Enzymatic antioxidants in tomatoes and sweet bell pepper fruits under abiotic factors

Olesia Priss, Valentina Kalytka

Tavria State Agrotechnological University, Melitopol, Ukraine

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Corresponding author:

Olesya Priss
E-mail:
olesyapriss@gmail.com

Abstract

Introduction. In order to maintain normal metabolism in the fruit separated from the mother plant and to protect plant tissues from oxidative damage the complex of high-molecular antioxidants is especially important.

Materials and methods. Fruits of tomatoes and sweet bell peppers grown under field conditions during the 2005 - 2012 years were studied. Catalase and peroxidase activity was determined by undecomposed rest titration of hydrogen peroxide. SOD activity was determined by its ability to inhibit the reaction of adrenaline auto-oxidation with a modification in the preparation of raw materials. The content of malondialdehyde was determined by thiobarbituric acid method.

Results and discussion. Activities of superoxide dismutase and catalase in both solanaceous vegetables are inversely correlated with the sum of temperatures of fruit development and ripening period. Rainfall induces activity of these enzymes in the pepper fruits, but do not affect on tomatoes.

Peroxidase activity inversely correlates with the sum of temperatures of fruit development and ripening period for both cultures (r=-0,63…-0,69). For tomatoes, from all of the above mentioned antioxidant enzymes, peroxidase is the most dependent upon weather factors. Peroxidase activity is directly dependent on the amount of rainfall during the development and ripening of the fruit for both cultures. Peroxidase activity is also dependent on rainfall of the whole vegetation period for pepper.

Conclusion. The sum of temperatures during fruit development and ripening has determining influence on the activity of antioxidant enzymes in tomatoes and pepper (r=-0,58…-0,76).

Introduction

Normal metabolism in living cells is represented by the set of the strongly connected biochemical processes. One of them is the reactive oxygen species (ROS) production. These short-lived radicals are involved in many physiological functions as well as in a number of pathological processes [1].To maintain the redox balance, the organism synthesizes a complex of high- and low-molecular bioantioxidants, which are able to stabilize the level of ROS. However, under the unfavorable conditions, dynamic balance between ROS and antioxidants (AO) may be perturbed. This leads to the oxidative stress, which is considered to be the root cause of many cardiac, cancer and other diseases. Epidemiological studies confirm that a diet rich in antioxidants is associated with a low incidence of degenerative diseases. Epidemiological studies confirm that a diet rich in antioxidants is associated with a low incidence of degenerative diseases [2]. Significant evidences of the effectiveness of food bioactive compounds in the maintenance of health and prevention of many diseases are the impetus for the antioxidant compounds in fruits and vegetables monitoring.

Complex of tissue antioxidants in solanaceous vegetables includes a strong system of low-molecular compounds [3, 4]. Low-molecular AO intercept free radicals, thus reducing ROS and products of oxidative modification. Formation of the complex of low-molecular antioxidants in solanaceous fruits under the influence of abiotic factors was investigated by many authors [5, 6]. However, to maintain normal metabolism in the fruit separated from the mother plant and to protect plant tissues from oxidative damage, the enzymatic complex of AO is especially important. It is known that resistance to low temperatures during storage and slowing senescence is closely related to the high-molecular antioxidants [7, 8]

Three main enzymes - superoxide dismutase (SOD), catalase (CAT) and peroxidase (PO) - ensure enzymatic system of tissues defense from oxidative damage [9].

SOD is a key enzyme of the first line of antioxidant defense in the cells of all aerobic organisms [10].That is why some authors propose to evaluate the antioxidant properties of plant material by determination of SOD activity [11]. SOD catalyzes dismutation of superoxide radicals. The result of superoxide anions dismutation is hydrogen peroxide. According to that, a group of enzymes, which can utilize hydrogen peroxide is a necessary element of antioxidant defense in plants. In the plant cell these enzymes are catalase and peroxidase that work in the second line of defense. Catalase catalyzes the conversion of H_2O_2 into two molecules of water and O_2 [12]. However, in some cell compartments catalase is almost absent, thus the functioning of other enzymes, which are involved in detoxification of hydrogen peroxide becomes necessary. Peroxidases react with hydrogen peroxide to form oxidation products of the enzyme and water [13].

The activity of antioxidant enzymes of raw fruits and vegetables varies widely and depends on many biotic and abiotic factors [7-10]. The formation of the enzymatic antioxidant complex in tissues of solanaceous fruits under the influence of abiotic factors remains open. Aim of this work was to determine the influence of hydrothermal conditions on the formation of complex of high-molecular antioxidants in fruits of tomato and pepper. To achieve this goal it is necessary to estimate the level of enzyme activity of SOD, CAT and PO for different weather conditions of vegetation period.

Materials and methods

Two cultivars of tomatoes Rio Grande Original (the Rio Grande) and Novachok and two cultivars of bell pepper fruits Nikita F1 and Hercules F1 grown under field conditions in Melitopol district during the 2005 - 2012 years were studied. The amount of temperatures during growing season, the sum of temperatures during fruit development and ripening (30 days before harvest for peppers and 40 for tomatoes), and the Seljanin's hydrothermic coefficient (HC) as an integrated index of hydrothermic parameters were calculated according to meteorological data collected in Melitopol.

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*]]. 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*]]. 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 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. 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* {*ektrofotometrychni metody v praktytsi fiziolohiyi, biokhimiyi ta ekolohiyi roslyn*}].

Results and Discussion

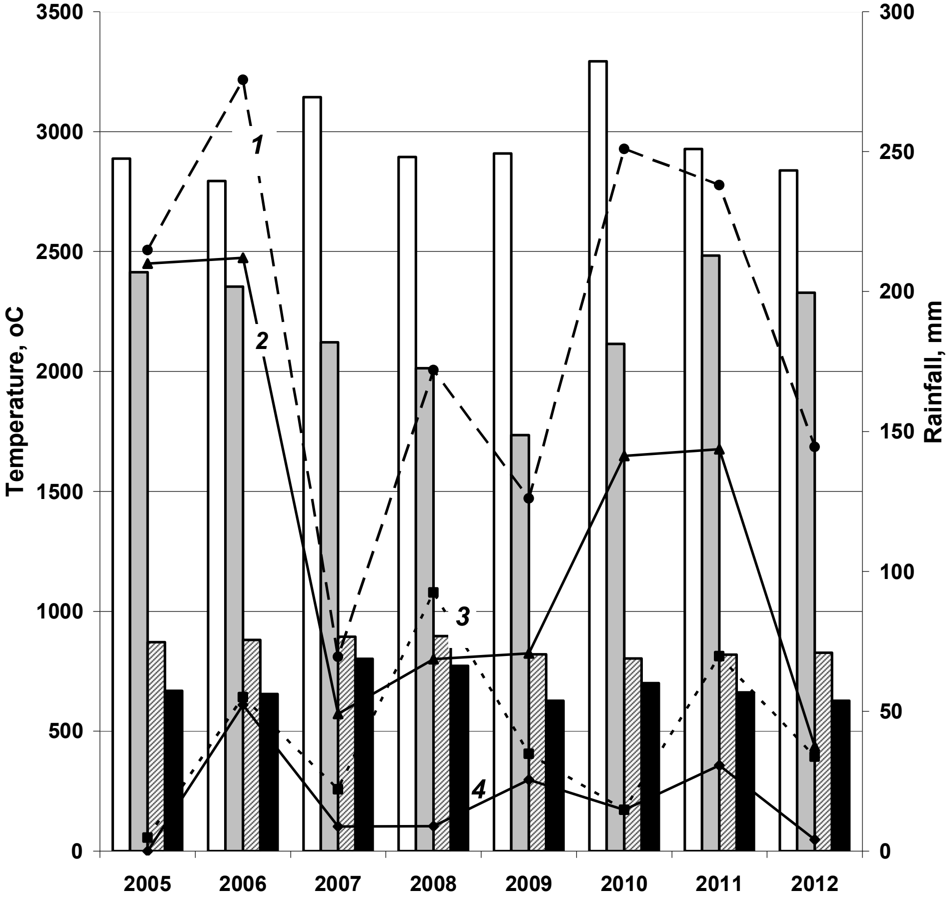
Weather conditions of growing of solanaceous crops during the studies varied in a wide range (Fig 1).

There were hot and dry years (2005, 2007), as well as cool (2009) and moderately moist (2006). Three of the eight years of research (2007, 2009, 2012) can be characterized as a strictly dry as HC was below 0,5 (Tab. 1).

Table 1

Hydrothermic coefficients during growing period of solanaceous vegetables

Number	Fruit	Years of research							
		2005	2006	2007	2008	2009	2010	2011	2012
HC of growing season	pepper	0,87	0,90	0,23	0,34	0,41	0,67	0,58	0,16
	tomato	0,74	0,99	0,22	0,59	0,43	0,76	0,81	0,48
HC of fruit development and ripening period	pepper	0,00	0,80	0,11	0,12	0,41	0,21	0,46	0,07
	tomato	0,06	0,62	0,25	1,03	0,42	0,18	0,85	0,41



– the sum of temperatures during the growing season of tomatoes
– the sum of temperatures during the growing season of pepper;
– the sum of temperatures during the development and ripening of tomatoes fruit;
– the sum of temperatures during the development and ripening of pepper fruit;
1 – rainfall during the growing season of tomatoes;
2 – rainfall during the growing season of pepper
3 – rainfall during the development and ripening of tomatoes fruit
4 – rainfall during the development and ripening of pepper fruit.

Fig. 1. Weather growing conditions of solanaceous fruits (2005 ... 2012)

HC of solanaceous growing season approached to the optimum only in 2006, reaching to 0,99 for tomatoes and 0,90 for peppers. Other years can be characterized from severely to moderately dry in terms of moistening. During fruit development and ripening it wasn't raining at all (2005 pepper), or it was raining very slightly.

SOD activity in both solanaceous crops during years of research ranged from 72 to 95 CU. Variability of SOD for both tomatoes and pepper was insignificant - variation coefficient didn't exceed 10%. A varietal difference in SOD activity is insignificant for both solanaceous fruit (Fig. 2).

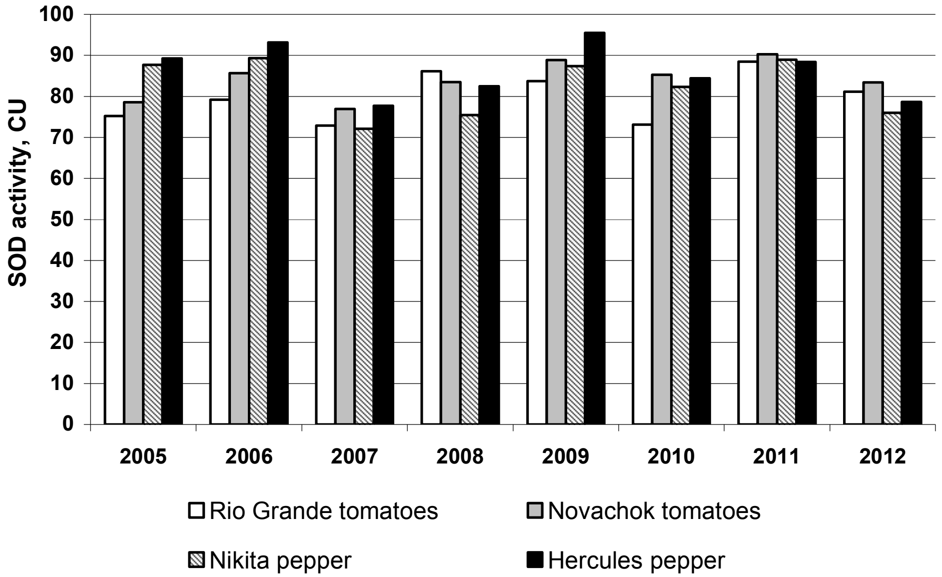


Fig. 2. SOD activity in solanaceous fruits, CU

Maximum of SOD activity was fixed in 2006 and 2009 for peppers and in 2011 for tomatoes. In the same years during fruit development and ripening the minimal sum of temperatures in combination with sufficiently large rainfall was observed. The lowest SOD activity was observed in 2007 in hot and arid conditions (HC = 0,22 ... 0,31).

The analysis of pair correlations confirms the inverse dependence of SOD activity on sum of temperatures during fruit development and ripening of solanaceous crops: r=-0,58; -0,60 (Fig. 3).

Unlike in tomatoes, in peppers SOD activity is closely dependent on other hydrothermal indicators. As shown in Fig. 3, weather factors associated with rainfall induce a SOD activity in fruits of pepper, but have no effect on this enzyme in tomatoes. This fact indicates higher tolerance of studied tomatoes to the lack of moisture and supports the idea of reduced SOD activity in response to drought in sensitive plants [14].

Variability of CAT activity in tomato in years of research was high: *V*=28,54; 28,74 %, while in the fruit of pepper it remained average: *V*= 18,32; 18,67 %. Varietal differences in CAT activity in the fruits of both cultures are insignificant. In the most dry years (2005, HC of fruit development and ripening period 0,06 (see tab. 1)) the activity of CAT in tomatoes is 2-3 times lower than in pepper. In years with sufficient moisture during the period of formation and ripening (in 2008, HC of the period of development and ripening is 1,03) catalase activity is lower than in pepper only by 32 ... 43% (Fig. 4).

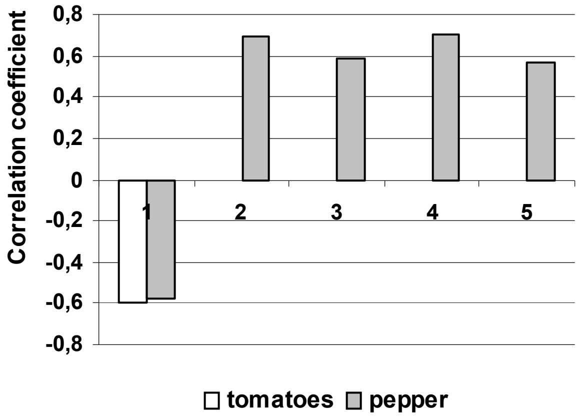


Fig. 3. Significant relationship between weather conditions and the SOD activity in solanaceous fruits, p<0,05:

1 – the sum of temperatures during fruit development and ripening;
2 – rainfall during the growing season;
3 – rainfall during fruit development and ripening;
4 – HC of growing season;
5 – HC of fruit development and ripening period.

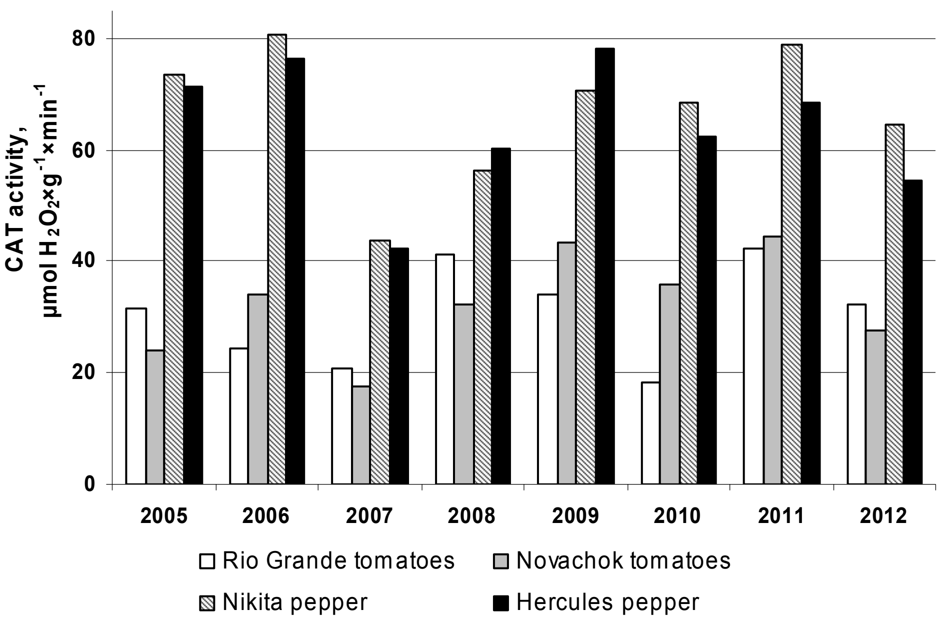


Fig. 4. CAT activity in solanaceous fruits

CAT activity remain low in both solanaceous vegetables in 2007, when HC of growing season and HC of fruit development and ripening period were low. Pepper shows high CAT activity in years with more rainfall during fruit development and ripening period (2006, 2009 and 2011). Maximum of catalase activity in tomato was recorded in 2011, when the sum of temperatures during fruit development and ripening was the lowest.

Analysis of pair correlation dependencies of CAT activity from weather factors allowed us to establish existence of the strong inverse correlation with the sum of temperatures during fruit development and ripening for both cultures: r=-0,65…-0,76 (Fig.5).

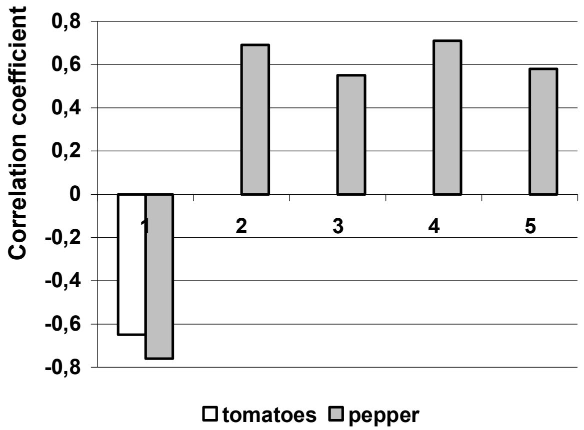


Fig.5. Significant relationship between weather conditions and the CAT activity in solanaceous fruits, p<0,05:

1 – the sum of temperatures during fruit development and ripening;
2 – rainfall during the growing season;
3 – rainfall during fruit development and ripening;
4 – HC of growing season;
5 – HC of fruit development and ripening period.

The CAT activity in pepper closely depends on factors related to the rainfall. Rainfall and HC directly influence the induction of CAT activity in fruits of pepper, but have no effect on this enzyme in tomatoes. As it was noted by some researchers, stress induced by drought causes a decrease in catalase activity in susceptible varieties, but increase in tolerant [15].

Peroxidase activity in solanaceous crops differs significantly. PO activity in tomato is approximately 2.5 times lower than in pepper. The variability of this enzyme is high in both solanaceous fruits (V = 33,73…51,41 %). However, especially significant differences are in Rio Grande tomato cultivar - more than 5 times over the years studies (Fig. 6).

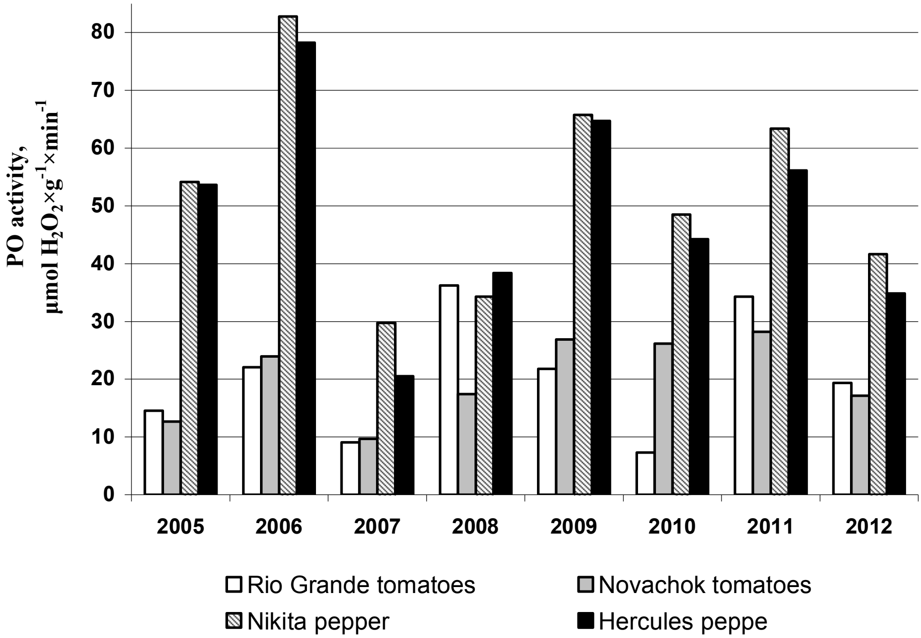


Fig. 6. PO activity in solanaceous fruits:

Maximum of PO activity in different varieties of tomatoes was fixed in different years. For tomatoes Rio Grande that was 2008, for Novachok - 2011. Both years could be characterized by high temperatures and rainfall during growing season and rainfall during the fruit development and ripening period (Fig.1). Peaks of PO activity in pepper fruits were observed when the highest rainfall fell out during the growing season (2006), and in 2009, while lowest temperatures of the growing season. Minimal PO activity was observed in 2007 for both species of solanaceous vegetables. The sum of temperatures during development and ripening of pepper fruit was the highest among other years and also growing season for tomatoes was characterized by high temperatures that year.

Analysis of pair correlations indicates the presence of a strong significant relation between peroxidase activity and the sum of temperature during fruit development and ripening for both cultures (r = -0,63; -0,69) (Fig. 7).

In tomatoes peroxidase is the most related to weather factors comparing to the other examined antioxidant enzymes. Peroxidase activity in fruits of both cultures shows a significant direct relationship with rainfall and HC of fruit development and ripening period. Activity of this enzyme in pepper is also dependent on rainfall and HC of growing season. It can be associated with a shorter vegetation period in pepper.

The level of malondialdehyde, which is a marker of oxidative stress [16], in peppers is 5-6 times higher than in tomatoes (Fig. 8).

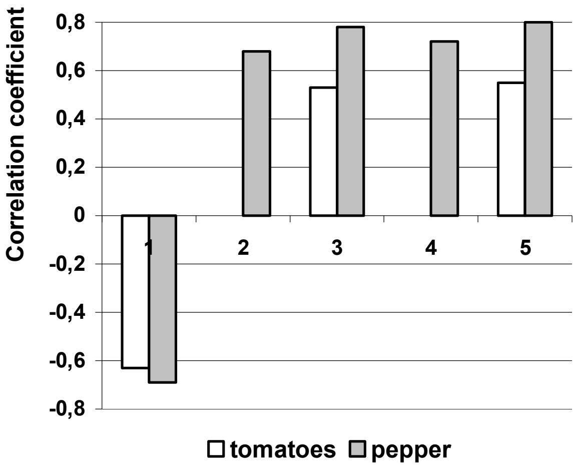


Fig.7. Significant relationship between weather conditions and the PO activity in solanaceous fruits, p<0,05:

1 – the sum of temperatures during fruit development and ripening;
2 – rainfall during the growing season;
3 – rainfall during fruit development and ripening;
4 – HC of growing season;
5 – HC of fruit development and ripening period.

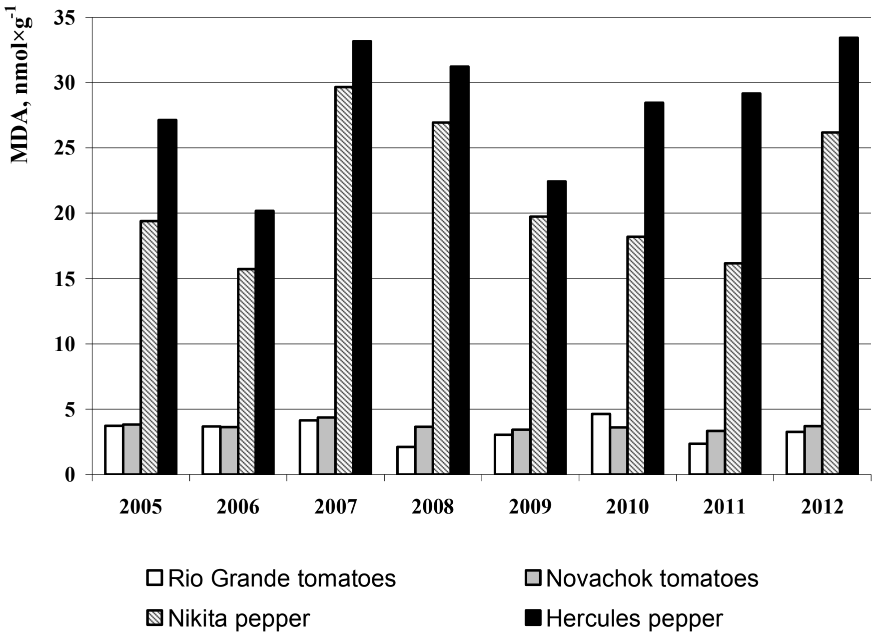


Fig. 8. Level of MDA in solanaceous fruits

Maximal levels of MDA in pepper fruits were fixed in dry years (2007, 2008, 2012). Maximum of MDA in tomato fruit was observed in 2007 and 2010, when the HC of fruit development and ripening period did not exceed 0,25.

Enzymatic antioxidants are very strongly dependent on each other and explicitly inversely correlated with the level of MDA (Tab. 2).

Table 2

Pair correlations of enzymatic antioxidants

Fruits	Enzyme	CAT	PO	MDA
Tomato	SOD	0,90	0,87	-0,71
	CAT	1	0,89	-0,80
	PO	0,89	1	-0,90
Pepper	SOD	0,86	0,85	-0,57
	CAT	1	0,92	-0,81
	PO	0,92	1	-0,80

Activity of SOD in tomatoes and peppers strongly directly correlated with activity of CAT and PO (r=0,85…0,90). The same strong correlation is also observed between CAT and PO: r=0,89; 0,92. Obviously the MDA level increases due to the dismutation of $O_2^{\cdot -}$ and harmful activity of produced H_2O_2, which is utilized by catalase and peroxidase. In tomatoes PO shows the highest correlation to the level of MDA, thus indicating its crucial role in the enzymatic antioxidant defense. In pepper correlations both between CAT and MDA and between PO and MDA are equally strong. This fact indicates the equal importance of these enzymes for protection of tissues from oxidative damage.

Conclusions

The sum of temperatures during fruit development and ripening period has the determining influence on the activity of antioxidant enzymes in tomatoes and pepper. (r = -0,58 ... -0,76). Rainfall induces activity of superoxide dismutase and catalase in fruit of pepper, but has no effect on these enzymes in tomatoes. Peroxidase activity in tomato fruit is the most related to the hydrothermal factors comparing to other investigated antioxidant enzymes.

Peroxidase activity in both cultures depends on rainfall and hydrothermic coefficient of the period of fruit development and ripening. Peroxidase activity in pepper fruit also depends on rainfall and hydrothermic coefficient of growing season. Enzymatic antioxidants of tomatoes and peppers dependent strongly one another (r = 0,85 ... 0,92) and are explicitly inversely correlated with level of MDA (r = -0,57 ... -0,90).

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The possibilities of using of essential oils in dairy products. 2. Dill (*Anethum Graveolens*)

Iliana Kostova[1], Dimitar Dimitrov[1], Mihaela Ivanova[2], Radka Vlaseva[2], Stanka Damyanova1, Nastya Ivanova[1], Albena Stoyanova[2], Oleksii Gubenia[3]

1 - Ruse University „A. Kanchev", Branch - Razgrad, Bulgaria
2 - University of food technologies, Plovdiv, Bulgaria
3 – National university of food technologies, Kyiv, Ukraine

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Corresponding author:

Stanka Damyanova
E-mail:
sdamianova@uni-ruse.bg

Abstract

Introduction. The possibility of using of the essential oil of dill (Anethum graveolens) in dairy products has been studied. The composition, antimicrobial properties and the effect of the essential oil of dill on the microorganisms of starter cultures for dairy products has been studied.

Materials and methods. The chemical composition of the oil is determined chromatographically. Antimicrobial effect of the essential oil of dill is determined against Gram-positive, Gram-negative bacteria, yeasts, fungi and two cultures for white brined cheese using the agar diffusion method.

Result and discussion. The analyses of the chemical composition of the essential oil of dill show that monoterpenes hydrocarbons (47.97%) dominate, followed by monoterpenes oxygen (37.52%). Considerably less is the quantity of sesquiterpenes, aliphatic and aromatic hydrocarbons.

The studies of the antimicrobial activity of the essential oil of dill show that there is weak antibacterial and high antifungal activity.

The antimicrobial effect of the oil against the lactic acid bacteria included in the composition of the starter culture is weak. The minimum inhibitory concentration is 0.05% and the minimum bactericidal concentration is 0.5%. These concentrations are higher than the concentrations that can be used in food products.

Conclusion. The essential oil of dill exhibits antimicrobial activity but does not inhibit the development of the lactic acid bacteria in the dairy starter cultures. It is a suitable natural addition to dairy products.