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## THE INFLUENCE OF ANTIOXIDANT HEAT TREATMENT ON UTILIZATION OF ACTIVE OXYGEN FORMS DURING STORAGE OF CUCUMBERS

*Досліджено вплив теплової обробки антиоксидантними композиціями на зниження симптомів окисного стресу, зумовленого охолодженням в огірках. Виявлено, що використання запропонованої обробки зменшує ступінь пошкодження холодом під час зберігання огірків. Комбінування теплової обробки та антиоксидантів сприяє індукуванню ензиматичного комплексу антиоксидантного захисту та зниженню рівня пероксидації тканин під час зберігання огірків.*

**Ключові слова:** зберігання огірків, тепла обробка антиоксидантами, малоновий діальдегід, супероксиддисмутаза, каталаза, пероксидаза.

### 1. Introduction

The analytical review of literature sources, as well as their own experience in the study of the influence of negative factors on the preservation of fruit and vegetable products indicates that oxidative stress is a key link in the pathogenesis of pathological processes in fruits during refrigeration [1–6]. The most significant factor leading to oxidative stress during storage of fruit and vegetable products is low temperatures. Storage in the cooled state makes it possible to significantly reduce the metabolism and continue the post-harvest life of the fruit. However, in thermophilic crops of tropical and subtropical origin, which include cucumbers, the adaptive capacity for cold is extremely limited. Storage at temperatures below the sensitivity limit leads to functional disorders and a rapid loss of quality [7].

To prevent losses during cold storage, slow the metabolism and maintain the high value of vegetable products, in the world practice widely use post-harvest processing [8]. Despite the efforts of researchers, effective solutions to eliminate chilling injury during storage of fruit vegetables have not been found to date. Preventing oxidation reactions with antioxidants (direct approach) can be very effective in combination with measures of an indirect (non-chemical) approach. To better understand the responses of antioxidant defense systems to the effects of various direct and indirect treatments, further research is needed. The obtained information will be important in development of combined post-harvest methods for their practical application. These considerations allow to consider the chosen direction of research as relevant.

### 2. The object of research and its technological audit

*The object of research* is the process of utilization of active forms of oxygen by cucumber tissues during storage.

According to DSTU 3247-95, fresh cucumbers from protected ground can be stored for up to 15 days at

a temperature of 10–14 °C, and from open ground at 7–10 °C [9]. Cucumbers, like other physiologically immature vegetables, quickly respond to a drop in temperature below optimal regimens by the appearance of physiological disorders [10]. Symptoms of chilling injury (CI) in them are the appearance of small dark dregdes with a softened watered surface with time rapidly increase and mollify. It has been established that their development is primarily due to damage to the structure of cell membranes, and the key motive for the degradation of membrane compartments is lipid peroxidation (LPO), which is activated by intense generation of reactive oxygen species (ROS) [11]. The product of free radical oxidation and the biological marker of oxidative stress is malonic dialdehyde (MDA) [12]. Its dynamics during refrigeration is a reflection of the level of oxidative damage of cells and the reserve-adaptive potential of fruits. An increase in the MDA level indicates a strengthening of LPO processes [13].

ROSs are generated in the respiratory chain and other normal metabolic processes. The main ROS utilizers are antioxidant enzymes of superoxide dismutase (SOD), catalase (CAT), peroxidase (PO) [14].

SOD performs a key function in protecting plant organisms from oxidative stress [15]. Preventing the oxidation of cellular macromolecules, SOD is the first line of protection against oxidative damage. Its role is to disproportionate superoxide radicals with the formation of hydrogen peroxide, for the utilization of which other antioxidant enzymes, CAT and PO, are activated.

During the storage of fruits, the synthesis of substances necessary for stable metabolism stops, the mechanism of antioxidant control over the formation of free radicals functions correctly only for a limited time. When irreversible aging processes develop, the content of free radicals rapidly increases [16], the resources of antioxidant protection are expended. This results in a number of metabolic abnormalities and leads to cell death. Therefore, the disposal of excess amount of ROS during storage of cucumbers is the key to maintaining their quality.

### 3. The aim and objectives of research

The aim of research is determination of heat treatment effect with solutions of antioxidant compositions on the level of ROS utilization by cucumber tissues.

The objectives of research:

1. To consider the degree of chilling injury during storage of cucumbers.
2. To analyze the dynamics of the malonic dialdehyde content when storing cucumbers.
3. To analyze the dynamics of activity of superoxide dismutase, catalase and peroxidase when storing cucumbers.

### 4. Research of existing solutions of the problem

To counter internal destructive processes, it is advisable to use a number of mechanisms that inhibit the oxidative stress induced by cooling.

There are various post-harvest technologies for delaying the CI development during the storage of sensitive products. Some of these technologies are of a physical nature and consist mainly of changes in temperature, relative humidity or gas composition of the atmosphere when storing fruits and vegetables. However, storage in a controlled atmosphere, depending on the type of product, can be useful, inefficient or even harmful in reducing chilling injury. Storage in a regulated gas environment has advantages for courgettes, does not affect tomatoes and enhances the symptoms of hypothermia in cucumbers and sweet pepper [17]. It is known that the use of a modified atmosphere helps to mitigate the effects of low temperatures. Cucumbers packed in low density polyethylene have less natural loss of weight and a delay in the development of chilling injury to storage at 5 °C. However, the shelf life of such products does not exceed 15 days [18].

The possibility of using cold acclimatization before storing cucumbers is being studied [19]. It is reported that the previous cold acclimatization allows to reduce the MDA level when storing cucumbers at 5 °C. However, the dynamics of the activity of antioxidant enzymes and the effectiveness of cold acclimatization depended on the duration of the effect of previous acclimatization. In addition, the shelf life of cucumbers did not exceed 12 days.

Most often at the industrial level, conditioning at moderate temperature [20], previous heat treatments (HT) at high temperatures [21, 22] or interruption of cold storage for temporary warming of production (one-time or intermittently) [23, 24]. The positive effect of thermal procedures is associated with the formation and protective action of heat shock proteins (HSP). HSPs are involved in the regulation of ROS formation, protecting cell compartments from oxidative stress [25]. Thermal tolerance, induced by heat stress, can provide protection against cold stress [25, 26]. HT after harvest can not only delay the development of chilling injury, but also modulate the rate of maturation and aging products [21].

Among the possible ways to enhance the tolerance to cold exposure, in addition to the application of physical methods is the use of chemicals with biological activity (growth regulators, antioxidants, microelements) [27, 28]. Fruit processing by compounds that can act as antioxidants and reduce the oxidative damage induced by cool-

ing is widely used in the storage of fruit and vegetable products. Antioxidants such as diphenylamine, dimethylpolysiloxane, ethoxyquin, safflower oil or mineral oils are well known [28, 29]. The antioxidant Xedefon reduces the intensity of respiration and chilling injury to cucumbers [1].

Various external stressors produce similar mechanisms to increase oxidative stress. If two or more stressors are used simultaneously, the stress of cross-tolerance develops more widely in plant tissue [30]. Therefore, a promising strategy for further reducing oxidative damage in fruits during storage is the combination of heat treatment with antioxidants, since there is a high likelihood of synergistic action of both elements of the technology to increase the stress resistance of the system.

### 5. Methods of research

#### 5.1. Plant materials, growing and harvesting conditions.

The experiments were carried out in 2005–2012 on the basis of the laboratory of technology for processing and storing agricultural products, the Institute of Agrotechnology and Ecology of the Tavria State Agrotechnological University (Melitopol, Ukraine). The fruit of cucumbers Masha F1 and Aphina F1 was examined from the open ground. Fruits were stored without unbroken stem, intact, 11...14 cm in size. Cucumbers were treated with solutions of antioxidant compositions with a temperature of 42 °C for 10 minutes by immersion. Compositions consisted of components of bactericidal and antioxidant action: chlorophyllipt (Chl), ionol (I) and lecithin (L) [31]. Fruits were dried and packaged in boxes lined inside with plastic wrap. Storage temperature 8±0.5 °C, relative humidity 95±1 %. The control was taken by untreated fruit.

**5.2. Methods for assessing chilling injury, determination of MDA and enzyme activity.** CI degree was determined at the end of storage under the following regimens and holding fruit for 24 hours at a temperature of 21±2 °C. Repeat five times, with 20 fruits in each.

Evaluation of CI development during storage was carried out on a subjective scale from 0 to 3 points and reflected through the chilling injury index (I), which was calculated by the formula:

$$II = \frac{N_1 \times 1 + N_2 \times 2 + N_3 \times 3}{S}, \quad (1)$$

where  $N_1$ ,  $N_2$ ,  $N_3$  – the number of fruits from the corresponding scale of chilling injury;  $S$  – the total number of fruits in repetition.

Scale parameters: 0 – no damage; 1 – minor damage (≤10 % of fetal surface); 2 – moderate damage (10–30 % of fetal surface), and 3 – significant damage (≥30 %).

MDA concentration is determined by the thiobarbituric method [32].

SOD activity was established with its property to slow down the reaction of self-oxidation of adrenaline in alkaline medium [33] with modification in the part of preparation of raw materials for research. To measure the SOD activity, 0.5 g of plant material was taken, 5 ml of phosphate buffer pH=10.65 was added and ground in a mortar with glass on ice. Then transferred to centrifuge tubes, 0.3 ml of chloroform and 0.6 ml of alcohol were added and centrifuged at 8,000 rpm during 20 minutes.

For the spectrophotometry, a supernatant centrifugate was selected,  $\lambda=347$  nm. SOD activity was expressed in conventional units, showing the percent inhibition of adrenaline autooxidation.

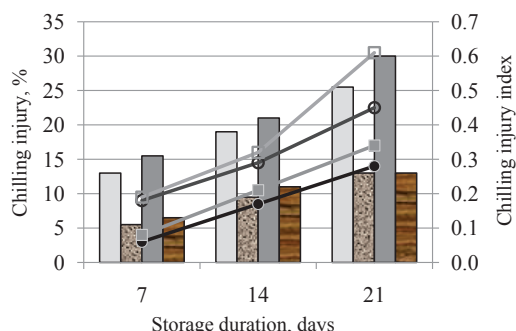
CAT activity was established by titration of the non-deposited residue of hydrogen peroxide with sodium thio-sulfate [34].

PO activity was established by titration of the non-deposited residue of hydrogen peroxide during oxidation of pyrocatechol [35].

## 6. Research results

**6.1. Chilling injury.** When storing cucumbers at a temperature of 8 °C, the initial signs of supercooling were manifested by depressed dark spots over 3 mm in the control lot already on the 7th day of storage. However, intensive damage development occurs from 14 days.

One of the effective measures to reduce the sensitivity to cooling during storage is preliminary heat treatment [21, 36]. The conducted studies confirm the following data [4, 6]. Immersion of cucumbers in water with a temperature of 42 °C for 10 minutes (regimes are selected on the basis of literature sources [36, 37], before depositing for storage, significantly reduces the percentage of cold-affected specimens and reduces the severity of hypothermia symptoms (Fig. 1)).

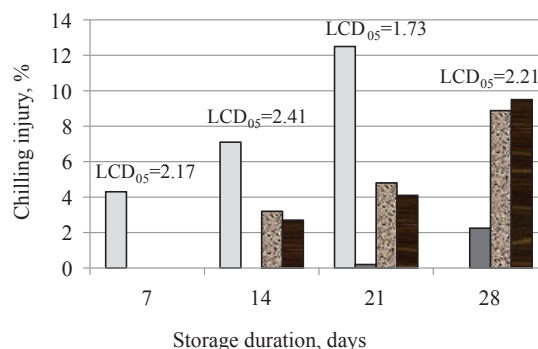


**Fig. 1.** Chilling injury during storage of cucumbers: □, ○ – Masha without treatment; ■, ● – Masha with treatment; □, ○ – Aphina without treatment; ■, ● – Aphina with treatment

As can be seen from Fig. 1, preliminary heat treatment almost doubles the number of fruits with chilling injury. In addition, the severity of hypothermia symptoms decreases, especially for the more susceptible cucumbers of Aphina, where the index of damage reaches 0.6. The difference in the severity of hypothermia symptoms increases with increasing shelf life.

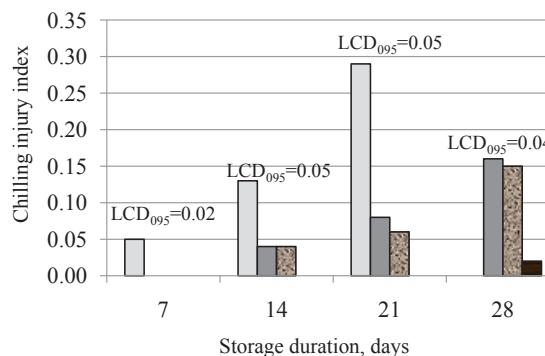
To induce the protection of tissues from stress and prevent losses from low-temperature damage, the joint effect of heat treatment and antioxidant compositions was investigated. Antioxidant substances were used at concentrations of 0.036 I; 0.50 Chl; 4 L.

Post-harvest heat treatment with antioxidant compounds induces cold cucumber tolerance. In the fruits treated with Chl+L and Chl+I and the first minor signs of hypothermia were noted 7 days later than in fruits with heat treatment (day 14). Fruits treated with a composition of antioxidants Chl+I+A, showed sensitivity to low temperatures already at the end of storage (Fig. 2).



**Fig. 2.** Chilling injury during storage of cucumbers, average data for two grades: □ – heat treatment with water; ■ – heat treatment with antioxidant Chl+L; ■ – heat treatment with antioxidant Chl+I; ■ – heat treatment with antioxidant Chl+I+L

As can be seen from Fig. 3, antioxidants also contribute to alleviating chilling injury.



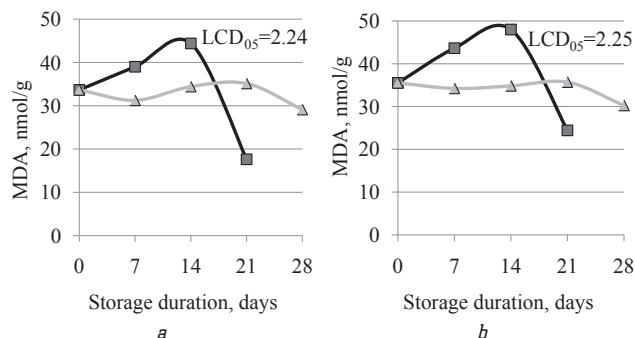
**Fig. 3.** Chilling injury index, average data for two grades: □ – heat treatment with water; ■ – heat treatment with Chl+L; ■ – heat treatment with Chl+I; ■ – heat treatment with Chl+I+L

On the 21st day, when the fruits with heat treatment were removed for storage, the severity of the chilling injury in cucumbers treated with Chl+L was 3.6 lower than in the control ones. CI index in cucumbers treated with Chl+I was 4.8 times less than in fruits with heat treatment. In fruit treated with a three-component antioxidant, practically no fruit was shown with a score of damages 2 and 3, significantly reducing the CI index.

**6.2. ROS utilization level.** MDA background level in cucumbers, on the average for the years of research and hybrids is 35 nmol/g (Fig. 4). Since oxidative stress develops immediately after separation from the mother plant, continues during cooling and deepens during storage, then the growth of LPO products is natural. Such increase in the MDA amount is observed after a week of storage of control samples.

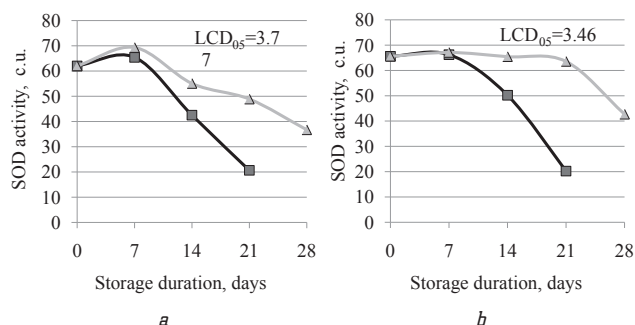
The increase in MDA amount in control samples occurs up to 14 days, when their commercial quality implies removal from storage. Further storage leads to a sharp decrease in the MDA level. Such decrease in MDA is natural when the cucumber fruits are ripe. As noted by Chinese researchers, MDA level decreases in cucumbers with the first manifestations of yellowing [38].

In research fruits, regardless of the hybrid, for the entire storage period, the deviations from the baseline MDA value are minimal, which, according to the results, reflects the stable functioning of the antioxidant system.



**Fig. 4.** Dynamics of MDA quantity: *a* – Aphina; *b* – Masha; —■— – control; —▲— – heat treatment with Chl+I+L

**6.3. ROS utilization through a system of high-molecular antioxidants. Superoxide dismutase.** At the beginning of the storage of cucumbers, some increase in the SOD activity occurs (Fig. 5). This can be explained by a response to cold stress. Many researchers describe the SOD induction under stress conditions [15].

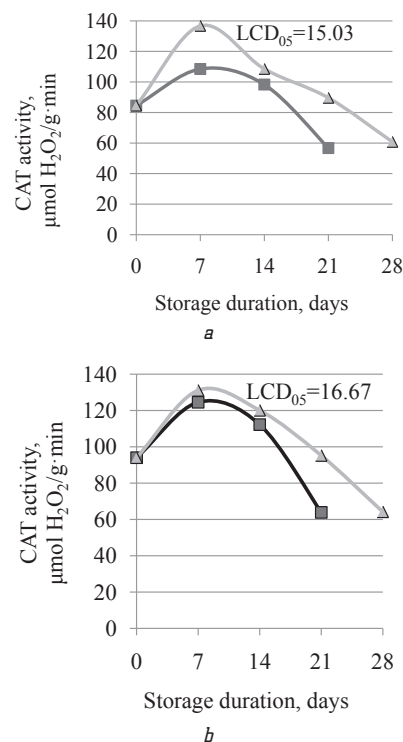


**Fig. 5.** Dynamics of SOD activity: *a* – Aphina; *b* – Masha; —■— – control; —▲— – heat treatment with Chl+I+L

Since the cucumbers of the Masha hybrid have a high cold tolerance in comparison with the Aphina hybrid, they demonstrate a much higher level of SOD activity. Further dynamics of SOD activity in control samples, regardless of varietal features, is similar. After 7 days, a sharp decrease in the activity of the enzyme occurs. After 21 days, SOD activity is three times less than the initial value.

Varietal specificity is preserved during research of cucumbers. The cold-tolerant Masha supports the initial SOD activity up to 21 days, and then it goes down by one third. Aphina hybrid shows the SOD dynamics activity close to the control ones. However, the rate of decline in enzyme activity is much lower, and this contributes to the SOD activity at a level of 60 % of the initial value after 28 days of storage.

**Catalase.** At the beginning of storage of cucumbers, catalase activity increases in control and test samples (Fig. 6). This increase in the activity of this enzyme during the first stage of storage is natural, because, according to various studies, CAT is activated in response to cooling [39]. More sensitive to cooling, Aphina cucumbers are characterized by low levels of CAT activity from begin of storage. After a week of storage, the activity of the enzyme increased by an average of 28 % in Athens and by 34 % in Masha relative to the initial value that confirms the theory of the catalase's main role in protecting against chilling injury [39].



**Fig. 6.** Dynamics of catalase activity: *a* – Aphina; *b* – Masha; —■— – control; —▲— – heat treatment with Chl+I+L

In the experimental samples, the exogenous treatment with antioxidants is particularly noticeable in the heat-sensitive fruit of the Aphina hybrid. CAT activity of the processed cucumbers of Aphina increases on the seventh day on average by 60 % of the initial value, which is 32 % more than in the control. At the same time, for cold-tolerant Masha, this growth is about 40 %, and in comparison with the control, it is only 8 % more. Therefore, heat treatment with antioxidants strengthens the protection of the endogenous system of maintaining normal metabolism only when necessary.

During further storage, through the development of oxidative stress, CAT activity decreases gradually in all groups of cucumbers. The difference is only in the rate of decline, just as in the SOD activity. Decreased SOD activity may also indirectly affect the decrease in CAT activity, since there is evidence of the sensitivity of catalase to a superoxide anion radical that can inhibit the enzyme [40].

**Peroxidase.** Variety features in the activity of peroxidase in cucumbers practically do not manifest. Unlike catalase, at the beginning of storage of cucumbers peroxidase activity is sharply inhibited in all variants (Fig. 7).

The decrease in the PO activity during the first stage of storage of cucumbers is fixed by other authors [41, 42], which is typical for cooling sensitive cucumbers and is a reaction to a decrease in temperature. In addition, heat treatment can also reduce the PO activity [21].

However, in spite of the multifunctionality of this enzyme, there may be other causes of changes in its activity.

After 7 days of storage, PO activity is not only restored, but also grows relative to the initial value. An increase in peroxidase activity is a normal process in aging of tissues [41, 42].

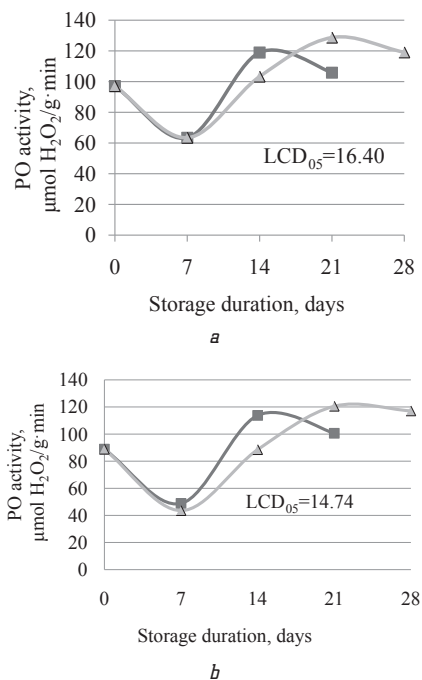


Fig. 7. Dynamics of peroxidase activity: a – Aphina; b – Masha; —■— – control; —▲— – heat treatment with Chl+I+L

Further, the dynamics of peroxidase control and experimental fruit is different. Processed cucumbers continue to increase the PO activity, while control samples already demonstrate a decrease in enzyme activity that may be evidence of catabolic processes. In addition, it has been proven that cell wall peroxidase, which is capable of forming hydrogen peroxide at neutral pH, restores the peroxidase property when pH is shifted to the acid side [43]. It is at this time there is an increase in acidity in research fruits that may affect the increase in PO activity. On the 28th day of storage in prototypes, there is also a gradual inhibition of PO activity.

## 7. SWOT analysis of research results

**Strengths.** The strong side of this research is the results of the effect of heat treatment with antioxidants on the degree of ROS utilization in the tissues of cucumbers. In favor of this statement is the lack of such information in contemporary world literature sources. The obtained results allow to understand the mechanism of increasing the stress resistance of cucumber during storage. This significantly reduces the percentage of cold-affected specimens and reduces the severity of hypothermia symptoms. Compared to other methods that induce cold tolerance, heat treatment with antioxidants can be easily integrated during the preparation stage of storage without significant increase in logistical costs, since the cost of antioxidants is 80.2 UAH/ton (3.1 USD/ton). At the same time, losses from physiological disorders are reduced, mass losses are reduced and the yield of standard products is increased after storage by 14 % compared to the control. The shelf life of the product is increased by 5 days. In addition, due to the inhibition of metabolism in cucumbers with heat treatment with antioxidants, valuable phytonutrients are better preserved, which will have a social effect on the health of the population.

**Weaknesses.** The weak side of this research is analysis of the effect of this treatment on the exclusively enzymatic complex of antioxidant protection, without encompassing non-enzymatic antioxidants.

**Opportunities.** However, the analysis of the reaction of each element of the cucumber antioxidant system will lead to an overload and a heavy perception of this work. To prevent this, it is advisable to include material on the effect of post-harvest treatment on non-enzymatic antioxidants of cucumbers in a separate work, and it will be a prospect of further research.

The introduction of cucumber storage technology with the use of heat treatment with antioxidants due to lengthening the shelf life, reducing the natural loss of weight and maintaining high product quality provides an increase in net profit by 5624.4 UAH/ton (217 USD/ton) and profitability level of storage by 107.7 %.

**Threats.** The difficulty in adjusting the symptoms of the oxidative stress of cucumber is the unstable content of endogenous antioxidants, the annual fluctuations of these indicators depending on the agro-climatic growing conditions.

With the introduction of cucumber storage technology with the use of antioxidant heat treatment, the actual costs of the enterprise will increase by 272.8 UAH/ton (10.5 USD/ton) or 80 %.

## 8. Conclusions

1. It is established that the use of exogenous biologically active substances induces cold cucumber tolerance. Depending on the used antioxidants, the severity of hypothermia symptoms decreases in 3.6...4.8 times compared with the fruits with heat treatment. Appearances of chilling injury appear 7 days later when applying Chl+I and Chl+L.

The combination of heat treatment and the antioxidant composition Chl+I+A makes it possible to avoid chilling injury until the end of storage.

2. It is shown that cucumbers with heat treatment with antioxidants for the entire storage time demonstrate minimal deviations from the background value of malonic dialdehyde. This reflects the stable functioning of the antioxidant system.

3. It is established that heat treatment with antioxidants allows to slow down the rate of decrease in superoxide dismutase activity. This contributes to SOD activity at a level of 60 % of the initial value after 28 days of storage.

The use of heat treatment with antioxidants induces catalase activity in cucumbers. The catalase activity of the processed cucumbers grows according to the degree of their cold tolerance. The maximum induction of catalase activity is observed in the hybrid Aphina, which more sensitive to cold. Therefore, heat treatment with antioxidants strengthens the protection of the endogenous system of maintaining normal metabolism only when necessary.

Heat treatment with antioxidants regulates the peroxidase activity in cucumbers, which is evidence of the slowing down of aging processes.

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#### **ВЛИЯНИЕ ТЕПЛОЙ ОБРАБОТКИ АНТИОКСИДАНТАМИ НА УТИЛИЗАЦИЮ АКТИВНЫХ ФОРМ КИСЛОРОДА ПРИ ХРАНЕНИИ ОГУРЦОВ**

Исследовано воздействие тепловой обработки антиоксидантными композициями на снижение симптомов окислительного

стресса, обусловленного охлаждением в огурцах. Установлено, что применение такой обработки снижает степень повреждения холодом при хранении огурцов. Комбинирование тепловой обработки и антиоксидантов способствует индуцированию энзиматического комплекса антиоксидантной защиты и снижению уровня перекисидации тканей при хранении огурцов.

**Ключевые слова:** хранение огурцов, тепловая обработка антиоксидантами, малоновый диальдегид, супероксиддисмутаза, каталаза, пероксидаза.

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