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The Impact of γ-Irradiation, Essential Oils and Iodine on Biochemical Components and Metabolism of Potato Tubers During Storage

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Abstract

Several methods have been suggested as effective for inhibition of sprouting of potato tubers during storage. Three methods; γ-irradiation, volatile oils and iodine vapor were used for the inhibition of potato tuber cv. 'Diamond'. Gamma irradiation, essential oils (caraway, clove, carvone, eugenol) and Iodine vapor were used to achieve the purpose. The results proved that γ-irradiation and essential oils maintain potato as well as inhibit sprouting for 9 weeks while iodine vapor maintain potato for six weeks. Alpha amylase activity showed an increase after six weeks and then reduced to lower value compared to control. During the metabolic pathway the concentration of lactate was decreased and reached to the level of control when potato tuber treated even with essential oils, radiation as well as with iodine vapor. The levels of NADP and NADPH+H were decreased during potato storage proving that synthesis of this metabolite were very low. The level of glycoalkaloids was fluctuated during storage depending on the treatments.

Keywords: essential oils; gamma irradiation; iodine; metabolism; potato tubers

Introduction

Potato (*Solanum tuberosum* L.) is the world's most important non-grain food crop and is central to global food security (Xun *et al.,* 2011). The tuber is usually dormant upon harvest, which means that the bud meristems are in an arrested state and visible bud sprout growth (sprouting) thereby blocked. Potato tuber dormancy is associated with down-regulated cell cycle genes and increased ABA contents in the bud meristems when compared with the non-dormant state (Campbell *et al.,* 2010; Destefano-Beltran *et al.,* 2006 a, b). Potato tubers could be stored in controlled or rudimentary conditions such as both temperature and humidity or either one of them could be controlled whereas under rudimentary conditions, such as store rooms of small farmers or houses; control of humidity and temperature could not be achieved. Respiration of tubers during storage and breakdown of dormancy during storage result in sprouting and loss of nutritive value of tubers (Suhag *et al.,* 2006). Sprouting reduces the weight, the nutritional and processing quality of tubers and the number of marketable potatoes, being responsible for important economic losses during potatoes storage (Delaplace *et al.,* 2008 a, b). These physiological changes affect the internal composition of the tuber and destruction of edible material and changes in nutritional quality (De Carvalho and Fonseca, 2006). Nowadays it's very important to use natural products compounds such as essential oils as well as the pure compound derived from essential oils or alcoholic extracts (Afify *et al.,* 2011 a; b, 2012 a; Ali *et al.,* 2011). On the same time it's not advisable to use pesticides because of its bad impact to the health of consumer (Afify *et al.,* 2010; Afify and El-Beltagi, 2011). Naturally occurring compounds could be used as anti-sprouting agents in potatoes, based on the common idea that natural products are less harmful to the environment than chemical products. Carvone was obtained from the essential oil of caraway seed (*Cuntm carvi* L.) such as caraway is one of several ancient cultivated species of the Umbelliferue. R-carvone inhibits sprouting by causing cell membrane damage mainly at the meristem tips of the tuber buds (Teper-Bamnolker *et al.,* 2010). Irradiation is a physical process that could be applied to harvested fruits to eliminate microorganisms, insects and plagues as well as delay ripening or spoilage, in turn lengthening its shelf life (Afify *et al.,* 2011 c; Aly and El-Beltagi, 2010). The radiation dose of 150 Gy was the most effective dose that gave the lower percentage of rotting and completely inhibition sprouting during storage (Afify *et al.,* 2012 b). The dose of 150 Gy exerts the best results in the biochemical changes during storage by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissue (Ahmed, 2004; Gunckel and Sparrow, 1961). Also, it has been shown to enhance the production of reactive oxygen species (ROS) in a variety of cells resulting oxidative stress (Alaoui *et al.,* 1992). Recent evidence suggests that reactive oxygen spe-

cies play an important role in the action of ionizing radiation (Xienia *et al.,* 2000). ROS are the byproducts of many degenerative reactions in crop plants, which will affect the regular metabolism by damaging the cellular components (Foyer and Noctor, 2002). Extensive study on oxidative stress has demonstrated that exposure of plants to adverse environmental conditions induces the over production of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\cdot-}$), H_2O_2 and hydroxyl radical ($HO^{\cdot}$) in plant cells (Afify *et al.,* 2011 d, 2012 c; Wise and Naylor, 1987). They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage, particularly when plants are exposed to stress conditions such as salt, Fe deficiency, drought, Cadmium stress, lead toxicity, roasting, ionizing radiation, nematode infection, organisms and micro-organisms (El-Beltagi, 2011; El-Beltagi *et al.,* 2008; 2010; 2011 a, b; 2012; El-Beltagi and Mohamed, 2010; Ibrahim *et al.,* 2011; Kesba and El-Beltagi, 2012; Kobeasy *et al.,* 2011; Mohamed *et al.,* 2009; Salama *et al.,* 2009; Shehab *et al.,* 2010).

Amylase is a carbohydrate splitting enzyme, which hydrolyzes starch to yield monomeric carbohydrates. Several authors reported that, activities of amylase were found to be increased from harvesting to cold stored conditions in different variety of potatoes (Karim *et al.,* 2008). The NADH concentrations demonstrated seasonal variations, and mean levels were not significantly different between cold-tolerant and cold-sensitive tubers in three of potato varieties (Blenkinsop *et al.,* 2003). Given that NADH is formed and oxidized at various stages of the respiration process, overall changes in the levels of NADH may not necessarily reflect a specific event, but rather general changes in metabolism. Glycoalkaloids are normal constituents of conventional potato varieties and contribute in small amounts to the typical potato flavour. Higher amounts make the potato taste bitter and might cause discomfort or illness (Knuthsen *et al.,* 2009). The amount of glycoalkaloids depends on various factors, such as potato cultivar, soil and weather conditions during growing season, fertilizer use, potato maturity at harvest time, tuber sizes, mechanical damage, storage conditions and access to light (Machado *et al.,* 2007; Tajner-Czopek *et al.,* 2006). Glycoalkaloids in potato leaves provide natural protection against pests. Glycoalkaloids; solanine and chaconine were detected in potato tubers with an acceptable level (under 20 mg/100 g of FW) in the cv. 'Satu', whereas concentration in cv. 'Sini' was 23 mg/100 g FW (Vaananen, 2007). The irradiation of tubers, bulbs, and rhizomes, prevents sprouting but this effect is irreversible (Molins, 2001).

The aim of the present investigation is to study the effect of two essential oils and its main components (Caraway, Carvone, Clove and Eugenol), iodine vapor and gamma irradiation for inhibiting sprouting of potato tubers (*Solanum tuberosum* L.) cv. 'Diamond' during storage and extending its shelf life. As well as to determine the changes in metabolic pathway in α-amylase activity, lactate, NADP, NADPH and solanine levels of potato tubers during storage period.

Materials and methods

Potato tubers (*Solanum tuberosum* L. ssp. tuberosum) cv. 'Diamond' of a uniform size (60-65 mm) was obtained at harvest time from the farm of faculty of Agriculture Cairo University. They were washed and allowed to dry at room temperature then divided to groups according to the different treatments.

Sprout inhibitor treatments

The essential oils treatments

Twenty potato tubers were put into air tight stored boxes (32.5 × 32.5 × 39.5 cm), 20 per cage and were kept in dark conditions. The essential oil (Caraway and Carvone) was placed in a beaker, inside boxes quantities of 10.4 µl, 20.8 µl and 31.25 µl corresponding to 100 ppm, 200 ppm and 300 ppm of vapor, respectively. Clove and eugenol were placed in a beaker, inside the cage in quantities of 9.4 µl, 18.9 µl and 28.3 µl corresponding to 100 ppm, 200 ppm and 300 ppm of vapor, respectively. Throughout the experimental period, the temperature in the dark boxes, varied from 27°C to 36.5°C. The application of the essential oil treatment was based on the technique of Sorce *et al.* (1997) and Klinge and Palomino (2010).

Iodine treatments

Twenty potato tubers were put into air tight wooden cages 32.5 cm × 32.5 cm × 39.5 cm, 20 per cage and were kept in dark conditions. The iodine was placed in a beaker, inside the cage in quantities of 0.39, 0.78, 1.56 and 3.129 g corresponding to 9.375, 18.75, 37.5 and 75 g/m^2, respectively. Throughout the experimental period, the temperature in the dark cages, were potatoes were stored, varied from 27°C to 36.5°C (Pifferi, 2001).

Irradiation treatments

Potato tubers cv. 'Diamond' packed in polyethylene high density bags and irradiated at different dose levels of γ-irradiation (0.0, 30, 50, 100 and 200 Gy) at room temperature (25 ± 1°C). γ-Irradiation was performed using a Gamma cell 200 apparatus equipped with a Cobalt 60 source (dose rate, 6.5 kGy/h) at the National Center for Irradiation Research and Technology, Cairo, Egypt. Packed tubers samples without irradiation served as the control (Blessington *et al.,* 2007).

Chemical analysis

α amylase determination

Amylase was determined using Biodiagnostic Kit. The test is based on the hydrolysis of starch by amylase and the blue-black complex that forms when iodine reacts with

starch. The amount of starch which remained at the end of incubation period. The amylase activity is measured by the difference in absorbance of the starch-iodine complex in which is there is no hydrolysis α- amylase was determined using Biodiagnostic Kit. α-amylase was expressed as (U/100 g tuber f.w.).

Lactate determination

Lactate was determined using Greiner kit Diagnostic GmbH (Kaplan *et al.,* 1998) and expressed as (µmol/g tuber f.w.).

Determination of NADP and NADPH

$NADP^+$/NADPH was determined using Bio Assay Systems EnzyChrom™ $NADP^+$/NADPH Assay Kit (ECNP-100) NADP and NADPH were expressed as (mmol/g tuber f.w.) (Zhao *et al.,* 1987).

Total gycoalkaloids determination

Preparation of potato extracts

Potato extract was prepared by blending 50 g tuber tissue with a 100 ml of 5% TCA in 75% methanol and centrifuged at 5000 rpm for 30 min. The supernatant was separated and used for determination.

Procedure

One ml of potato extract and 4 ml of antimony trichloride reagent were mixed in a test tube. The absorbance at 500 nm was determined 15 min after mixing (Smittle, 1971). blank and a complete set of standards containing 1, 5, 10, 15 and 20 mg of solanine per 100 ml of 5% acetic acid were run with each batch of antimony trichloride reagent. Total glycoalkaloid content was expressed as µg/g tuber F.W.

Statistical analysis

All analysis 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 significant.

Result and discussion

α-Amylase activity

Data presented in Tab. 1 revealed that α-amylase activity decreased compared to control all over treatments such as caraway 100 ppm , 183.7(U/100 g F.W.) and 89.6 after 3 and 9th week respectively. While the activity of α-amylase after 9th week with concentration of 200 and 300 ppm represents 98 and 90.8 respectively. Activity of amylase after gamma radiation was inhibited and the activity of enzyme was reached maximum decrease 177.0 with 200 Gy after 9 weeks. This is logic due to sprouting inhibition effect of radiation. The only difference was in iodine treatment in which is been found an increase in amylase activity with 226.4, 347.13 and 224.4 corresponding to 18.75 g/m^3, 37.5 g/m^3 and 75 g/m^3 respectively. Is been notice that iodine treatment inhibit sprouting only for 6 weeks and it could be extended to more weeks if we could maintain iodine vapor saturation to their corresponding concentration. Irradiation at higher doses (5-6 kGy) caused the amylase content to exhibit a decreasing trend. When amylase activity was compared between the control (non-irradiated) seed samples, maximum activity was noted in *C. arietinum,* while 'Shankar' cultivars showed the least (Maity *et al*., 2009). However, variation of such activity was noted to be maximally pronounced in the case of 'Shankar' as a function of radiation exposure. In C. arietinum, the maximum activity of amylase (48%) was observed when exposed to an absorbed dose of 3 kGy, whereas all other seed types showed maximum amylase activity at 4 kGy. While, noticed increase in α-amylases in the sub-eye tissue after the start of sprouting (Biemelt *et al*., 2000). Thus, there is no clear-cut evidence for an increase in starch-degrading enzymes around the time of tuber sprouting. On the other hand, α-amylases and starch phosphorylase increased rapidly at the time of sprouting and new isoenzymes of α-amylases also have been identified during sprouting (Panneerselvam *et al*., 2007). Germination also increases the activities of the enzymes α-amylase and various proteases which result in the degradation of both starch and protein, as well as a loss of total dry matter (Koehler *et al*., 2007). Different concentrations of eugenol may inhibit the production of amylase and also previous studies found that there's no significant correlation was detected between barley α-amylase activity and germination percentage (Lin *et al*., 2008; Silva and Fernandes, 2010). In addition, GA-induced dormancy release is associated with tissue specific regulation of α and β-amylases (Rentzsch *et al*., 2012). Carvone interacts with the GA-mediated accumulation of α-amylase transcripts. Low Carvone concentrations enhance the accumulation of α-amylase transcripts type 2, but do not affect the α-amylase-type 1 transcripts. Low Carvone concentrations also enhance the accumulation of α and β-amylase enzyme activity in sprouts, but not in 'sub-eye' tissues. In contrast, high Carvone concentrations have no appreciable effect in sprouts on the enzyme activities and the α-amylase transcript abundances of either group. Essential oils such as monoterpenes therefore may have specific targets for their bioactive interaction with biochemical components in the cell (Afify *et al*., 2012 a).

Lactate

The data in Tab. 2 proved that treatments with essential oils inhibit sprouting for six to nine weeks by maintaining the concentration of the lactate in lower concentration in the range of control or lower. Most of the values got lower than control, like caraway 100 ppm (lactate) 8.8 after 3 weeks were observed, compared to Control with concen-

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Tab. 1. Levels of α-amylase (U/100g f.w.) activity in stored potato tubers treated with different treatments

Treatments	Storage time (weeks)			
	Zero	3	6	9
	α-amylase activity (U/100 g F.W.)			
Caraway (ppm)				
Control	217.03±2.31	-	-	-
100	-	183.7±1.37^{a}	573.9±2.40^{a}	89.6±0.45^{h}
200	-	128.3±0.61^{e}	142.8±1.12^{h}	98.0±0.53^{g}
300	-	72.67±0.53^{o}	179.1±1.41^{g}	90.8±0.63^{h}
Carvone (ppm)				
100	-	134.9±1.26^{d}	357.8±1.78^{b}	88.47±0.76^{h}
200	-	147.5±1.41^{c}	117.2±1.13^{kl}	63.37±0.59^{i}
300	-	155.2±0.85^{b}	125.9±0.56^{j}	97.07±1.15^{g}
Clove (ppm)				
100	-	68.0±0.20^{p}	143.9±1.13^{h}	44.3±0.38^{k}
200	-	122±0.49^{g}	80.92±1.35^{m}	62.6±0.56^{i}
300	-	66.0±0.51^{pq}	260.4±1.71^{d}	53.3±0.44^{j}
Eugenol (ppm)				
100	-	115.3±0.52^{h}	206.7±1.11^{f}	187.4±1.40^{c}
200	-	86.6±1.19^{l}	62.43±0.78^{n}	170.4±0.95^{d}
300	-	94.9±0.35^{k}	63.2±1.03^{n}	152.8±1.41^{f}
Iodine (g/m^3)				
9.375	-	44.35±0.66^{q}	112.4±0.75^{l}	-
18.75	-	67.32±1.05^{p}	226.4±1.26^{e}	-
37.5	-	44.93±1.33^{q}	347.13±1.32^{c}	-
75	-	100.8±0.61^{j}	224.4±1.15^{ef}	-
Gamma irradiation (Gy)				
10	-	111.7±0.85^{i}	120.4±1.08^{k}	-
30	-	78.0±0.58^{n}	134.9±1.31^{i}	-
50	-	81.0±0.35^{m}	80.43±1.32^{m}	197.2±2.15^{a}
100	-	66.0±1.51^{pq}	224.7±1.49^{ef}	191.4±1.79^{b}
200	-	78.0±1.11^{n}	205.37±0.78^{f}	177.0± 0.81^{e}
LSD at 0.05	-	2.50	1.63	1.05

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

tration 17.0. Treatments of potato tuber with high concentration of essential oil and its pure compounds (300 ppm) gave the high concentration of lactate and represents (Caraway 34.34, Carvone 41.4, Clove 31.5, Eugenol 28.34) respectively. On the other hand concentration lower than 300 ppm increase the level of lactate (200 pmm) or decrease the concentration to reach the concentration of control or lower. Lactate value increased after treatment with essential oils pure clove and reached 27.9, 26.9 and 31.5 corresponding to 100, 200 and 300 ppm respectively which maintain potato tuber during storage.

Iodine vapor treatment gave low values of lactate with concentration 13.81, 18.7 and 19.1 corresponding to 9.375 g/m^3, 18.75 g/m^3 and 37.5 g/m^3 saturation respectively. Treatment with iodine vapor (75 g/m^3) showed that lactate increased to 68.1 on the 3rd week and 95.0 on the 6th week and could not save potato from sprouting until the week 9.

Treatment with gamma radiation ranged from 10 to 200 Gy showed that the level of lactate was inhibited in all potato tuber treated with gamma radiation except the dose 100 Gy increased the level of lactate after three weeks (34.4) and the level was decreased to the maximum minimum level after 9 weeks (9.6) with 50 Gy.

Products of anaerobic metabolism, such as lactate, acetaldehyde and ethanol, are not considered as normal products of plant metabolism under aerobic conditions, and their presence suggests a substantial alteration of respiratory metabolism. The alteration in respiratory metabolism as a result of potato tuber treatment with essential oils, iodine vapor as well as radiation will be involved in inhibition of respiration and sprouting activity at buds (Afify *et al.,* 2012 d). Previous studies found that, concentrations of lactate in cold-tolerant and cold-sensitive tubers did not show a significant changes over-all increase or decrease over the duration of storage in any of the four years of studied (Blenkinsop *et al.,* 2003). The anaerobic respiratory enzymes including L-lactate dehydrogenase contribute to Low-temperature tolerance (Pinhero *et al.,* 2007).

Tab. 2. Levels of lactate (µmol/g f.w.) in stored potato tubers treated with different treatments

Treatments	Storage time (weeks)			
	Zero	3	6	9
	Lactate (µmol/g f.w.)			
Caraway (ppm)				
Control	17.0±0.27	-	-	-
100	-	8.8±0.24^{k}	4.6±0.20^{n}	4.13±1.13^{k}
200	-	10.8±0.27^{i}	16.7±0.42^{f}	31.0±0.56^{c}
300	-	6.3±0.14^{m}	18.6±0.26^{e}	34.34±0.27^{b}
Carvone (ppm)				
100	-	23.2±0.43^{c}	5.8±0.21^{m}	7.16±1.15^{j}
200	-	13.3±0.38^{g}	7.8±0.14^{k}	13.41±0.36^{f}
300	-	11.6±0.31^{h}	12.0±0.16^{g}	41.4±1.21^{a}
Clove (ppm)				
100	-	5.0±0.16^{n}	3.6±0.19^{o}	27.9±0.27^{cd}
200	-	5.7±0.18^{mn}	2.6±0.16^{p}	26.9±0.34^{d}
300	-	3.4±0.17^{p}	5.61±0.94^{m}	31.5±1.47^{b}
Eugenol (ppm)				
100	-	4.2±0.23^{o}	5.8±0.24^{m}	18.18±0.37^{e}
200	-	19.9±0.44^{d}	8.5±0.23^{j}	18.38±0.35^{e}
300	-	9.8±0.40^{j}	39.3±1.16^{b}	28.34±0.21^{c}
Iodine (g/m^3)				
9.375	-	13.8±0.39^{g}	16.78±1.33^{d}	-
18.75	-	18.7±0.38^{e}	9.70±0.18^{i}	-
37.5	-	19.1±0.47^{d}	7.16±0.24^{l}	-
75	-	68.1±0.99^{a}	95.0±0.90^{a}	-
Gamma irradiation (Gy)				
10	-	5.9±0.11^{mn}	9.8±0.24^{i}	-
30	-	6.9±0.19^{l}	23.0±0.39^{d}	-
50	-	15.9±0.54^{f}	34.8±0.60^{c}	9.6±0.30^{i}
100	-	34.4±1.64^{b}	10.7±0.41^{h}	11.2±0.31^{h}
200	-	8.98±0.26^{k}	11.7±0.25^{g}	11.8±0.12^{g}
LSD at 0.05	-	0.25	0.26	0.22

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

Total glycoalkaloid

The data in Tab. 3 proved that most of the values got lower than control, like treatment of clove affect concentration of total glycoalkaloid to give 16.5, 19.4 and 28.2 corresponding to essential oils concentration 100, 200 and 300 ppm, respectively compared to control activity 45. Moreover total glycoalkaloid value decreased with iodine treatment 22.0, 8.80 and 12.80 corresponding to 9.375 g/m^3, 18.75 g/m^3 and 37.5 g/m^3 respectively, but increased with concentration of 75 g/m^3 to be around the control value. Different dose of gamma radiation ranged from 10-200 Gy inhibit the synthesis of total glycoalkaloid except dose of 100 Gy which increased the amount of total gylcoalkaloid (88.3) after three weeks. The level of total gylcoalkaloid after 9 weeks of treatment were much closed to the control and still lower than the control.

The general decrease in total glycoalkaloids in present study results could be due to storage conditions in dark which could be attributed to previous studies findings about the rule in the case when mother tuber is sprouted (usually at room temperature) under alternating dark and light conditions, e.g. 1 week darkness, 1 week indirect light (Lommen, 2007; Lommen and Struik, 2007). In the darkness the sprouts elongate and during the light phases, elongation is suppressed and anthocyanin and glycoalkaloids can be formed that make the sprouts less susceptible to attack by diseases after planting. An elevated temperature (10°C) during long-term storage without sprouting inhibitors led to an increase in SGA contents (up to 518 mg SGA kg^{-1} dry matter) in two of three investigated cultivars independent of the sprouting level. Cold storage (4°C) slightly enhanced SGA contents in two cultivars (Haase, 2010). Sprout control resulted in a tendentious decrease in Steroidal glycoalkaloids (SGA) contents in a set of three cultivars. Potatoes also produce biologically active secondary metabolites, which may have both adverse and beneficial effects in the diet. These include glycoalkaloids (GAs), calystegine alkaloids, protease inhibitors, lectins, phenolic compounds, and chlorophyll (Afify *et al.,* 2012 a; Friedman, 2006). Glycoalkaloids in potatoes indicated

Tab. 3. Levels of total glycoalkaloids (µg/g) in stored potato tubers treated with different treatments

Treatments	Storage time (weeks)			
	Zero	3	6	9
	Total glycoalkaloids (µg/g f.w.)			
Caraway (ppm)				
Control	45.0 ±0.46	-	-	-
100	-	61.1±0.33^{e}	18.6±0.15^{l}	25.6±0.35^{k}
200	-	18.2±0.21^{no}	30.8±0.21^{g}	80.7±1.20^{b}
300	-	58.2±0.29^{f}	24.3±0.37^{j}	16.5±0.28^{n}
Carvone (ppm)				
100	-	95.0±1.48^{a}	27.7±0.26^{h}	58.7±0.45^{c}
200	-	36.2±0.44^{h}	38.0±0.44^{c}	42.5±0.40^{g}
300	-	24.3±0.32^{k}	20.2±1.76^{ij}	65.7±1.05^{d}
Clove (ppm)				
100	-	16.5±0.15^{o}	22.3±0.32^{k}	28.4±0.26^{j}
200	-	19.4±1.40^{l}	24.9±0.15^{i}	23.0±0.20^{l}
300	-	28.2±2.23^{h}	30.8±0.16^{g}	78.2±0.47^{c}
Eugenol (ppm)				
100	-	28.2±0.34^{ijk}	24.1±0.25^{j}	57.0±0.25^{f}
200	-	84.7±0.60^{c}	42.73±0.27^{d}	143.3±1.21^{a}
300	-	82.3±0.42^{d}	42.13±0.36^{d}	42.4±0.31^{g}
Iodine (g/m^3)				
9.375	-	22.0±0.23^{l}	46.5±0.31^{c}	-
18.75	-	8.8±0.18^{q}	55.2±1.40^{a}	-
37.5	-	12.8±0.31^{p}	49.0±0.39^{b}	-
75	-	44.1±0.36^{g}	54.5±2.15^{a}	-
Gamma irradiation (Gy)				
10	-	18.8±0.31^{n}	30.6±0.19^{gh}	-
30	-	20.9±0.25^{m}	30.6±0.27^{gh}	-
50	-	28.8±0.26^{j}	31.5±0.22^{f}	40.6±0.30^{h}
100	-	88.3±0.59^{b}	27.5±0.19^{h}	30.6±0.40^{i}
200	-	32.5±0.30^{i}	49.5±0.35^{b}	18.0±0.29^{m}
LSD at 0.05	-	0.34	0.29	29.7

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

that very few potato samples contained more than 200 mg TGA/kg (Machado *et al.,* 2007). However, as levels of glycoalkaloids in potato tubers differ between varieties and are affected by environmental factors during growth, harvest and storage. It's very important to note that amount of solanine increases in mature tuber while they are greening and germinating, as well as they is exposed to light.

NADP

It is very important to note that the concentration of NADP in potato tubers after treatments kept around the value of control. By looking at clove treatments is been found that it gave the lower level values on the 9 week of NADP as 6.8, 19.4 and 16.5 corresponding to 100, 200 and 300 ppm respectively, comparing to 50, 100 and 200 Gy, caraway, carvon and even the eugenol. Clove results promise a longer shelf life to the potato tuber. The small doses of gamma radiation 10, 30 and 50 Gy gave very low values of NADP comparing to control as 12.9, 18.1 and 20.5 respectively. On the other hand the higher doses 100 and 200 Gy gave a close values to control as 33 and 34. Iodine treatments gave a low values among treatments 15.5, 14.2, 17.7 and 21.5 corresponding to 9.375, 18.75, 37.5 and 75 g/m^3 but potato sprouted by the end of six week.

The key enzymes, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were increased even before the visible appearance of sprouting and their activities were at their maximum during sprouting (Panneerselvam *et al.,* 2007). And that agrees with us, proves that NADP as a co-factor to these enzymes in pentose phosphate pathway, didn't increase in present results because the treatments led to sprouting inhibition (Firenzuoli *et al.,* 1968). In higher plants, the OPPP is a major source of reduction power (NADPH) required for anabolic biosyntheses and assimilatory processes in the cytosol, as well as in plastids, providing key intermediates for the shikimate pathway and nucleic acid biosynthesis (Neuhaus and Emes, 2000). The maintenance in the dark of a consistently high rate of pyruvate kinase, phosphoglycerate kinase, and glyceraldehyde 3-P dehydrogenase NAD dependent

Tab. 4. Levels of NADP (mmol/g f.w.) in stored potato tubers treated with different treatments

Treatments	Storage time (weeks)			
	Zero	3	6	9
	NADP (mmol/g f.w.)			
Caraway (ppm)				
Control	37.8±0.60	-	-	-
100	-	3.40±0.02^{o}	15.6±0.10^{p}	29.6±0.24^{b}
200	-	29.0±0.14^{e}	26.5±0.26^{f}	20.8±0.23^{c}
300	-	31.4±0.22^{c}	16.5±0.26^{n}	17.7±0.16^{f}
Carvone (ppm)				
100	-	12.6±0.21^{n}	13.1±0.17^{r}	8.7±0.16^{l}
200	-	23.3±0.20^{h}	22.6±0.19^{i}	17.1±0.17^{g}
300	-	24.7±0.41^{g}	21.2±0.19^{k}	15.2±0.11^{i}
Clove (ppm)				
100	-	29.2±0.19^{e}	26.0±0.21^{f}	6.8±0.30^{d}
200	-	22.9±0.30^{hi}	28.0±0.16^{d}	19.4±0.22^{e}
300	-	31.6±0.35^{c}	29.6±0.22^{c}	16.5±0.17^{h}
Eugenol (ppm)				
100	-	24.3±0.29^{g}	22±0.14^{j}	13.7±0.11^{j}
200	-	29.8±0.27^{d}	27.2±0.18^{e}	12.1±0.03^{k}
300	-	25.4±0.14^{f}	27.2±0.19^{e}	12.1±0.03^{k}
Iodine (g/m^3)				
9.375	-	15.5±0.21^{l}	20.7±0.22^{l}	-
18.75	-	14.2±0.08^{m}	23.2±0.18^{h}	-
37.5	-	17.7±0.18^{kl}	17.2±0.20^{m}	-
75	-	21.5±0.23^{i}	16.0±0.04^{o}	-
Gamma irradiation (Gy)				
10	-	12.9±0.16^{n}	14.5±0.13^{q}	-
30	-	18.1±0.23^{k}	24.2±0.73^{g}	-
50	-	20.5±0.15^{j}	27.7±0.20^{d}	20.3±0.11^{d}
100	-	33.0±0.15^{b}	34.2±0.17^{b}	15.1±0.13^{i}
200	-	34.0±0.17^{a}	35.3±0.25^{a}	39.5±0.32^{a}
LSD at 0.05	-	0.25	0.21	0.22

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

can be explained by their relationship to the metabolism of glyceraldehyde-3-P that derives from the pentose phosphate shunt. Glyceraldehyde 3-P dehydrogenase NAD dependent also necessary for the pentose phosphate catalytic cycle for photosynthesis, remain present, although at a lower level (Kruger and von Schaewen, 2003).

NADPH

It is very important to note that the concentration of NADPH logically kept around the value activity of control and maybe these values in potato tubers after treatments was small decreased (Tab. 5). Those results were expected due to decrease in NADP which will inhibit the phosphate pentose pathway which produce NADPH. For essential oil treatments with caraway 100, 200 and 300 ppm gave 29.4, 39.1 and 31.1 respectively and gamma radiation the doses 10, 30, 50, 100 and 200 Gy gave 44.5, 42, 34.7, 34 and 25.3 respectively. Iodine treatments gave the same trend of lower NADPH+H values than control although it couldn't save potatoes from sprouting more than 6 weeks its values on 6 weeks were 30.3, 30.9, 35.2 and 25.9 corresponding to 9.375, 18.75, 37.5 and 75 g/m^3. No significant increase or decrease in all treatments even potato maintained for 6 or nine weeks. These results proved that activity of NADP is not a major factor to inhibit potato sprouting.

Soluble sugars can feed the oxidative pentose phosphate pathway, a pathway producing NADPH which can scavenge ROS via the ascorbate-glutathione cycle, or accumulation of sugars may lead to ROS production (Blenkinsop *et al.,* 2003; Foyer and Noctor, 2009). Results from this study did not show significant differences in NADPH accumulation between cold-tolerant and cold-sensitive tubers (Barichello *et al.,* 1990). The key enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were increased even before the visible appearance of sprouting and their activities were at their maximum during sprouting (Panneerselvam *et al.,* 2007). In agreement whit this study, proves that NADPH when results out of reaction to these enzymes in PPP, didn't in-

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Tab. 5. Levels of NADPH (mmol/g f.w.) in stored potato tubers treated with different treatments

Treatments	Storage time (weeks)			
	Zero	3	6	9
	NADPH (mmol/g f.w.)			
Caraway (ppm)				
Control	65.4±0.20	-	-	-
100	-	40.5±0.25^{d}	29.4±0.37^{j}	34.1±0.22^{g}
200	-	43.8±0.11^{b}	39.1±0.21^{d}	18.0±0.01^{j}
300	-	29.8±0.11^{k}	31.1±0.22^{h}	34.3±0.14^{g}
Carvone (ppm)				
100	-	36.5±0.15^{fg}	34.1±0.19^{g}	33.3±0.19^{h}
200	-	35.0±0.21^{h}	18.1±0.38^{h}	35.1±0.15^{f}
300	-	37.2±0.16^{f}	31.2±0.11^{h}	29.7±0.42^{i}
Clove (ppm)				
100	-	40.7±0.04^{d}	34.1±0.19^{g}	33.3±0.19^{h}
200	-	34.5±0.13^{i}	18.1±0.18^{m}	35.1±0.15^{f}
300	-	35.0±0.28^{h}	31.2±0.13^{h}	29.7±0.42^{i}
Eugenol (ppm)				
100	-	34.8±0.18^{h}	88.8±0.18^{a}	41.7±0.29^{b}
200	-	35.2±0.18^{h}	32.4±0.29^{e}	37.8±0.25^{d}
300	-	42.1±0.24^{c}	22.7±0.23^{l}	42.5±0.10^{a}
Iodine (g/m^{3})				
9.375	-	36.0±0.21^{g}	30.3±0.22^{i}	-
18.75	-	39.5±0.02^{c}	30.9±0.08^{h}	-
37.5	-	33.2±0.19^{j}	35.2±0.08^{e}	-
75	-	36.8±0.21^{f}	25.9±0.48^{fg}	-
Gamma irradiation (Gy)				
10	-	53.7±0.35^{a}	44.5±0.07^{b}	-
30	-	42.1±0.27^{c}	42.0±0.12^{c}	-
50	-	40.0±0.22^{d}	34.7±0.10^{f}	39.9±0.17^{c}
100	-	37.0±0.28^{f}	34.0±0.14^{g}	36.0±0.14^{e}
200	-	33.2±0.23^{j}	25.3±0.29^{k}	34.4±0.12^{g}
LSD at 0.05	-	0.26	0.21	0.18

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

crease in study results because the treatments led to sprouting inhibition. It well known that NADPH oxidation is much more sensitive to inhibition by diphenyleneiodonium than is NADH oxidation and suggested that diphenyleneiodonium preferentially inhibits flavoenzymes that function by one-electron transfer subsequently the iodine vapor expected to have the same reaction mechanisms and inhibit potato tuber germination (Afify *et al.,* 2012d; O'Donnell *et al.,* 1994; Roberts *et al.,* 1995). As proved in this study carvone was found to be a good potato sprouting inhibitor when compared to the traditional chemical mixtures of isopropylphenylcarbamate and isopropyl-3-chlorophenylcarbamate. Carvone was as good or even better during long-term storage, also showing antifungal activity against *Fusarium sulphureum*, *Phoma exigua* var. *foveata* and *Helminthosporium solani* (Hartmans *et al.,* 1995). As proved in this study carvone was found to be a good potato sprouting inhibitor when compared to the traditional chemical mixtures of isopropylphenylcarbamate and isopropyl-3-chlorophenylcarbamate.

There are argues against the proposal that the cold liability of enzymes catalyzing the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate is a major cause of sugar accumulation at low temperature. Such a conclusion is consistent with the results of others who found that transfer of tubers to low temperature results in an immediate inhibition and subsequent delayed stimulation of sucrose synthesis over the course of several days. Nevertheless, temperature coefficients of changes in metabolic flux over the range 4-25°C for tubers stored at either the lower or higher temperature confirms that there is a preferential decrease in carbohydrate oxidation relative to other aspects of sugar metabolism as temperature is lowered. Furthermore, the yield of CO_2 release from specific carbon positions within metabolized substrate suggests that the oxidative pentose phosphate pathway accounts for an increasing proportion of this flux in the cold (Jacob *et al.,* 2006). Taken together, these results support the notion that glycolysis is constrained in tubers in the cold. It is concluded that the cold liability of enzymes catalyz-

ing the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate is not a major factor in cold-induced sweetening in plants and that this widely held hypothesis should be abandoned .Therefore the level of NADP and NADPH+H are constant at room temperature ranged from 20-25°C as reported in this investigation.

In conclusion, essential oils (caraway, clove, carvone, and eugenol) and gamma irradiation recorded the most suitable methods for inhibiting sprouting of potato tubers (*Solanum tuberosum* L.) cv. 'Diamond' during storage for 9 weeks and extending its shelf life. This results provide some evidences to the sprout inhibitors treatments of some essential oils, γ-irradiation and iodine as alternatives to pesticides because of its bad impact to the health of consumer for 9 weeks, which need further investigation in the future for longer periods of time.

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