

EFFECT OF LONG-TERM STORAGE OF PEPPER (*Capsicum annuum* L.) SEEDS ON THEIR VIABILITY MEASURED BY SELECTED THERMODYNAMIC PARAMETERS

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Abstract. The main goal of the present study was to investigate the changes in viability potential and selected thermodynamic parameters of peppers seeds in aging during storage as well as the relationship between them. The water content and storage temperature of seeds were used for determining differential thermodynamic parameters: Gibbs free energy, entropy, and enthalpy. The experiments were carried out with aging pepper seeds stored in ambient (uncontrolled) conditions for 132 months. It was found that the viability potential deteriorated more rapidly after 4–5 years of storage. Thermodynamic parameters – differential enthalpy and entropy, and free energy decreased with decline of germination. Therefore these parameters can be used as means for better understanding of aging processes and this is of great importance for agricultural practice because it allows more detailed description and prediction of the deterioration of pepper seeds during long-term storage.

Key words: seed germination, enthalpy, entropy, Gibbs free energy, seed longevity, seed moisture

INTRODUCTION

Seed deterioration has important economic and ecological consequences. Lowered seed quality on one hand influences the reproduction and productivity of agricultural crops, affects stand establishment and the ability of seeds to persist in soil, on the other hand, it affects the population dynamics of ecosystems [Justice and Bass 1978, Black et al. 2006]. Therefore, the studies about seed aging are very significant and actually contribute to better understanding of this process. Seed quality evaluation can be conducted through physical and germination vigour tests that provide information about viability potential of the seeds under various period of storage [Mc Donald 1994, Akbudak and Bolkan 2010].

The research and measurements of seeds viability according to Roberts [1972] is very important and interesting about the seed producer, agronomist and during storage period about trader and also it is in relationship with preserve of cultivars and genetically diversity. Author told that there is another aspect that is essential for studies of seed viability, which concerns to their aging and has a great scientific and practical significance. There are methods establishing the viability of seeds that are not based on the determination of their germination. That is the TTC test, which is authorized by ISTA (2003) and it also sets the viability status without being placed the seeds for germination.

Temperature and moisture play a fundamental role in determining the storage longevity of seeds [Krishnan et al. 2004]. They underlined that during storage there are critical thermodynamic parameters of water, controlling seed aging reaction kinetics and that the thermodynamic properties are better parameters for characterization of the seed deterioration. Wendell [1997] founded that the critical temperatures for long-term storage of the seeds were near or below their glass transition temperatures, which indicates the requirement for the presence of the glassy state for long-term seed storage. He pointed out also that water content and storage temperature are two major factors affecting the stability of intracellular glasses in seeds which formation is important for seeds surviving during longtime storage. According to Leopold et al. [1994] thermodynamic properties of seed water determine the reaction kinetics during seed deterioration. Investigation of thermodynamic processes, particularly the free energy jointly with germination contributes to better understanding of the aging of seeds [Dragicevic et al., 2004].

Enthalpy represents the total energy available to do work, while entropy at any temperature provides lost work (the energy not available to perform work); thus the energy available to do work is the difference between these two quantities – namely Gibbs free energy [Aviara and Ajibola 2002, Krishnan et al. 2004]. For this reason the thermodynamic parameters: Gibbs free energy (ΔG), entropy (ΔS) and enthalpy (ΔH) (chosen in the present investigation) are appropriate to be included in studies for evaluation the changes occurring in the seed deterioration and aging. As already established [Dragicevic et al. 2004] the aging process, associated with lower water intake, reflects on the differential values of the indicated thermodynamic parameters. Buitink et al. [1998] and Black et al. [2006] also pointed out that the term ΔG is useful to determine how changes in storage conditions or seed quality affect the overall reaction.

According to Li et al. [2005] the main mechanism for aging of pepper seeds is associated with increased peroxidation of lipid membranes. The loss in total phospholipids can be used as a first signal for reduction of the seed viability [Salama and Pearce 1993]. Tang and Song [1999] established that the aging process is connected with reduction of the vigour index. Through accelerated aging of pepper seeds in temperature regime 30–40°C and relative moisture from 55 to 100%, Rota [1986] observed deterioration of the sowing quality accompanied with increase of chromosomal aberrations. In long term storage of pepper seeds, Passam et al. [1997] reported about differences between studied varieties as well as harvesting year. The investigations on the aging of pepper seeds, in the ambient conditions, i.e. condition that they usually storage in the storehouse, are very limited and insufficient. Therefore, the further scientific researches to reveal the specific features during the aging are needed to supplement the scientific information as well as for agriculture practice.

The main goal of the present study was to investigate the relationship between the changes in viability potential and differential thermodynamic parameters: Gibbs free energy (ΔG), entropy (ΔS) and enthalpy (ΔH) of peppers seeds in aging during long-term storage.

MATERIALS AND METHODS

Plant material. Long-term experiments were carried out in Department of Horticulture and in Department of Mathematics and physics at the Agricultural University, Plovdiv, Bulgaria, with pepper (*Capsicum annuum* L.) seeds from typical Bulgarian cultivars 'Kurtovska Kapia 1619' (with conic shape fruit) and 'Bulgarski Rotund' (with flat-oval shape fruit). Both cultivars are for middle early field production. The seeds were long-term stored in paper bags under ambient (uncontrolled) conditions for period of 132 months. Every 12 months, the viability status and seed parameters: seed moisture, germination, moisture content and fresh weight of seedling, and thermodynamic parameters were monitored. One month before assessment of viability potential, the seeds were placed at 16.5°C in order to equilibrate and equalize their temperature, which was necessary for accurate calculation of thermodynamic parameters: free energy of Gibbs (ΔG), entropy (ΔS) and enthalpy (ΔH).

Seed moisture. Moisture content was determined in 4 replications according to the commonly applied ISTA [2003] procedures. Assessment was done with the drying-oven method: glass containers with 4.0 g seeds, previously weighted with precision of 0.01 g, and were placed at 103 ± 2°C for 17 ± 1 hours. At the end of this period the containers were covered and placed in a desiccator to cool for 30–45 minutes and then were weighted again. The glass containers and its cover before and after filling were weighted. The difference between the first and the second weight determines average percentage moisture content on a fresh weight basis.

Germination test. The first count (germination energy) and final count (germination capacity, germination) were assessed in 4 replications, each of them by 100 seeds taken randomly, according to ISTA [2003] prescriptions. The first count and final count were established at the 6th and 14th days, respectively after the placement for germination. At the final day (14th) of germination (according to ISTA prescriptions) the fresh weight of the seedlings per seed was measured on 10 normally developed seedlings from each 4 replications on a scale which has a precision of 0.01 g.

Moisture content of the seedlings. The moisture of the seedlings that developed from germinated seeds was established at the 14th day after the placement for germination in 4 replications each of them from 2.0 g seedlings, which represented the initial weight. The samples then were put in a glass container (with previously known weight) and placed in the oven, at 105°C for 4 hours. After they were put in a desiccator to cool for 30–45 minutes, weighed and dried again for 30 minutes at the above mentioned temperature and consecutive oven-drying and weighing until reaching a constant weight in 2 consecutive measurements. The difference between the first and the last weight determined average percentage of moisture content on a fresh weight basis [Stambolova et al. 1978].

Calculation of the thermodynamic parameters. The differential values of the free energy of Gibbs (ΔG), entropy (ΔS) and enthalpy (ΔH) in kJ mol^{-1} were calculated according to the relations [Sun 2002]:

$$\Delta H = [RT_1T_2 \ln(W_{c1}/W_{c2})] / (T_2 - T_1) \quad (1)$$

$$\Delta G = RT [\ln W_{c1} - \ln W_{c2}] \quad (2)$$

$$\Delta S = (\Delta H - \Delta G) / T \quad (3)$$

where R is the universal gas constant; W_{c1} and W_{c2} are water contents in seed and seedling; T_1 and T_2 are temperatures of seed storage (289.5 K) and germination (298 K).

Statistical analysis. The statistical analysis was done according to ANOVA. The data of the study was subjected to analysis of variance, and the least significant differences between means were calculated by the Fisher test at $p = 0.05$. Linear correlation (r) and regression were calculated according to the description of Fowel and Cohen [1992].

Seeds harvested in the year of initiation of the experiments were used as a control group, labelled as "one year old seeds".

RESULTS

The moisture content of naturally aging seed (tab. 1) changed during different years of long-term storage, as well as compared to the control, between 0.2–1.2% for 'Kurtovska Kapia 1619' and between 0.2–0.8% in the latter cultivar. This change was more clearly expressed at the first cultivar. The biggest moisture content was found for both cultivars in the 10th and 11th year of storage, and values for 'Kurtovska Kapia 1619' indicated more considerable difference in comparison with the control seeds. Aging of seeds during long-term storage caused changes in their vital potential. Sharp drop in germination energy – below 30% was observed after the third year for 'Kurtovska Kapia 1619', and after the fifth one – for 'Bulgarski Rotund'. Minor values for this index were reported after the seventh year of storage.

The seeds of Kurtovska kapia 1619 demonstrated germination around 70% till the 5th year, and those of 'Bulgarski Rotund' – till the 4th one. Comparatively high percentages of germination for the seeds of the second and third year were observed. The highest values for both cultivars were accounted for the one year old seeds. A deterioration of seed quality began most sharply from the seventh year, with a decrease towards the previous year for 'Kurtovska Kapia 1619' with 14.67%, for the other variety – with 13.33%. In these variants, the decreases in comparison to the control seeds values (one year old seeds) were with 31.33% and 37.66%, respectively.

Comparing the both investigated cultivars, it can be pointed out that the seedlings of 'Bulgarski Rotund' had bigger fresh weight (tab. 2). The variation of this parameter was lower to the third year of aging: between 53.52 mg (1st year) and 50.84 mg (3rd year). After this period, a stronger decrease of seedlings fresh weight began, and for eleventh years old seedlings the weight reached 16.60 mg, which was with 68.99% less than the

control. For 'Kurtovska Kapia 1619' the seedling with the biggest fresh weight was developed by the seeds of two year age – 34.52 mg. Till the fifth year of aging the changes of the fresh weight of the seedlings were relatively weak, but after that it considerably decreased and for the seedling of the 11th year old seeds the weight reached a value of 7.31 mg, this was with 25.43 mg less than of the initial (control) value of this indicator, which represents 22.33%. Mathematical significances for 8-, 9-, 10- and 11-years old seeds from 'Kurtovska Kapia 1619' and from 5- to 11-years old seeds from 'Bulgraski Rotund' were established. The relationship between the germination and the fresh weight was considerable, which was confirmed by the correlation coefficient, that was high and positive for both cultivars, being for 'Kurtovska Kapia 1619' $r = 0.78$ and for 'Bulgarski Rotund' $r = 0.72$, respectively.

Table 1. Effect of long-term storage of pepper seeds on the viability parameters

Variants (age, years)	'Kurtovska Kapia 1619'			'Bulgarski Rotund'		
	moisture content (%)	germination energy (%)	germination capacity (%)	moisture content (%)	germination energy (%)	germination capacity (%)
11	6.9*	0.0*	7.33*	6.8*	0.0*	11.33*
10	7.1*	0.0*	10.67*	6.8*	0.0*	24.0*
9	6.3	2.00*	37.33*	6.1	0.67*	41.33*
8	6.1	1.33*	45.33*	6.0	2.0*	42.67*
7	6.2	10.67*	52.0*	6.4	7.33*	48.67*
6	6.3	24.67*	66.67*	6.2	22.0*	62.0*
5	6.4	26.00*	76.67	6.4	36.67*	64.0*
4	6.2	28.67*	79.33	6.4	40.67*	70.0*
3	5.9	32.0*	80.0	6.2	50.67*	80.34
2	5.9	46.67*	82.0	6.1	58.67	82.33
1	5.9	66.67	83.33	6.3	70.0	86.33
LSD $p = 0.05$	0.6	17.23	15.73	0.5	15.83	11.15

* Seed samples of a given cultivar with significant differences values compared to the control (one year old seeds)

The differences in moisture content of the seedlings between both cultivars were comparatively small. Seedlings received from the seeds in the initial stages of storage, had lower water content and with aging it increased gradually. The increase after the fifth year was bigger than the previous year with 46.21% and 15.44% for 'Kurtovska Kapia 1619' and 'Bulgarski Rotund', respectively. In comparison to the control, in 'Kurtovska Kapia 1619' the changes were higher, at the seven year old seeds, the increase was with 27.6%. It could be noted some variation of this parameter, but the trend to higher values after the 5th year was kept for both genotypes. Statistically significant are the values about variants 9- to 11-years old seeds for both varieties.

The pattern of the thermodynamic parameters for both varieties was similar. The data was presented in tab. 3. Strong decrease of the free energy was observed after the 6th

Table 2. Effect of long-term storage of pepper seeds on the fresh weight of seedlings of one seed and moisture content of seedlings

Variants (age, years)	'Kurtovska Kapia 1619'		'Bulgarski Rotund'	
	fresh weight (mg)	moisture content (%)	fresh weight (mg)	moisture content (%)
11	7.31*	11.43*	16.60*	12.33*
10	17.28*	11.29*	21.19*	11.75*
9	19.56*	10.54*	20.01*	11.25*
8	24.27*	11.30*	31.89*	9.65*
7	27.57	9.70	22.04*	8.09
6	28.07	9.65	36.18*	7.85
5	30.78	6.60	44.59*	6.80
4	32.14	8.85	49.21	7.15
3	32.07	6.50	50.84	6.75
2	34.52	7.80	53.12	7.15
1	32.74	7.60	53.52	7.70
LSD p = 0.05	6.06	2.2	5.97	1.69
r with germination capacity	0.78		0.72	

* Seed samples with significant differences values compared to the control one (one year old seeds)

Table 3. Effect of long-term storage of pepper seeds on the their thermodynamic parameters

Variants (age, years)	'Kurtovska Kapia 1619'			'Bulgarski Rotund'		
	ΔG	ΔS	ΔH	ΔG	ΔS	ΔH
11	-0,046	-0.19	-55.77	-0,047	-0.19	-56.63
10	-0,045	-0.18	-54.73	-0,046	-0.18	-55.25
9	-0,040	-0.16	-48.93	-0,042	-0.17	-50.25
8	-0,042	-0.17	-50.62	-0,029	-0.12	-34.63
7	-0,013	-0.05	-15.59	-0,005	-0.02	-6.34
6	-0,030	-0.12	-35.96	-0,002	-0.07	-19.90
5	-0,004	-0.02	-5.27	-0,004	-0.02	-5.11
4	-0,026	-0.11	-31.38	-0,012	-0.05	-14.78
3	-0,002	-0.08	-2.63	-0,007	-0.03	-8.54
2	-0,007	-0.03	-7.93	-0,012	-0.05	-14.78
1	-0,007	-0.03	-8.14	-0,017	-0.07	-21.04
r with age	-0.85	-0.82	-0.86	-0.73	-0.77	-0.77
r with germination	0.84	0.82	0.84	0.87	0.79	0.80

and mostly after the 8th year of storage for the seeds of ‘Kurtovska Kapia 1619’ and ‘Bulgarski Rotund’, respectively. Same changes in values of entropy and enthalpy were observed at the same years of storage. The changes in the values of free energy and enthalpy after the 6–8 year of storage are 2–3 times bigger than those of their entropy. These changes in values of investigated thermodynamic parameters allow using them for a better evaluation of the seed germination.

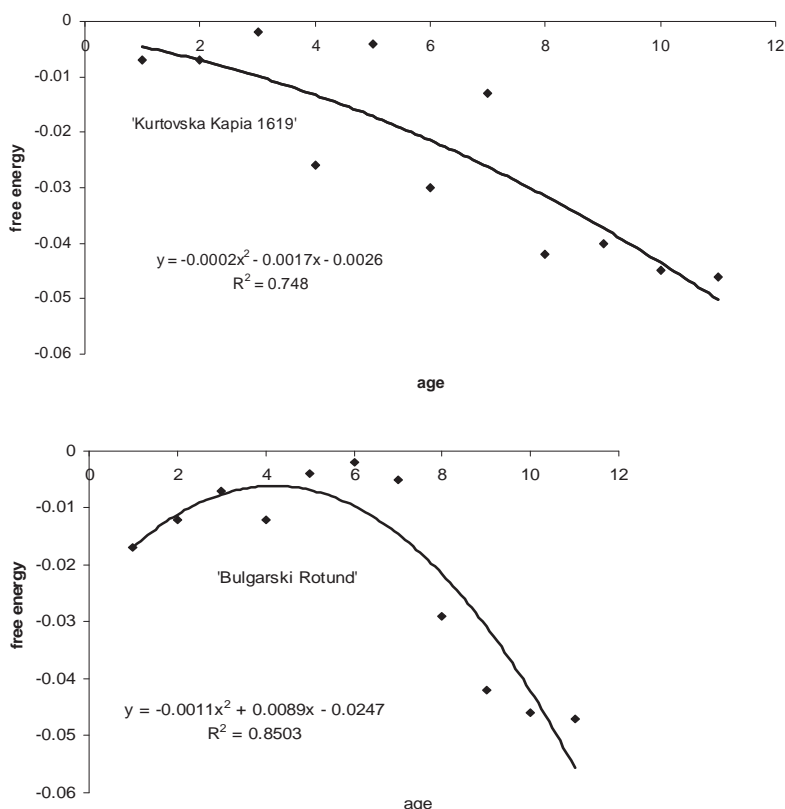


Fig. 1. Regression dependence between age and differential free energy (ΔG) of pepper seeds during long-term storage

The images in fig. 1 show, that both compared indicators – age and free energy, have the same course. This is confirmed by the regression dependence, which is polynomial with high determination coefficients $R^2 = 0.74$ and $R^2 = 0.85$ for ‘Kurtovska Kapia 1619’ and ‘Bulgarski Rotund’, respectively, which means that in 74–85% of cases the changes in seeds age will provoke a respectively decrease of the free energy. Correlation coefficients for both varieties were high but negative: $r = -0.85$ and $r = -0.73$, but the correlation between the germination and the free energy was also strong and positive, for ‘Kurtovska Kapia 1619’ being $r = 0.84$ and for ‘Bulgarski Rotund’ was $r = 0.87$.

The changes of the differential entropy and enthalpy followed almost the same course. The values of ΔS varied in ranges from -0.03 to -0.19 for ‘Kurtovska Kapia 1619’ and from -0.02 to -0.19 for ‘Bulgarski Rotund’. In the case of differential enthalpy, the variation was between $-2.63 \div -55.77$ and $-5.11 \div -56.63$, for both varieties, respectively. For entropy and enthalpy, as well as for the free energy, the correlation with germination capacity was strong positive with correlation coefficients: $r = 0.82$ and $r = 0.79$ for ΔS , and $r = 0.84$ and $r = 0.80$ for ΔH , respectively for ‘Kurtovska Kapia 1619’ and ‘Bulgarski Rotund’. Correlation of thermodynamic parameters with the aging was also strong, for ΔS being high but negative $r = -0.82$ and $r = -0.77$, and for ΔH $r = -0.86$ and $r = -0.77$ for above mentioned cultivars, respectively.

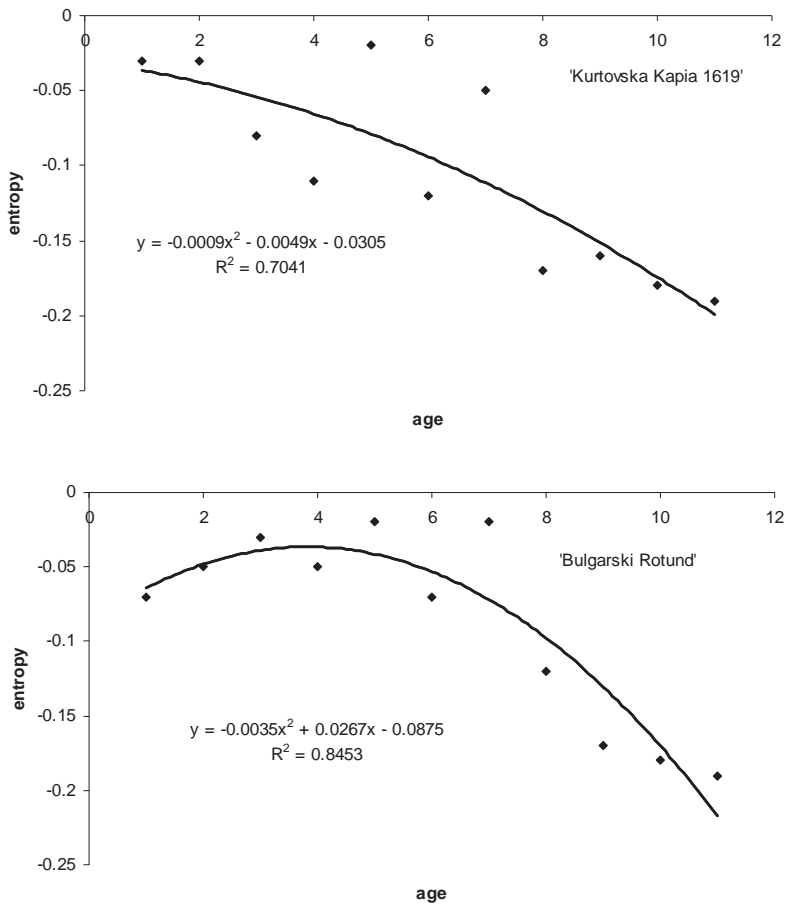


Fig. 2. Regression dependence between age and differential entropy (ΔS) of pepper seeds during long-term storage

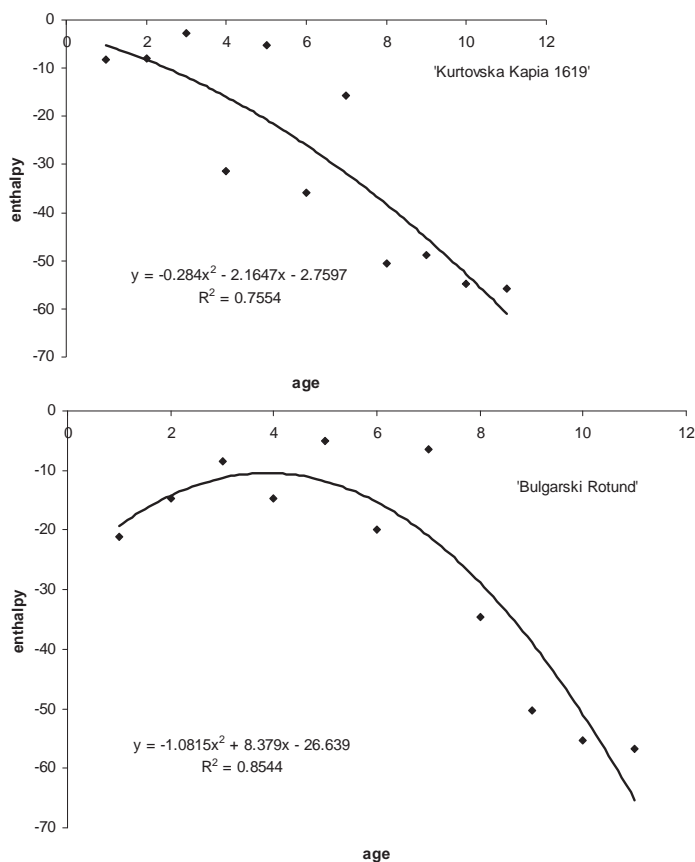


Fig. 3. Regression dependence between age and differential enthalpy (ΔH) of pepper seeds during long-term storage

The regressions between the entropy and enthalpy and the age (figs 2, 3) were also polynomial. More clearly and with higher coefficients of determination it is represented for 'Bulgarski Rotund'. These coefficients show that in 70–84% of cases the increase of age will be followed by the respectively decrease in entropy, about enthalpy this trend will be presented in 75–85% of the cases.

DISCUSSION

Black et al. [2006] reported that research on seed deterioration often distinguishes between 'natural' and 'accelerate' aging. This terminology reflects the conditions of storage more than mechanisms of deterioration. Moreover the natural aging is reportedly relatively slower (many months to years) and asynchronous within a given population of seeds. This highlights the existence of two lines of researches, related to seed aging. The natural aging has not yet been enough profoundly investigated; therefore this

study brings more information for clarifying this process. According to Wendell [1997] seed storage stability and the kinetics of seed viability loss are interlinked and largely dependent upon seed moisture content and storage temperature. The thermodynamic processes also depend on both parameters – water content and temperature. The established regressions between storage stability and thermodynamic parameters for pepper seeds help to predict the start of deterioration. Furthermore the obtained results provide additional more precise idea and information about the viability status of the seeds during long-term storage. The way of defining is cheap and easy to be carried out. However for wider application in practice, the further study with seeds from other crops and additional standardization are needed.

Strongly expressed trend in the changes of seed moisture content of different years of aging has not been established in our investigation. It is noteworthy, however, that with aging, i.e. with the increase of the storage period, although slightly, moisture content increased. The established slight increase of moisture content with increase of the storage period may be related to the storage conditions, especially environmental atmosphere humidity, which affects absorption processes, typical for the seeds with porous seed-coat structure as pepper seeds, but also have had an impact the biochemical processes occurring in the seeds [Panayotov 2010].

Normal vital status of the seeds was observed till 4th and 5th year of storage. These results conform to those of Priestley [1986] who announced that the seeds of a majority of species, even under uncontrolled storage conditions, are able to survive for a period of four years. The performed experiments revealed that in the beginning of the storage the changes of germination were weaker, but after some period of aging and at the end of investigation the worsening of the vitality was more expressive. Similar assertion was communicated also by Black et al. [2006] as they have established that deterioration courses followed a sigmoidal curve with an initial lag period and then, despite the seeds germination, the changes were very clear. The authors noted also the fact that the pattern of viability loss with storage time is consistent among species and regime of storage.

The fresh weight of the seedlings is one of the characteristics of the vigour. Through its measurement it is more appropriate to evaluate the vital status of seeds and the development of seedlings [Copeland and Mc Donald 2001]. The changes of seedling fresh weight depended on the variety and were the sharpest after four – five years of storage and coincided with the above mentioned variation of germination.

Aviara and Ajibola [2002] and Krishnan et al. [2004] determine the thermodynamics as one of the three approaches used to understand the properties of water and calculate the energy requirements of heat and mass transfer in biological systems. The water content in seedlings is a very important parameter for the characterization of biophysical thermodynamic processes. The significance of the water content for the development of these processes has been reported also by Tang and Song [1999].

The increase of the storage period resulted in a decrease of the values of free energy. This coincided with a reduction in the overall life-potential and functions of seeds and with clearly seem smaller development of seedlings. Similar observations, reported by Krishnan et al. [2004], point out, that thermodynamic parameters corresponding to the start of seed deterioration during storage can be identified as the critical upper limit for that parameter for seed storage at particular temperature. According to these authors, for

any particular period of storage, the thermodynamic properties of water generally decrease as the temperature increases. The interaction between seed moisture and temperature of seed deterioration in our research was very clear. The results can be used as additional possibilities to describe the effect of these two parameters on the kinetics of seed aging during storage.

CONCLUSIONS

1. The aging of pepper seeds, during long term storage, deteriorated their vital status which was expressed in changes in their moisture content, decreasing of their sowing qualities and development of weaker seedlings with higher water content.

2. Lower vital potential of pepper seeds corresponded to changes in thermodynamic parameters such as differential entropy, enthalpy and free energy of Gibbs. Their values decreased with the increase of seeds age and the loss of vital potential. The regression dependences between the age, on one hand, and the free energy, entropy and enthalpy on the other hand, have polynomial character and high determination coefficient. Strong positive correlation was observed also between the germination and the fresh mass of seedlings on one side, and the differential thermodynamic parameters (ΔG , ΔS and ΔH), on the other.

3. The demonstrated in obtained results changes of the thermodynamic parameters can be used as a reliable mean for better description, understanding and prediction of processes ongoing with aging and deterioration of pepper seeds during long-term storage which has great significance for agriculture practice.

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BADANIE WPLYWU DŁUGOTERMINOWEGO PRZECHOWYWANIA NA ŻYWOTNOŚĆ NASION PAPRYKI (*Capsicum annuum* L.) ZA POMOCĄ TERMODYNAMICZNYCH PARAMETRÓW

Streszczenie. Głównym celem doświadczenia było zbadanie zmian w wybranych parametrach termodynamicznych nasion papryki podczas długotrwałego przechowywania. W tym celu badano zawartość wody w nasionach, jak również temperaturę osiągniętą w czasie ich przechowywania. Doświadczenie przeprowadzono z nasionami papryki przechowywanymi w temperaturze pokojowej przez okres 132 miesięcy. Stwierdzono, że żywotność nasion pogarsza się bardzo szybko, po 4–5 roku przechowywania. Parametry termodynamiczne – entropia i entalpia, jak również energia Gibbsa – pogorszą się wraz z obniżaniem kiełkowania nasion. Stwierdzono, że wskazane parametry mogą być wykorzystywane jako wskaźniki procesu starzenia się nasion. Jest to ważne dla praktyki rolniczej, ponieważ pozwala na dokładniejszy i bardziej szczegółowy opis oraz przewidywanie degradacji nasion papryki podczas procesu przechowywania.

Słowa kluczowe: kiełkowanie nasion, entalpia, entropia, swobodna energia Gibbsa, wilgotność nasion, liger nasion