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THE INFLUENCE OF STORAGE TEMPERATURE AND TIME ON TOTAL PHENOLIC CONTENT IN A SOFT WHEAT CULTIVAR

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Abstract: *The influence of storage temperature and time on Total Phenolic Content (TPC) in a soft wheat cultivar, was the purpose of this paper. The biological material was represented by soft wheat grains from which there were formed control and working samples (grains, flour and bran). The control samples were stored at 10°C, and the working ones at four thermal thresholds (40ºC, 60ºC, 120ºC and 180ºC) for 15 days. Aftet 5, 10 and 15 days, Total Phenolic Content (TPC) has been estimated by a colorimetric assay, by measuring the ability of phenols to reduce Folin-Ciocalteu reagent. In all analyzed samples, compared to controls (samples stored at 10ºC), the largest decreases of Total Phenolic Content were registered after 15 days of storage, with significant percentage reductions at 180°C and 120°C, in flour type 480 (WF), and in whole-wheat flour (WWF). After 10 days of storage, significant percentage reductions of TPC were registered in flour type 480 (WF), and in whole-wheat flour (WWF), and after 5 days in WF (in all cases in samples stored at 180°C and 120°C). The percentage decrease of TPC values was emphasized more when the storage temperature increased from 120°C to 180°C, compared with the thresholds 40-60°C or 60-120°C. The keeping of soft wheat samples under high temperature conditions led to a greater reduction in the total content of phenolic compounds,as compared to grains or bran.*

Keywords: *storage, temperature, wheat, grains, flour, bran, total phenolic content*

1. Introduction

Phenolic compounds are organic substances widespread in plants. Present in more than 4000 different types of plants [1], these compounds highlight various physiological properties such as: anti-allergenic, antioxidant, anti-artherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects [2-7].

Wheat is an important component of the human diet, and an important source of phenolic antioxidants [8], wheat cultivars having a wide variation of phenolic content [9]. In cereal grains, the phenolic acids are found in three forms: free, soluble conjugate and insoluble bound [9]. Bound phenolics are considered to have have more health benefits because they escape from the upper gastrointestinal digestion conditions with cell wall materials, and after they are absorbed into blood plasma previous hydrolysis from esterases present in the intestinal microflora [10 ct. by 8]. Thus, wheat bran consumption increases total phenols and antioxidant capacity in plasma to a comparable extent to some other phenol-rich foods [11].

It is known that heat processing influences

the concentration and quality of food nutrients (carbohydrates, lipids, proteins), but there are less information, in this regard, on natural antioxidants and their activity. It seems that thermal treatment significantly reduces concentration of natural antioxidants, but the overall antioxidant properties of food products were maintained or even enhanced by the development of Maillard products [12].
Because of vulnerability to rancidity or for reducing number of microorganisms, sometimes is necessary a preheat treatment of wheat bran, germ or flour. The temperature used in the preheat treatment is relatively low not exceeding 100°C, and heat exposure time is short [13].
In this paper it has searched the influence of storage temperature and time on Total Phenolic Content (TPC) in four samples, belonging to a soft wheat cultivar, stored 15 days at four different thermal thresholds.

2. Experimental

The biological material, supplied by Suceava Agricultural Research and Development Station, was represented by grains (WG), belonging to a local cultivar of soft wheat (*Triticum aestivum* L. spp. *aestivum*), from which, by grinding, there were formed the other samples of this research, such as: integral flour made from ground beans (whole-wheat flour, WWF), bran (WB) and flour type 480 (WF). Type 480 signifies the maximum content of ashes in flour (0,480) multiplied by 1000. Before the beginning of experiment, the moisture content of wheat samples was between 8 and 10%. Control samples were stored at 10ºC, and work samples at four different thermal thresholds (40ºC, 60ºC, 120ºC and 180ºC) for 15 days, using thermostats and ovens set at temperatures above mentioned.
To determine Total Phenolic Contents (TPC), first it was obtained an extract for each sample, weighting each 1 g of grain (flour or bran) which were finely ground and subjected to extraction with a mixture methanol and water 80:20 (v/v), by stirring, centrifuging and recovering the supernatant [14].
The estimation of Total Phenolic Content in seed extracts was carried out through a colorimetric assay, by measuring the capacity of phenols to reduce Folin-Ciocalteu reagent. For this pupose, it has generated a standard curve (Fig.1) representing the absorbance values of Gallic Acid standard solutions in relation to their concentrations [15]. TPC was expressed as mg Gallic Acid Equivalent per gram dry matter (GAE/g d.m.). The data obtained from four replications were analyzed using Statistical Package for Social Science software, version 16.0. The correlation analyses were performed at the probability levels of 95% and 99%.
The differences between mean values of TPC were tested using Analysis of Variance ANOVA One-Way. In order to highlight the degree of influence of storage temperature and duration and the interaction between them on TPC in each wheat samples, there was applied factorial Analysis of Variance [16].
Further, in Tab. 1 there are shown mean values of Total Phenolic Content in the 4 samples of soft wheat, kept at 10ºC (control samples = blank).

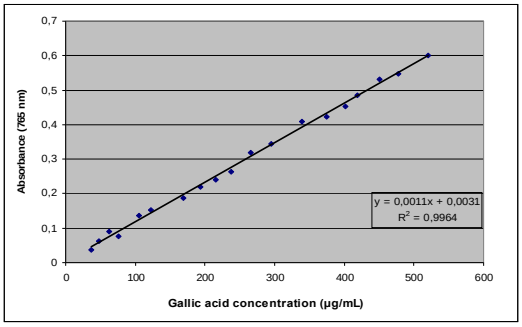

Fig. 1. Standard curve for TPC, using Gallic Acid

Table 1

Total phenolic content mean values (±SD) of the control wheat samples

Test	**TPC (mg GAE/g d.m.)**			
Wheat samples	WG	WWF	WB	WF
	1.95±0.035	2.01±0.03	2.76±0.08	0.79±0.03

SD=standard deviation; TPC=total phenolic content; GAE=gallic acid equivalent; WG=wheat grains; WWF=whole-wheat flour; WB=wheat bran; WF=white flour (type 480)

3. Results and discusion

From Table 1 it can observe that the highest mean values of TPC were in bran samples, WB (2.76 ± 0.08 mg GAE/g), and the lowest ones in flour type 480 samples, WF (0.79 ± 0.03 mg GAE/g).

Fig. 2 reproduces the distribution of TPC mean values in the four samples of soft wheat, compared to average values of the control (blank) samples, after 5 days of storage at thermal thresholds analyzed. Compared with the control (1.95 ± 0.035 mg GAE/g), TPC of wheat grain (WG) has changed very little, dropping only 2% at 180°C (1.91 ± 0.03 mg GAE/g) .

TPC mean values in whole-wheat flour samples (WWF) has decreased by 2%, compared to the control, at 40°C (1.97 ± 0.024 mg GAE/g), by 5.5 % at 120°C (1.90 ± 0.08 mg GAE/g), and by 8.4% at 180°C (1.84 ± 0.05 mg GAE/g). During the five days, TPC correlated negatively with storage temperature ($r = –0.494$, $p>0.05$).

After 5 days of storage, TPC mean values from bran (WB) fell by 2.2% at 120°C (2.70 ± 0.06 mg GAE/g), and by 5.4% at 180°C (2.61 ± 0.045 mg GAE/g) ($r = –0.363$, $p>0.05$).

TPC mean values in flour (WF) were reduced, compared to the blank, by 8.8% at 120°C (0.72 ± 0.04 mg GAE/g) and by 21.5% at 180°C (0.62 ± 0.02 mg GAE/g).

During the five days, in WF samples, the correlation analysis between TPC and temperature has shown negative correlations ($r = –0.892$, $p<0.05$).

Fig. 3 plays the mean values of TPC in the four samples of soft wheat, compared to average values of the control (blank samples), after 10 days of storage.

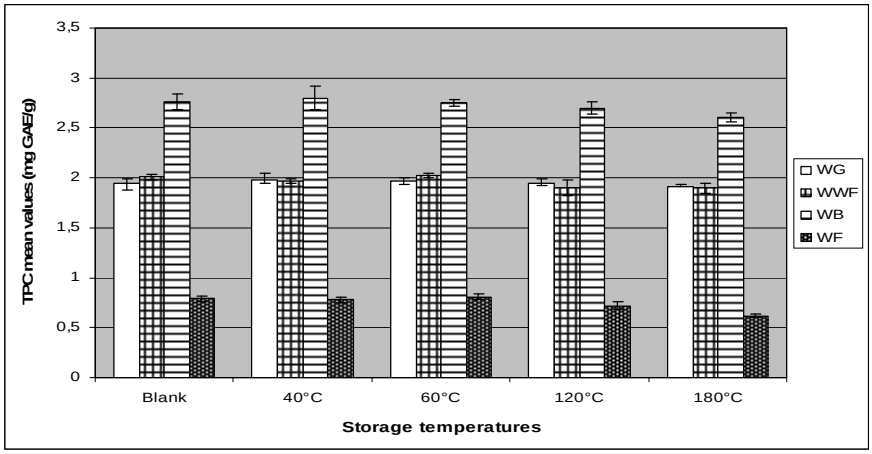

Fig. 2. Mean values of phenols total content after 5 days keeping of soft wheat samples WG=wheat grains; WWF=whole-wheat flour; WB=wheat bran; WF=white flour (type 480)

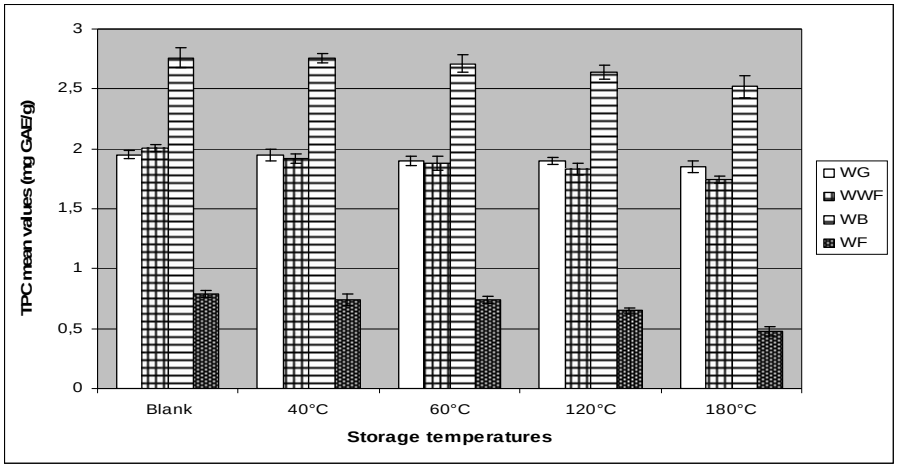

Fig. 3. Mean values of phenols total content after 10 days keeping of soft wheat samples WG=wheat grains; WWF=whole-wheat flour; WB=wheat bran; WF=white flour (type 480)

Compared with the control, after 10 days TPC of wheat grains (WG) fell by 2.5% at 60°C (1.90 ± 0.04 mg GAE/g) and 120°C (1.90 ± 0.03 mg GAE/g) and by 5.1% at 180°C (1.85 ± 0.05 mg GAE/g) ($r = -0.347$, $p>0.05$).

TPC mean values in whole-wheat flour samples (WWF) have decreased by 4.5% at 40°C (1.92 ± 0.04 mg GAE/g), by 6.5% at 60°C (1.88 ± 0.06 mg GAE/g), by 8.9% at 120°C (1.83 ± 0.05 mg GAE/g), and by 13.4% at 180°C (± 1.74 0.03 mg GAE/g).

In WWF samples, the correlation analysis between TPC and temperature has shown negative correlations ($r=-0.865$, $p<0.05$). After 10 days of storage, TPC mean values in bran (WB) fell by 1.8% at 60°C (2.71 ± 0.075 mg GAE/g), by 4.3% at 120°C (2.64 ± 0.06 mg GAE/g), and by 8.7% at 180°C (2.52 ± 0.09 mg GAE/g). During the ten days of storage, TPC correlated negatively with storage temperature ($r = -0.548$, $p>0.05$). Compared to the control, TPC mean values in flour (WF) were reduced by 6.3% at 40°C (0.74 ± 0.045 mg GAE/g) and 60°C (0.74 ± 0.03 mg GAE/g), by 17.7% at 120° C (0.65 ± 0.025 mg GAE/g), and by 39.2% at 180°C (0.48 ± 0.04 mg GAE/g) ($r = -0.947$, $p<0.01$).

In Fig. 4 are reproduced mean values of TPC in wheat samples after 15 days of storage.

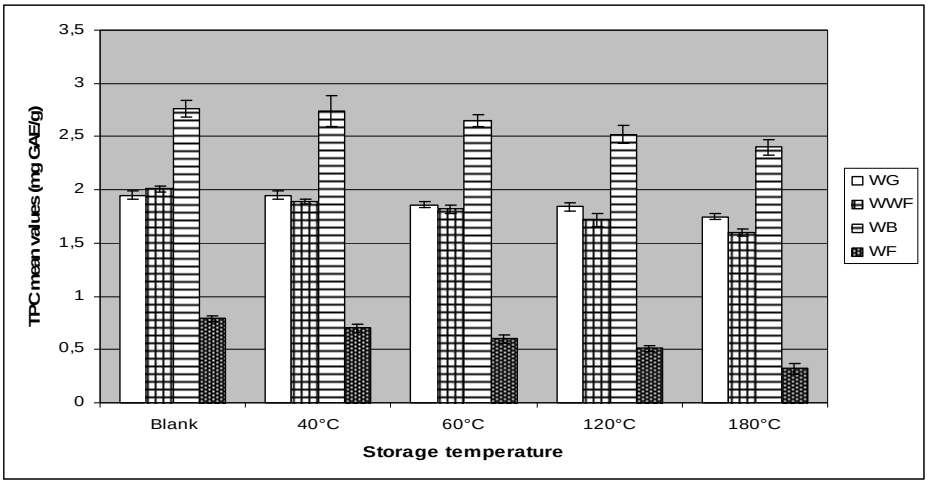

Fig. 4. Mean values of phenols total content after 15 days keeping of soft wheat samples WG=wheat grains; WWF=whole-wheat flour; WB=wheat bran; WF=white flour (type 480)

After 15 days, compared with the control, TPC in wheat grains (WG) fell by 4.6% at 60°C (1.86 ± 0.03 mg GAE/g), by 5.6% at 120°C (1.84 ± 0.04 mg GAE/g) and by 12.8% at 180°C (1.75 ± 0.025 mg GAE/g). In WG samples, TPC correlated negatively with storage temperature ($r = –0.624$, $p>0.05$).

Compared with the control, TPC mean values in whole-wheat flour (WWF) has decreased by 5.9% at 40°C (1.89 ± 0.025 mg GAE/g) by 9.4% at 60°C (1.82 ± 0.04 mg GAE/g), by 14.4% at 120°C (1.72 ± 0.06 mg GAE/g) and by 20.4% at 180°C (± 1.60 0.03 mg GAE/g). After 15 days of storage, in WWF samples, the correlation analysis between TPC and storage temperature has shown significant negative correlations ($r = –0,925$, $p<0,01$).

After 15 days of storage, TPC in bran samples (WB) decreased by 4% at 60°C (2.65 ± 0.06 mg GAE/g), by 8.7% at 120°C (2.52 ± 0.08 mg GAE/g) and by 13% at 180°C (2.40 ± 0.07 mg GAE/g) ($r = –0.597$, $p>0.05$).

After 15 days, TPC mean values in flour samples (WF) were reduced compared to the control, by 11.4% at 40°C (0.70 ± 0.035 mg GAE/g), by 24% at 60°C (0.60 ± 0.04 mg GAE/g), by 35.4% at 120°C (0.51 ± 0.025 mg GAE/g) and by 59.5% at 180°C (0.32 ± 0.05 mg GAE/g). In WF samples, the correlation analysis between TPC and storage temperature has shown significant negative correlations ($r = –0,995$, $p<0,01$).

The value of F test has indicated a significant cumulative effect ($p=0.000$) of the factors temperature and storage time on TPC in WF and WWF. TPC mean values in blank differ significantly ($p<0.01$) of those ones from WF samples (stored 5, 10 and 15 days), anf from WWF (stored 10 and 15 days), the storage temperature significantly influencing these values.

In the Table 2 are centralized the decreasing percentages of TPC mean values in wheat samples.

Table 2

Percent reduction of TPC mean values in wheat samples kept at different temperatures

Test	TPC reduction percent											
Duration	5 days				10 days				15 days			
ST*	WG	WWF	WB	WF	WG	WWF	WB	WF	WG	WWF	WB	WF
40°C	-	2%	-	-	-	4.5%	-	6.3%	-	5.9%	-	11.4%
60°C	-	-	-	-	2.5%	6.5%	1.8%	6.3%	4.6%	9.4%	4%	24%
120°C	-	5.5%	2.2%	8.8%	2.5%	8.9%	4.3%	17.7%	5.6%	14.4%	8.7%	35.4%
180°C	2%	8.4%	5.4%	21.5%	5.1%	13.4%	8.7%	39.2%	12.8%	20.4%	13%	59.5%

ST*= storage temperature; WG=wheat grains; WWF=whole-wheat flour; WB=wheat bran; WF=white flour (type 480)

As it can see in all samples analyzed, the largest decreases of TPC were after 15 days of storage, with the largest percentage reductions at 180°C and 120°C, in WF, followed by WWF. Although TPC reductions were also in WB and WG (with higher values at 180°C and 120°C), they did not differ significantly from controls, and therefore could not be taken into account.

Compared to controls, after 10 days of storage, significant percentage reductions of TPC were in WF and WWF, but after 5 days only in WF (in the samples stored at 180°C and 120°C).

From Table 3 one can see that the percent decrease of TPC values was emphasized more when the storage temperature increased from 120°C to 180°C, compared with the thresholds 40-60°C or 60-120°C.

Except WG sample, the other samples (WWF, WB and WF) were stored at time intervals and working temperatures as powder with particles of different sizes.

Exposure of wheat bran to high temperature (100°C) resulted in partial loss of total phenolic compounds and significant decline in antioxidant activity [17]. According to Cheng et al., 2006 [17], the effect of temperature under storage on total phenolic compounds and antioxidant activity varied significantly depending on the material stored, particle size, wheat variety, or assay methods used for antioxidant activity

Reduction of wheat bran particle size facilitates phytochemical release, thus enhancing the available and maybe bioavailable antioxidant activity [18]. If are not protected, phytochemicals are exposed (through grinding) to oxidation, resulting in shorter shelf life and loss of antioxidant activity [17].

In this paper, of the wheat samples exposed for 15 days at the four thermal thresholds, WF (wheat flour type 480) had the smallest particle size, which made that the phenolic acids from its composition to be exposed to oxidation, accelerated once with increasing of temperature.

The phenolic compounds from whole wheat grain were the most protected, being less exposed to the direct oxidation process, but in the other two samples (milled) the phenolic compounds have not undergone major changes, during storage at thermal thresholds and intervals analyzed (WWF – after 5 days, and WB – after 5, 10 and 15 days).

Cheng et al., 2006 [17] have stored wheat grains under different temperatures (25, 60, and_100°C) for 9 days, and found that TPC and antioxidant activity have not changed during the whole tested period, regardless of the assay methods used.

4. Conclusion

The storage at four different thermal thresholds (40°C, 60°C, 120°C and 180°C) of some hard wheat samples belonging to a Romanian cultivar for 5, 10 and 15 days, has revealed changes in the total content of phenolic compounds, depending on storage temperature, duration, and type of sample.

In all analyzed samples, compared to controls (samples stored at 10ºC), the largest decreases of Total Phenolic Content were after 15 days of storage, with significant percentage reductions at 180°C and 120°C, in flour type 480 (WF), and in whole-wheat flour (WWF).

After 10 days of storage, significant percentage reductions of TPC were in flour type 480 (WF), and in whole-wheat flour (WWF), and after 5 days in WF (in all cases in samples stored at 180°C and 120°C).

The percentage decrease of TPC values was emphasized more when the storage temperature increased from 120°C to 180°C, compared with the thresholds 40-60°C or 60-120°C.

Keeping of soft wheat samples under high temperature as flour caused a greater reduction in the total content of phenolic compounds, compared to grains or bran.

4. Acknowledgments

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