-
75 g/m^3		91.98±13.54^{gh}	500±54.57^{b}	-
LSD at 0.05		95.18	53.00	-

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different ($P \leq 0.05$).

TABLE 6 - Influence of different treatments on sprout suppression effects during storage period (weeks) and on MDA (nmol.g^{-1} tuber f.w.) content.

Treatments	Storage time			
	Zero week	3 weeks	6 weeks	9 weeks
Control	23.5±2.13			
Iodine (g/m^3)				
9.375 g/m^3		16.0±1.21^{fghi}	13.0±2.54^{ik}	-
		19.6±2.22^{efgh}	21.7±0.24^{ghij}	-
37.5 g/m^3		16.0±2.87^{fghi}	29.0±4.38^{defgh}	-
75 g/m^3		56.0±12.67^{c}	22.0±0.24^{ghij}	-
LSD at 0.05		6.7	9.3	-

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different ($P \leq 0.05$).

3.5. Catalase enzyme activity

The data in Table 5 show that the activity of CAT decreased after 3 weeks when treated with iodine vapor at different concentrations (9.375, 37.5, 75 g/m^3), except iodine vapor concentration of 18.75 g/m^3 which increased activity of CAT (522.2±21.11). After 6 weeks, the activity of CAT increased to reach values between 300±33.68 (iodine, 37.5g/m^3) to 588.9±10.79 (iodine, 18.75 g/m^3) compared to control (191.4±18.64).The results proved that inhibition of CAT inhibited germination while increasing CAT activity broke potato tuber dormancy and initiated germination. Therefore, during 6-weeks storage of potato tubers, CAT activity progressively increased from second to fourth week of storage in the apical « buds/sprouts », in untreated potato tubers but was inhibited after treatment with iodine.

3.6. MDA and lipid peroxidation

Data in Table 6 show that MDA, which represents the oxidation process, was decreased after 3 weeks and reached 16.0±1.21 with iodine vapor of 9.375 g/m^3. The results showed that the 3 iodine vapor levels (9.375, 18.75, 37.5 g/m^3) slightly decreased activity of oxidative stress while highest concentration of iodine vapor increased oxidative stress. Therefore, it was preferred to use lower iodine vapor portions to store potato tubers. Peroxidation of unsaturated fatty acids in their membrane phospholipids may deteriorate the membrane. In the case of lipid peroxidation, damage occurred at the cellular membrane. In general, as MDA had a greater percentage increase, it appears likely that lipid peroxidation results in membrane damage, and so could be one of the early causes of tuber decay.

4. DISCUSSION

The changes in polyphenol oxidase, peroxidase, superoxide dismutase and catalase activities as well as MAD of lipids were studied in stored potato tubers treated with iodine vapor. The increases or inhibition of enzyme activities may reflect only change in constitutive levels of expression, or possibly, a generalized alteration in all tuber enzyme activities through iodine complex. Some of the enzyme activities exhibited no appreciable change during storage, and others demonstrated time-dependent increases or decreases in activity. As expected, the inhibition of sprouting was found to be dependent on the length of tuber exposure period to iodine atmosphere. The concentration of iodine was identified around the buds and increased as a function of the exposure over the entire time-length of the treatment with iodine in potatoes. Exposure of potatoes to iodine vapor suggests complex interactions between tuber metabolism and iodine concentration in tissues, finally inhibiting potato tuber germination by inhibiting most of antioxidant enzymes.

4.1. Peroxidase enzyme activity

Our results are in agreement with [21] demonstrating that the activity of free radical scavenging enzymes viz. POX, showed inverse relationships with ageing period and direct proportion to reductions in the seed germination. It was stated that long-term storage reduced the germination capability, and caused a delay in the germination [22]. In addition, from antioxidant enzymes, CAT, POX, and SOD activities were also low in the aged dry seeds. The decrease in germination capability of the aged dry seeds

of alfalfa was well correlated with the increased levels of lipid peroxidation and phenolic content, and the decreased activities of POX, CAT and SOD. However, the most noticeable result was high with POX but low with CAT activity during the germination.

Enhanced POX activity in germinating aged seeds was reported as an efficient growth signature since being involved in the control of cell activities and significantly low in aged dry seeds, compared to respective control seeds [22].

4.2. Polyphenol oxidases enzyme activity

Our results are in agreements with [9] who concluded that transcription of PPO coding genes in potato tubers is responsive to iodine treatments that inhibit sprouting, the inhibition being concomitant with the transient increase in the levels of PPO mRNAs. On the other hand, [23] cited that small changes in PPO activity were approved. In conclusion, our observations demonstrate that the activity of PPOs is responsive to the treatment of potato tubers with iodine, and that the induced suppression of sprouting is concomitant with a transient increase of the level of PPO. Nevertheless, the level of PPO could be associated with the activity of other enzymes to identify a biochemical pattern useful as a marker of sprouting inhibition.

4.3. Glutathione-S-transferase enzyme activity

The reduction rates in GST activity are in accordance with those obtained by [24] who reported on a transgenic rice plant that over-expressing the rice GST gene enhanced germination and growth at low temperature. Therefore, the inhibition in GST activity inhibited germination of potato tubers.

4.4. Superoxide dismutase enzyme activity

The main reduction in enzyme activity was noticed below the normal activity of SOD in potato control which proved that small changes were observed with POX activity. Our results were approved by [22] who cited that decreased germination ability of aged legume seeds were well correlated with the increase in lipid peroxidation levels and decreased ones in antioxidants. During germination, SOD activity did not show any significant differences between the aged and non-aged seeds as approved in beech [25]. However, activity of free radical scavenging enzymes viz. SOD also showed inverse relationships with ageing period and direct proportion to reductions in the seed germination [22]. Antioxidant enzyme activity of SOD was also enhanced during the advanced phase of aging [26].

4.5. Catalase enzyme activity

Our results were in agreement with those of [21] who found that the activity of free radical scavenging enzymes viz., POX, CAT and SOD, showed inverse relationships with ageing period and direct proportion to reductions in the seed germination. At the same time, dormancy breaking effects of thiourea were found to increase catalase activity. The results were in agreement to [26] who stated that antioxidant enzyme activities (SOD, CAT, ascorbate POX) were enhanced during the advanced phase of aging.

Ethanol promotes seeds germination in a wide range of species; however, its effect on tuber sprouting are slightly promotive, except in presence of anesthetic bromoethane where it considerably breaks dormancy in cultivar-specific manner. We do know that under anaerobic conditions, there is no accumulation in sugar, although there is evidence for production of volatile end-products of glycolysis, such as ethanol and acetaldehyde.

4.6. MDA and lipid peroxidation

Accelerated ageing due to increased lipid peroxidation, decreased activities of several free radical and peroxide-scavenging enzymes [21] because of main reasons like loss of storability, which occurs due to decreased levels of antioxidants, reduced activity of free radicals and peroxide-scavenging enzymes, and increased lipid peroxidation through MDA contents. The level of MDA, a product of the lipid peroxidation, was significantly ($P<0.01$) high in aged dry seeds, compared to controls [22]. High lipid peroxidation and oxidative stress have been observed during storage of various seeds and potato tubers, and have been widely proposed as the major cause of deterioration during seed aging [25, 27]. Therefore, our results show that high lipid peroxidation is one of the major results of natural aging in long-term stored potato tubers. One mechanism, by which plants defend themselves against free radical-mediated damage, is the induction of SOD, which catalyzes the dismutation of superoxide radicals to molecular oxygen and H_2O_2. POX and CAT then catalyze the breakdown of H_2O_2 to H_2O and O_2**.** By eliminating H_2O_2 accumulation, POX and CAT prevent the further formation of potent free radicals. The activities of free radical-scavenging enzymes (SOD, POX, and CAT) usually decrease during plant senescence.

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CORRESPONDING AUTHOR

Abd El-Moneim M.R. Afify
Department of Biochemistry
Faculty of Agriculture
Cairo University
P. Box 12613
Gamma st
Giza, Cairo
EGYPT

E-mail: moneimafify@yahoo.com