Effect of heat treatment with antioxidants on oxygen radical scavenging during storage of bell pepper fruits

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Keywords:

Storage
Pepper
Antioxidant
Treatment

Article history:

Received 19.12.2015
Received in revised form 20.02.2016
Accepted 24.03.2016

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Abstract

Introduction. Despite known effectiveness of heat preconditioning and antioxidant treatment for decreasing of oxidative damage induced by cooling, their combined effect on fruits of sweet bell pepper was never studied before.

Materials and methods. Fruits of sweet bell pepper Nikita F1 and Hercules F1, which were preconditioned with warm composition of antioxidants, were stored in 7± 0,5 ºC. Content of malondialdehyde (MDA) was determined by thiobarbituric method. Superoxide dismutase (SOD) activity was determined by estimation of its ability to inhibit the reaction of auto-oxidation of adrenaline in alkaline medium with a modification in the stage of raw materials preparation. Activity of peroxidase (PO) and catalase (CAT) was determined by titration of undecomposed rest of hydrogen peroxide.

Results and discussion. Without using additional methods of prevention of chilling injury after 24 days third part of fruit is damaged. In pepper, treated with heat and composition of antioxidants, chilling injuries are seen only on 21st day independently from hybrid. Chilling injuries decrease in 3,9…4,5 times in comparison with untreated fruits. During the storage of pepper in temperature conditions above the cold-sensitivity threshold content of MDA increases constantly. Heat treatment with antioxidants changes a dynamic of MDA in the sweet bell pepper fruit. Till the 12th day of study level of lipid peroxidation remains stable in treated samples. Then, on each stage of storage, level of MDA raises by 5...15% depending on hybrid. On the 18th day (loss of commercial quality of control samples) level of MDA in the studied samples is lower in 1,7…2 times. Heat preconditioning with antioxidants decreases speed of SOD deactivation by 25% and CAT by 30...50%. Activity of PO during storage of pepper decreases till the moment of commercial quality loss and then raises. In studied fruits growth of PO activity starts 12 days after such in the control group. Between the activity of studied enzymes and content of MDA strong reverse correlations were found (r=-0,81…≈-1) that testifies to the antioxidant functions of these enzymes.

Conclusions. Combination of heat preconditioning and antioxidant treatment for preparation of pepper to storage increases effectiveness of functioning of the system aimed on the reactive oxygen species utilization, which allows to minimize chilling injury.

Introduction

Fruit and vegetables during the periods of treatment, storage and sale can be potentially affected by the numerous negative factors (low temperature, storage condition, mechanical damage), which can lead to the oxidative stress. Oxidative stress occurs when partly reduced reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl ($OH^{\cdot}$) and peroxinitrite ($ONOO^-$) are overproduced, which leads to the failure of the organism ability to maintain a cell redox homeostasis [1]. Duration of the ROS affect on tissues is determined by antioxidant system, which is a set of cell, tissue and organism defense mechanisms that are aimed on the homeostasis maintenance. Endogenous antioxidants allow preserving low constant level of products of lipid peroxidation, thus preventing illnesses in the postharvest period [2].

Antioxidant system of plant tissues consists of non-enzyme (low-molecular) and enzyme (high-molecular) antioxidants [3]. Three enzymes are mainly responsible for the defense of the organism from oxidative damage: superoxide dismutase (SOD), catalase (CAT), peroxidase (PO) [4].

Superoxide dismutase plays a central part in the protection from oxidative stress in all aerobic organisms [5]. Dismutation of superoxide radicals is SOD function. Hydrogen peroxide is a result of superoxide anion dismutation. That is why the group of enzymes, which utilize hydrogen peroxide, are a necessary element of antioxidant defense of plants. Catalase and peroxidase are such enzymes and they act in a cell as a second defense line.

Due to the interruption of synthesis processes of substances, which are needed for the normal metabolism, system of the antioxidant control over ROS generation is acting properly limited time only. Level of ROS increases dramatically when the irreversible aging processes develop [6, 7]. Mechanisms of antioxidant protection exhaust, which leads to the number of metabolic disorders and cell death. Therefore utilization of ROS excess during the storage of fruit and vegetables is a key to preserve the quality of production.

Together with decreasing of storage temperature, the intensity of respiration decreases, production of ethylene and weight losses are reduced [8, 9]. This allows extension of shelf life of fruit and vegetables. Still for many species of horticultural crops low temperature is harmful as leading to the oxidative damage. Despite powerful endogenous antioxidant system [10], sweet bell pepper fruits are quite sensitive to the influence of cold. Level of sensitivity depends on variety: hot species of *Capsicum annuum* can withstand temperature decrease till 5 ºC, although "paprika" fruits immediately react on the temperature decreasing below 7 ºC with physiological disorders [11,12].

For protection of fruits from oxidative damage that was induced by cooling in industry heat treatment is often used [13]. Positive influence of heating procedures is connected with formation and protective action of heat shock proteins, which play a part in the regulation of ROS formation and protection of cell compartments from oxidative stress. Temperature preconditioning also decrease sensitivity of sweet bell pepper fruits to cold [14, 15]. Group of scientists from Israel investigated the influence of temperature preconditioning on pepper storing in the temperature diapason from 22 to 55 ºC [14]. They have found, that temperature conditioning above 44,5 ºC significantly reduce damage of fruits from cooling.

Another effective method to reduce oxidative damage is using of direct approach – antioxidants [16]. Combination of direct and indirect approaches can lead to the synergic effect of system stress resistance. However, analysis of antioxidant defense functioning in sweet bell pepper fruits during the heat treatment with antioxidants was newer studied, that is why area of research is of a great interest.

Materials and methods

Fruits of pepper hybrids Hercules F1 and Nikita F1, grown in open field conditions in agribusiness of Melitopol district of Zaporizhzhska region, were investigated. For storage fruits of technical stage of ripening were taken (fruits coloured in the main colour to 80-90%), uniform in size also. Fruits were placed in the prepared solutions of biologically active substances with a temperature of 45 ºC for 15min. Complex composition with bactericide and antioxidant activity was used. It is based on the approved for usage in food antioxidants: buthylhydroxitoluol (ionol), lecithine and water extract of horseradish root [Priss, O. P., Prokudina, T. F., Zhukova, V. F. Antioxidant composition for the treatment of fruit vegetables before storage [Antyoksydantna kompozytsiya dlya obrobky plodovykh ovochiv pered zberihannyam]. Pat. 59733 Ukraine, IPC A 23 7/14, 2011].

After drying fruits were put in boxes, lined with polyethylene wrap and stored at 7 ± 0,5 ºC and relative humidity 95 ± 1%. Control group consists of not treated fruits. Development of chilling injury (CI) was evaluated after storage under mentioned conditions and transferring for 1-day storage of pepper under the room temperature (21±2°C). That was done five times (20 pepper fruits in each group). Level of chilling injury during the pepper storage was evaluated with a subjective scale from 0 to 3 points and expressed through the chilling injury index [14].

The content of malondialdehyde (MDA) was determined by the thiobarbituric method [Musienko, M. M. et al. (2001) *Spectrophotometric methods in the practice of physiology, biochemistry and ecology of plants* [*Spektrofotometrychni metody v praktytsi fiziolohiyi, biokhimiyi ta ekolohiyi roslyn*]]. SOD activity was determined by estimation of its ability to inhibit the reaction of auto-oxidation of adrenaline in alkaline medium [Sirota T.V. (2000), *A method for determining the antioxidant activity of superoxide dismutase and chemical compounds* [*Sposob opredelenija antioksidantnoj aktivnosti superoksiddismutazy i himicheskih soedinenij*], Russian Federation Patent 2144674] (method was modified in the stage of preparation of raw materials for research). For the measurement of SOD activity to 0,5 g of plant material 5 ml of phosphate buffer pH=7,8 was added and substance was triturated in a mortar with glass (on ice). Next, homogenate was transferred to the centrifuge tubes with 0,3 ml of chloroform and 0,6 ml of alcohol and centrifuged at 8000 rpm. 20 minutes. For spectrophotometric measurements supernatant was used. SOD activity was expressed in conventional units (CU), which show the percentage of inhibition of adrenaline auto-oxidation. Catalase activity was determined by titration of the undecomposed rest of hydrogen peroxide with sodium thiosulfate [Hrytsayenko, Z.M. et al. (2003) *Methods of biological and agrochemical research plants and soils* [*Metody biolohichnykh ta ahrokhimichnykh doslidzhen roslyn i gruntiv*]]. Determination of peroxidase activity was conducted by titration of undecomposed rest of hydrogen peroxide in the reaction of pyrocatechol oxidation [Zemljanuhin, A. A. (1985) *Small workshop on Biochemistry* [*Malyj praktikum po biohimii*]].

Results and discussion

First signs of chilling injury in the control group were noticed on the 15th day of storage in pepper fruits from both hybrids. Without additional treatment aimed on prevention from chilling injury third part of fruits become damaged after 24th day of storage. Figure 1 shows that combination of heat and antioxidant treatment significantly induces tolerance to cold.

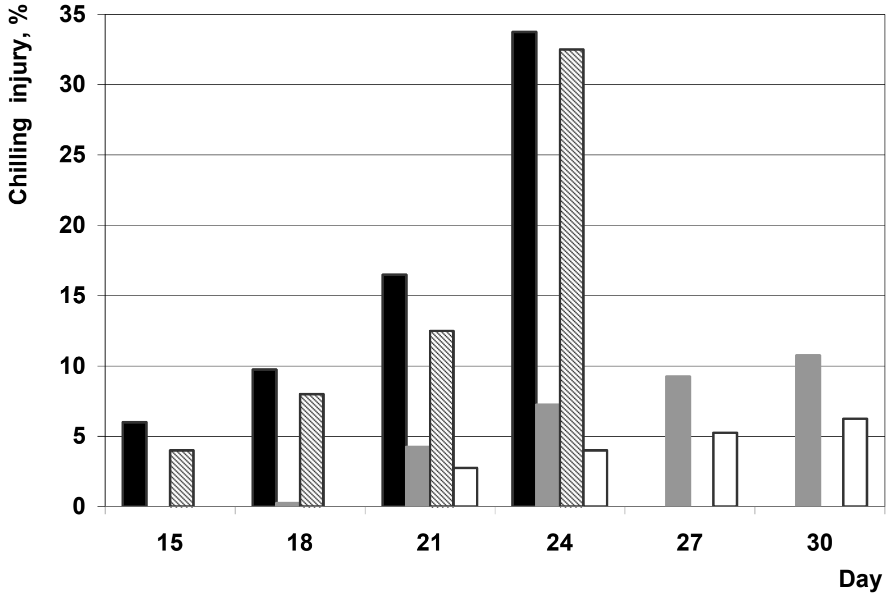

Fig. 1. Chilling injury in sweet bell pepper:
- Nikita hybrid, without treatment
- Temperature preconditioning with antioxidants of Nikita hybrid fruits
- Temperature preconditioning with antioxidants of Hercules hybrid fruits
- Hercules hybrid, without treatment

In the fruits of sweet bell pepper that was treated with the composition of antioxidants cold injury is seen only after 21^{st} day independently from the studied hybrid. Cold-induced damage decreases in 3,9...4,5 times in comparison to the fruits without any treatment. After 30 days of storage chilling injury of fruits, which were preconditioned with temperature and antioxidants, was on the level of 10%.

The degree of severity of chilling injury increases with the increasing of storage life. However, heat treatment with antioxidants leads to reduction of chilling injury index (Fig. 2).

The severity of chilling injury symptoms in studied fruits decreases in 8,8...13,2 times comparing to the fruits without treatment.

Increasing of malondialdehyde content is a direct indication of cell oxidative damage, and such a rising is natural during aging of fruit tissues. During the storage of pepper in the temperature conditions above the cold-sensitivity threshold content of MDA increases constantly [17, 18]. Same dynamics can be seen in our study (Fig.3). As You can see in Fig. 3, there is a significant difference in a background MDA content depending on the variety of pepper. Speed of lipid peroxidation processes in Nikita pepper is higher comparative to such in Hercules. That is why after storage content of MDA is same in both hybrids.

First six days of shelf life content of MDA in the control group of fruits has only a tendency to rise (some years studies show statistically insignificant difference). On twelfth day of storage content of MDA grows in 1,6 times in Hercules hybrid fruits and in 2,2 times in Nikita hybrid. This is an evidence of intensification of free radical processes in this stage of storage.

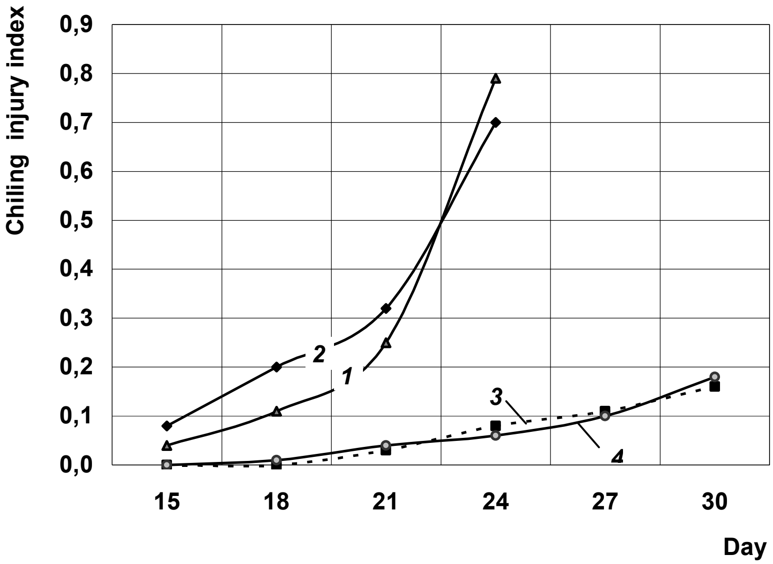

Fig. 2. Index of chilling injury:
1 - fruits of Nikita hybrid without treatment;
2 - fruits of Hercules hybrid without treatment;
3 - temperature preconditioning with antioxidants of Nikita hybrid fruits;
4 - temperature preconditioning with antioxidants of Hercules hybrid fruits.

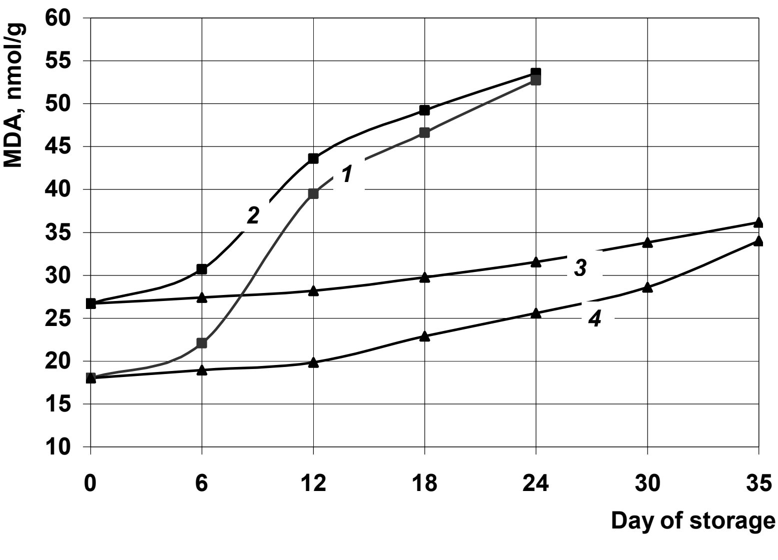

Fig. 3. MDA content in bell peppers during storage:
1 - fruits of Nikita hybrid without treatment;
2 - fruits of Hercules hybrid without treatment;
3 - temperature preconditioning with antioxidants of Nikita hybrid fruits;
4 - temperature preconditioning with antioxidants of Hercules hybrid fruits.

To the end of storage content of lipid peroxidation products in the control samples raises twice in Hercules hybrid and in 2,9 times in Nikita fruits. Differences in the speed of lipid peroxidation collection in different hybrids can be explained with lower antioxidant status of Nikita pepper.

As it is shown in Fig. 3, heat preconditioning combined with antioxidants changes the dynamic of MDA in sweet bell pepper fruit. Till 12th day of storage level of lipid peroxidation products stays almost stable in both hybrids. Then, on each stage of storage, level of MDA raises by 5...6% in Hercules hybrid and by 12...15% in Nikita hybrid. On the 18th day (loss of commercial quality of control samples) level of MDA in the studied samples is lower in 1,7 times for Hercules hybrid and twice for Nikita hybrid in average. That confirms an explanation that exogenous antioxidants inhibit processes of lipid peroxidation.

Development of lipid peroxidation and aging of pepper during storage is considered to be connected with decreasing of antioxidant enzymes (SOD, CAT and PO) activity [17]. Activity of SOD rises till pepper reaches full-red colour and decreases during over maturation [19]. Dynamic of SOD activity during pepper storage also differs in dependence on cultivar sensitivity to cold. Tolerant to cooling cultivars demonstrate increasing of SOD activity during decreasing of such in sensitive [20]. As far as studied hybrids have similar sensitivity to cooling, it is natural that dynamic of SOD activity during storage in both hybrids is same (Fig. 4).

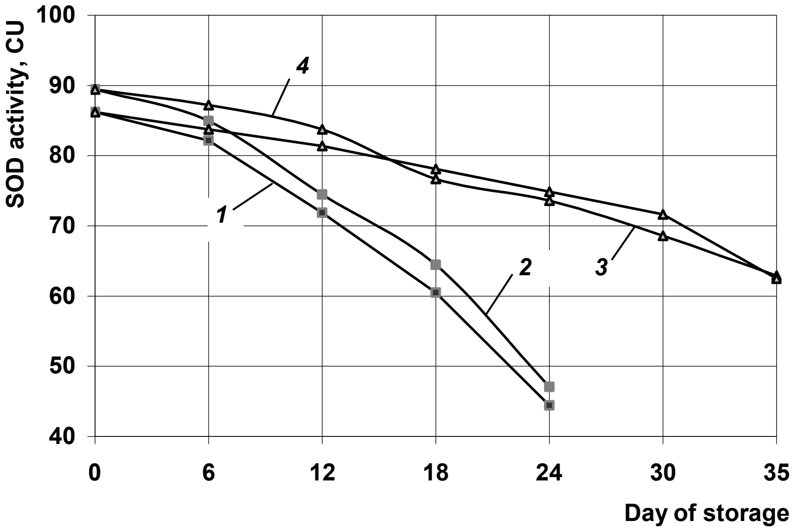

Fig. 4 . SOD activity in bell peppers during storage:
1 - fruits of Nikita hybrid without treatment; 2 - fruits of Hercules hybrid without treatment; 3 - temperature preconditioning with antioxidants of Nikita hybrid fruits;
4 - temperature preconditioning with antioxidants of Hercules hybrid fruits.

Activity of enzyme constantly decreases in control and studied samples of both hybrids. Heat preconditioning with antioxidants significantly decreases speed of SOD deactivation in comparison to the control group. Control samples in 18 days of storage loose 40% of enzyme activity in average. Slowdown of SOD activity in preconditioned fruits is seen only after 35 days of shelf life.

In studied groups probable decreasing of activity of SOD happens only after 12th day of storage. Partly such stabilization of SOD activity can be explained by heat preconditioning, which induces SOD activity directly after conditioning [21]. On 18th day of storage activity of SOD in fruits that were preconditioned with heat and antioxidants is higher by 25% in average in comparison with a control group. In the end of storage of studied fruits (30 days) activity of this enzyme stays on the same level that is in a control samples on 18th day.

These results testify to induction of SOD activity with heat preconditioning with antioxidants which enlarges its ability to dismutate highly toxic superoxide anions and prevents oxidative damage of cells during longer shelf time.

Activity of superoxide dismutase has high reverse correlation to the content of malondialdehyde in control and studied groups that testifies to ability of antioxidant defense system to self regulation and confirms high antioxidant status of fruits (table 1).

Activity of catalase during ripening and storage of pepper decreases independently from stage of maturation [22]. Same results were received in this study (Fig. 5).

Table 1

Correlation between SOD activity and content of MDA in sweet bell pepper fruits during shelf life

Year	Hercules		Nikita	
	Without treatment	Temperature preconditioning with antioxidants	Without treatment	Temperature preconditioning with antioxidants
2009	-0,90	-0,97	-0,95	-0,96
2010	≈-1,00	-0,97	-0,90	-0,96
2011	-0,93	≈-1,00	-0,99	-0,97

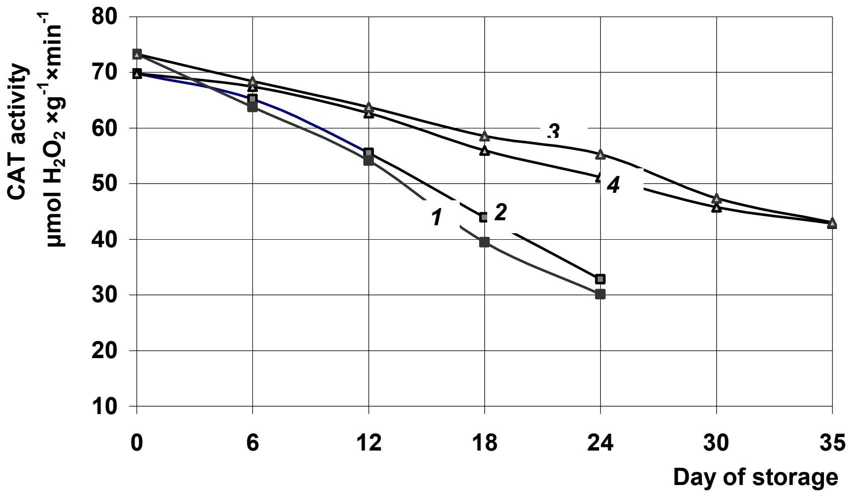

Fig. 5. CAT activity in bell peppers during storage:
1 - fruits of Nikita hybrid without treatment;
2 - fruits of Hercules hybrid without treatment;
3 - temperature preconditioning with antioxidants of Nikita hybrid fruits;
4 - temperature preconditioning with antioxidants of Hercules hybrid fruits.

Although values of CAT activity in studied hybrids differ statistically, these differences smoothen during storage. In the end of study catalase activity in control groups was lower than beginning values in 2,1 times for Hercules hybrid fruits and in 2,4 times for Nikita hybrid.

Heat preconditioning with antioxidants does not change a character of dynamic of catalase activity in pepper fruits. On the other hand, slow down of enzyme deactivation speed is noticeable. On the 18th day of shelf life catalase activity in treated peppers of Nikita hybrid is higher than in control in1,5 times. For Hercules hybrid this difference is 1,3 times. Catalase activity in studied variants to the end of storage is higher than in control group on 18th day by 5...20%, where value depends on the hybrid of pepper. Persistence of catalase activity on the high level in studied fruits preserves pepper tissues from peroxidative damage during longer period of storage.

During all years of study control and studied samples had strong direct correlation between CAT and SOD activities and reverse correlations with MDA (table 2) that testifies to normal functioning of antioxidant defense system.

Table 2

Correlation between activity of CAT, SOD and content of MDA in sweet bell pepper fruits during shelf life

Year	Hercules				Nikita			
	Without treatment		Temperature preconditioning with antioxidants		Without treatment		Temperature preconditioning with antioxidants	
	SOD	MDA	SOD	MDA	SOD	MDA	SOD	MDA
2009	0,97	-0,96	0,99	-0,97	≈1,00	-0,97	0,99	-0,96
2010	0,99	-0,97	0,99	-0,97	0,98	-0,96	0,94	-0,99
2011	0,98	-0,96	0,97	-0,97	0,98	-0,98	0,97	-0,97

Peroxidase activity during storage of pepper can increase during shelf life of fruits in green stage of maturity and decrease during the storage of fruits, which have reached main colour more than by 80% [23, 24]. Activity of this enzyme has a big part in defense from low-temperature stress, as far as gvayakol peroxidase activates with damage from cold temperature [24]. Character of graphic of peroxidase activity change during storage of different hybrids is similar (Fig. 6).

Peroxidase activity is slowly decreasing till 12th day, after that speed of enzyme inactivation grows twice and starting from 18th day activity of peroxidase increases. Similar character of pepper peroxidase dynamic in temperature around 8 ºC is described also in other works [17]. It is obvious that induction of peroxidase in the moment, when fruit lose their commodity quality and exhaust abilities of antioxidant defense mechanisms, are connected with growth of amount of unutilized H2O2. Another reason of peroxidase activity growth in pepper is a development of physiological disorders and microbiological illnesses of fruit [25].

Heat treatment with antioxidants changes a bit the dynamic of peroxidase activity in fruit of sweet bell pepper. Stable decreasing of peroxidase activity can be noticed till the 30th day. On the other hand, speed of activity deactivation is lower. Activity of enzyme in studied fruits of both hybrids on the 18th day is twice higher in comparison to the control. That allows to prolong maintenance of hydrogen peroxide concentration on the stationary level. Increasing of PO activity after 30th day of storage coincides with the loss of commercial quality of pepper.

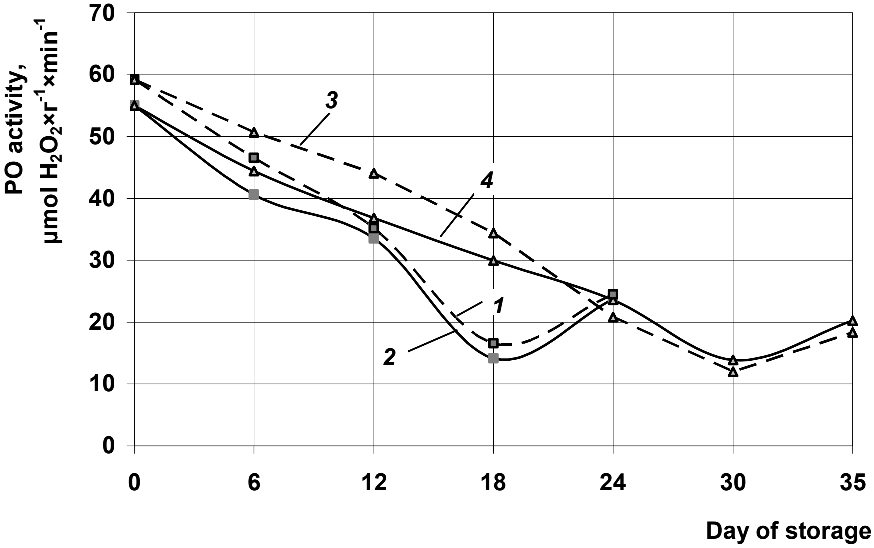

Fig. 6. PO activity in bell peppers during storage:
1 - fruits of Nikita hybrid without treatment; 2 - fruits of Hercules hybrid without treatment; 3 - temperature preconditioning with antioxidants of Nikita hybrid fruits;
4 - temperature preconditioning with antioxidants of Hercules hybrid fruits.

In pepper fruits peroxidase activity is directly correlated to the SOD and CAT activity and reverse correlated to the content of MDA (table 3).

Such correlation dependences testify to the direct influence of peroxidase on the degree of antioxidant defense of pepper tissues. In the conditioned samples strength of correlation between enzymes is higher than in control group, which shows increasing of antioxidant defense system balance for successful neutralization of ROS.

Table 3

Correlation between peroxidase, catalase, superoxide dismutase activity and content of MDA in sweet bell pepper fruits during shelf life

Year	Hercules						Nikita					
	Without treatment			Temperature preconditioning with antioxidants			Without treatment			Temperature preconditioning with antioxidants		
	SOD	CAT	MDA	SOD	CAT	MDA	SOD	CAT	MDA	SOD	CAT	MDA
2009	0,73	0,87	-0,89	0,90	0,92	-0,83	0,83	0,86	-0,92	0,88	0,93	-0,81
2010	0,87	0,82	-0,90	0,93	0,96	-0,90	0,84	0,93	-0,94	0,86	0,93	-0,92
2011	0,80	0,87	-0,92	0,94	0,93	-0,92	0,87	0,94	-0,92	0,91	0,97	-0,91

Conclusions

Using of heat preconditioning with antioxidants before storage of sweet bell pepper fruits leads to decreasing of oxidative damage induced by cooling. Heat preconditioning with antioxidants has a noticeable influence on the content of lipid peroxidation products. In studied groups of pepper level of malondialdehyde stays stable in both hybrids till 12th day of storage. If storage was continued, growth of lipid peroxidation products in conditioned samples has been maintained minimal. Level of MDA in studied samples is lower in 1,7 times in average for Hercules hybrid and twice for Nikita hybrid. This is a confirmation of lipid peroxidation inhibition by exogenous antioxidants.

Heat preconditioning with antioxidants decreases speed of SOD deactivation by 25% and CAT by 30...50%. Heat treatment with antioxidants decreases also speed of peroxidase deactivation in sweet bell pepper fruits and postpone a moment of increasing of its activity. Activity of this enzyme in studied fruits of both hybrids on 18^{th} day is twice higher compared to the control. This lets longer maintenance of hydrogen peroxide concentration on the stationary level. Strong reverse correlations between the content of malondialdehyde and activities of superoxide dismutase, catalase and peroxidase were found and testify to antioxidant functions of these enzymes in control and studied groups of fruits.

Combination of heat and antioxidant treatment for preparation to the storage of sweet bell pepper fruits lets prolong maintenance of functioning of the system responsible for reactive oxygen species utilization.

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