THE PHENOLIC COMPOUNDS EVOLUTION IN A DURUM WHEAT CULTIVAR DURING STORAGE AT DIFFERENT TEMPERATURES

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Abstract

The aim of this work was to investigate the influence of storage temperature and time on Total Phenolic Content (TPC) in a romanian durum wheat cultivar. The biological material was represented by fall durum 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 5 days. After 1, 3 and 5 days it has estimated Total Phenolic Content (TPC) through 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 after 5 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 1 and 3 days of storage, significant percentage reductions of TPC were only in flour type 480 samples stored at 180°C and 120°C too. Keeping of durum wheat samples under high temperature as flour caused a greater reduction in the total content of phenolic compounds, compared to grains or bran.

Key words: wheat, flour, bran, phenolic content

INTRODUCTION

Phenolic compounds are widely distributed in plants and they have recently gained much attention due to their antioxidant activity and free radical scavenging ability with potential beneficial implications in human health [5].

Cereals, especially whole-grain cereals, are important macronutrient sources that contain a wide range of antioxidant compounds [1].

In cereal grains, the phenolic acids are found in three forms: free, soluble conjugate and insoluble bound, wheat cultivars having a wide variation of phenolic content [8].

During food processing the exposure of phenolic acids to light, oxygen and heat may accelerate their destruction [7].

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 [9].

Considering these preheat treatments of wheat products, and knowing that during pastry baking the outer layers are exposed to about 160°C or higher (the inner layers not exceeding 100°C), in this paper it has searched the influence of different temperatures and exposure time on Total Phenolic Content (TPC) in four samples, belonging to a hard wheat cultivar, stored 5 days at four different thermal thresholds.

MATERIAL AND METHOD

Materials

The biological material was represented by fall durum wheat grains (WG), belonging to a Romanian cultivar of *Triticum durum* Desf., supplied by Suceava Genebank (AN: SVGB - 13191), from which, by grinding, there were formed the other samples of this research, such as: integral flour made from ground beans (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

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samples was between 7 and 10%. Control samples were stored at +10ºC, and work samples at four different thermal thresholds (+40ºC, +60ºC, +120ºC and +180ºC) for 5 days, using thermostats and ovens set at temperatures above mentioned.

Reagents preparation

Acording to Singleton et al. (1999) [12] cited by [11], there were prepared the following reagents needed for phenolic compounds determination: Folin-Ciocalteu (FC) reagent, Na_2CO_3 20%, 1 mg/mL Gallic Acid stock solution, prepared in the same solvent as samples solutions, and gallic acids working solutions for preparing standard curve, representing the absorbance values of Gallic Acid standard solutions in relation to their concentrations (Fig. 1), 10–550 µg/mL

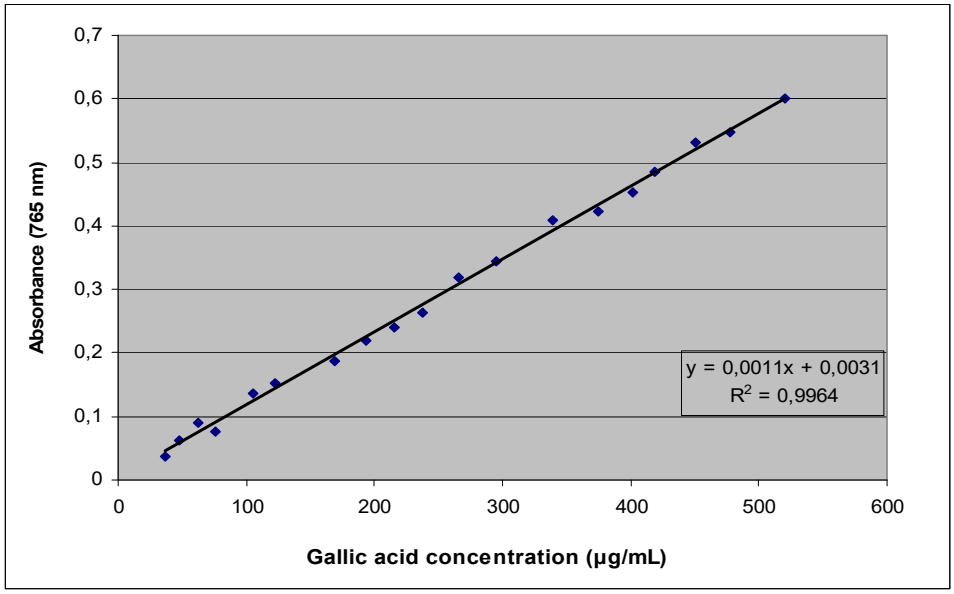

Fig. 1 Standard curve for TPC, using Gallic Acid

Phenols extraction

It has obtained an extract for each sample, weighting each 1 g of grain (flour or bran) which was finely ground and subjected to extraction with a mixture methanol and water 80:20 (v/v) at a solid-to-solvent ratio of 1:10 w/v, by stirring, centrifuging and recovering three times the supernatant [15], [1].

Total Phenolic Content (TPC) assay

The estimation of Total Phenolic Content in grain (flour or bran) extract was carried out through a colorimetric assay, by measuring the capacity of phenols to reduce Folin-Ciocalteu (FC) reagent. For this purpose, it has added 3 mL water to each test tube, 50 µL sample, standard, or solvent (blank), and 250 µL FC reagent, then vortexing for 5 s. It has waited at least 1 min, but not longer than 8 min, and after that it has added 750 µL Na_2CO_3 20% solution to each tube, and sealed test tubes were kept in dark, at ambient temperature for 2 h. It was added blank solution to cuvette and blank spectrophotometer was set at 765 nm, measuring the absorbance at 765 nm for all standard and sample solutions. For TPC estimation, µg Gallic Acid Equivalent (GAE)/mL was calculated for each sample using linear regression equation from standard curve (y = 0.0011x + 0.0031) as shown in Fig. 1.

TPC value of the samples, in mg GAE/g sample, was calculated as:

mg GAE/g = (µg GAE/mL)x(1mg/1000 µg)x($mL_{solvent}$ / g_{sample})

where $mL_{solvent}$ and g $_{sample}$ are the mL of solvent and grams of sample used for the sample extraction [12] cited by [11].

Finally, TPC was expressed as mg Gallic Acid Equivalent per gram dry matter.

Statistical analysis

The data obtained from four replications (for each sample) 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 [14].

RESULTS

In the Tab. 1 there are shown the mean values of Total Phenolic Content in the four samples of durum wheat, kept at 10ºC (control samples = blank).

From Table 1 one can see that the highest mean values of Total Phenolic Content (TPC) were in bran samples, WB (3.65±0.18 mg GAE/g), and the lowest ones in flour type 480 samples, WF (1.14±0.05 mg GAE/g).

In the Fig. 2 are reproduced the TPC mean values in the four samples of durum wheat, compared to average values of the control (blank) samples, after one day of storage at thermal thresholds analyzed.

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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
	3.06±0.1	2.98±0.12	3.65±0.18	1.14±0.05

TPC=total phenolic content; WG=wheat grains; WWF=whole-wheat flour; WB=wheat bran; WF= white flour (type 480);

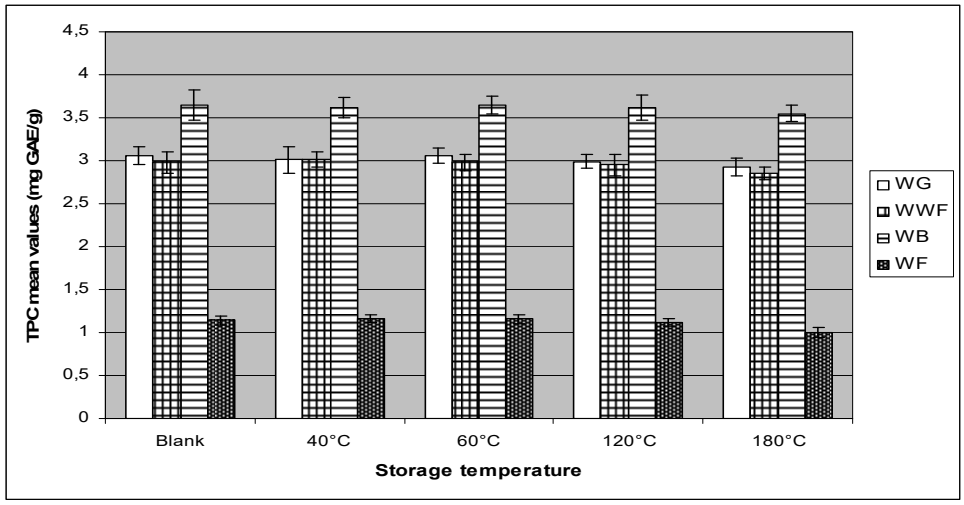

Fig. 2 Mean values of phenols total content after one day keeping of durum wheat samples WG=wheat grains; WWF=whole-wheat flour; WB=wheat bran; WF=white flour (type 480)

Compared with the control (3.06±0.1 mg GAE/g), TPC of wheat grain (WG) decreased by 1.6% at 40°C (3.01±0.15 mg GAE/g), by 2,3% at 120°C (2.99±0.08 mg GAE/g), and by

4,2% (2.93±0.1 mg GAE/g) at 180°C (r=–0,363, p>0,05).

TPC mean values in whole-wheat flour samples (WWF) decreased by 1% at 120°C (2.95±0.12 mg GAE/g), and by 4,3% (2.85±0.07 mg GAE/g) at 180°C (r=–0.394, p> 0.05).

Compared with the control, after one day of storage TPC mean values in bran (WB) fell by 2.7% (3.55±0.09 mg GAE/g) at 180°C (r=–0.425, p>0.05).

Compared to the control, TPC mean values in flour (WF) reduced by 1.8% at 120°C (1.12±0.04 mg GAE/g), and by 12.3% at 180°C (1.00±0.06 mg GAE/g). After one day of storage at 180°C, in WF samples, the correlation analysis between TPC and temperature has shown significant negative correlations (r=–0.8972, p<0.05).

In Fig. 3 is rendered the evolution of TPC mean values in wheat samples, compared to control (blank samples), after three days of storage at different temperatures.

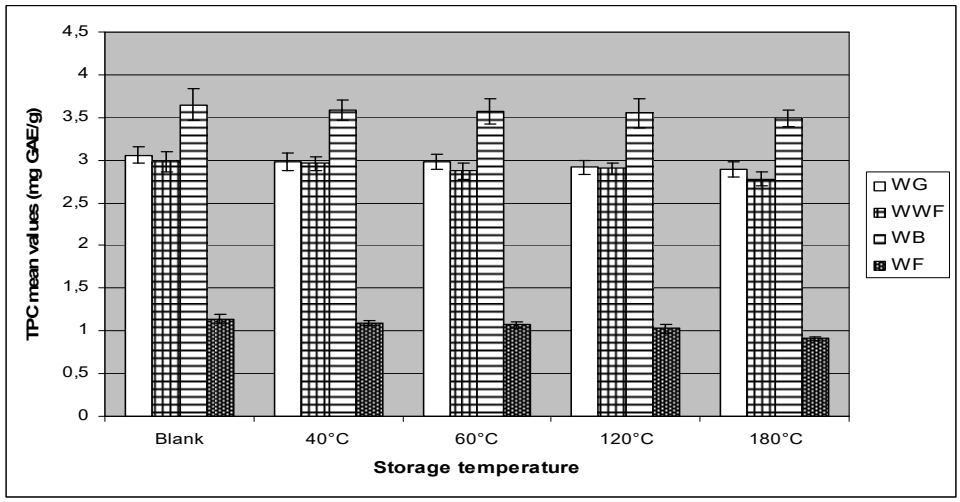

Fig. 3 Mean values of phenols total content after 3 days keeping of durum wheat samples
WG=wheat grains; WWF=whole-wheat flour; WB=wheat bran; WF=white flour (type 480)

As seen in the graph, TPC of wheat grains (WG) changed compared to the control, decreasing by 2.6% at 40°C (2.98±0.1 mg GAE/g) and 60°C (2.98±0.09 mg GAE/g), by 4.6% at 120°C (2.92±0.08 mg GAE/g), and by 5.5% (2.89±0.09 mg GAE/g) at 180°C (r=– 0,3451, p>0.05).

TPC mean values in whole-wheat flour samples (WWF) decreased by 3.7% at 60°C (2.87±0.09 mg GAE/g), by 2.7% at 120°C (2.90±0.07 mg GAE/g), and by 6.7% (2.78±0.08 mg GAE/g) at 180°C (r=–0.485, p>0.05).

Compared to the control samples, after three days of storage, TPC mean values in bran (WB) fell by 1.6% at 40°C (3.59±0.12 mg GAE/g), by 2.2% at 60°C (3.57±0.15 mg GAE/g), by 2.7% at 120°C (3.55±0.17 mg GAE/g), and by 4,4% (3.49±0.1 mg GAE/g) at 180°C (r=–0.597, p>0.05).

After three days of storage, TPC mean values in flour (WF) were reduced, compared to the control, by 4.4% at 40°C (1.09±0.03 mg GAE/g), by 6.1 at 60°C (1.07±0.03 mg GAE/g), by 9.6% at 120°C (1.03±0.05 mg GAE/g), and by 20.2% at 180°C (0.91±0.04 mg GAE/g). After ten days, the correlation analysis between TPC and storage temperature has shown significant negative correlations (r=–0.947, p<0.01).

Fig. 4 reproduces the mean values of TPC in wheat samples after five days of storage.

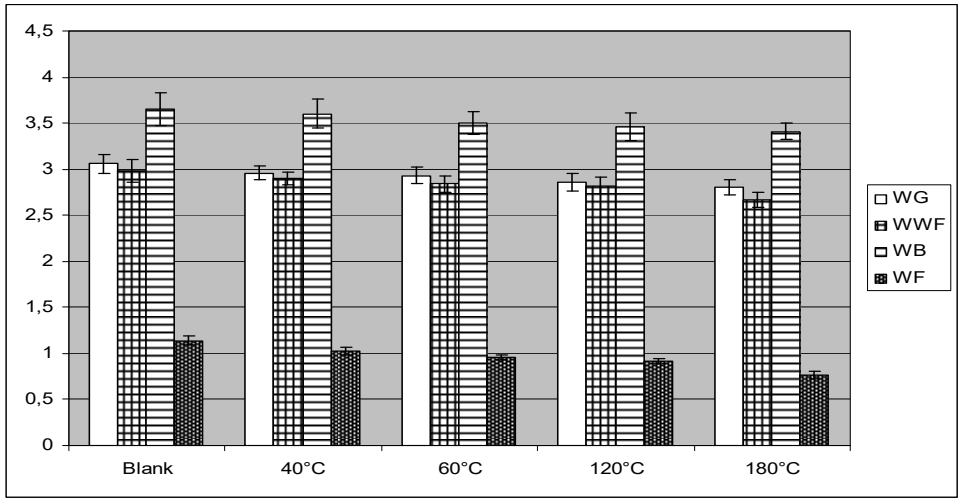

Fig. 4 Mean values of phenols total content after 5 days keeping of durum wheat samples
WG=wheat grains; WWF=whole-wheat flour; WB=wheat bran; WF=white flour (type 480)

After five days of storage, compared with the control, TPC in wheat grains (WG) fell by 3.3% at 40°C (2.96±0.07 mg GAE/g), by 4.2% at 60°C (2.93±0.09 mg GAE/g), by 6.5% at 120°C (2.86±0.1 mg GAE/g), and by 8.5% (2.80±0.08 mg GAE/g) at 180°C ($r = -0.418$, $p>0.05$).

Compared with the control samples, TPC mean values in whole-wheat flour (WWF) has decreased by 2.7% at 40°C (2.90±0.07 mg GAE/g) by 4.7% at 60°C (2.84±0.09 mg GAE/g), by 5.4% at 120°C (2.82±0.09 mg GAE/g) and by 10.4% at 180°C (2.67±0.08 mg GAE/g). In WWF samples, the correlation analysis between TPC and storage temperature has shown significant negative correlations ($r = -0,875$, $p<0,05$).

Compared with the control, TPC of bran samples (WB) decreased by 1.3% at 40°C (3.60±0.16 mg GAE/g), by 4.1% at 60°C (3.50±0.12 mg GAE/g), by 5.2% at 120°C (3.46±0.15 mg GAE/g), and by 6.6% (3.41±0.09 mg GAE/g), at 180°C ($r = -0.4081$, $p>0.05$).

After 5 days of storage, TPC mean values in flour samples (WF) were reduced, compared to the control, by 9.6% at 40°C (1.03±0.03 mg GAE/g), by 15.8% at 60°C (0.96±0.025 mg GAE/g), by 19.3% at 120°C (0.92±0.01 mg GAE/g) and by 32.4% (0.77±0.04 mg GAE/g) at 180°C). In WF samples, the correlation analysis between TPC and storage temperature has shown significant negative correlations ($r=-0,958$, $p<0,01$).

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. TPC mean values in blank differ significantly ($p<0.01$) of those ones from WF samples (stored 1, 3 and 5 days), and from WWF (stored 5 days), the storage temperature significantly influencing these values.

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

As it can see in all samples analyzed, the largest decreases of TPC were after 5 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 WG and WB (with higher values at 180°C and 120°C), they did not differ significantly from controls and therefore could not be considered.

Compared to controls, after 1 and 3 days of storage, significant percentage reductions of TPC were only in wheat flour (WF), in the samples stored at 180°C and 120°C.

Table 2 Percent reduction of TPC mean values in wheat samples kept at different temperatures

	TPC reduction percent (%)											
Time	1 day				3 days				5 days			
ST*	WG	WWF	WB	WF	WG	WWF	WB	WF	WG	WWF	WB	WF
40°C	1.6	-	-	-	2.6	-	1.6	4.4	3.3	2.7	1.3	9.6
60°C	-	-	-	-	2.6	3.7	2.2	6.1	4.2	4.7	4.1	15.8
120°C	2.3	1	-	1.8	4.6	2.7	2.7	9.6	6.5	5.4	5.2	19.3
180°C	4.2	4.3	2.7	12.3	5.5	6.7	4.4	20.2	8.5	10.4	6.6	32.4

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

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.

Heat treatment at 150°C for 40 min. liberated bound phenolics in citrus peels having as result a significant increasing of TPC after treatment [6].

Han and Koh (2011) [4], researching antioxidant activity of hard wheat flour, dough and bread prepared using addition of different phenolic acids, found that the antioxidant activity and residual free phenolic acid content of flour were reduced by mixing, but increased by fermentation and baking.

Acording to Cheng et al. (2006) [3], the thermal treatment causes phytochemical degradation, oxidation, and Maillard reactions resulting in changes in antioxidant property. Maillard reaction products may protect phytochemicals from oxidation [9], and those products, derived from mixtures of glucose or fructose and cysteine or glutathione, greatly inhibit activities of polyphenoloxidases and oxidoreductases [2].

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 [13].

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 [3].

Reduction of wheat bran particle size facilitates phytochemical release, thus enhancing the available and maybe bioavailable antioxidant activity [10]. According to Cheng et al. (2006) [3], if phytochemicals are not protected, grinding exposes them to oxidation, resulting in shorter shelf life and loss of antioxidant activity.

In this paper, of the wheat samples exposed for 5 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.

Although the phenolic compounds from whole wheat grain were the most protected, being less exposed to the direct oxidation process, however also in the other two samples milled the phenolic compounds have not undergone major changes during storage at thermal thresholds and intervals analyzed (WWF – after 1 and 3 days, and WB – after 1, 3 and 5 days).

Cheng et al. (2006) [3], 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.

CONCLUSIONS

The storage at four different thermal thresholds (+40°C, +60°C, +120°C and +180°C) of four durum wheat samples belonging to a Romanian cultivar for 1, 3 and 5 days, has revealed changes in the total content of phenolic compounds, depending on 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 5 days of storage, with significant percentage reductions at 180°C and 120°C, in flour type 480 (WF) and whole-wheat flour (WWF).

The percentage decrease of TPC values was emphasized more when the storage temperature increased from 120°C to 180°C.

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

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