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KINETICS OF THERMAL DEGRADATION OF ANTHOCYANINS IN CORRELATION WITH THE ANTIOXIDANT ACTIVITY OF BIOLOGICALLY ACTIVE COMPOUNDS IN THE EXTRACT OF PURPLE CORN (*ZEA MAYS L.)*

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Abstract: *Purple corn (Zea mays L.) is an excellent source of natural pigments due to its high content of phenolic compounds, namely anthocyains. The aim of the present study is the characterization of phytochemical profile of a purple corn extract in terms of total phenolic compounds, total flavonoid contents, total anthocyanins content, antioxidant activity and chromatographic profile. The kinetics of thermal degradation of anthocyanins in correlation with antioxidant activity was also evaluated. The thermal degradation parameters of antioxidant activity were significantly lower compared to those for thermal degradation of anthocyanins. The z-values started from 61.72 ± 2.28°C for anthocyanins and 75.75 ± 2.87°C for antioxidant activity. The simulated digestion showed that the heat treatment increased the degradation rate of anthocyanins in simulated intestinal juice. The thermal degradation of anthocyanins was positively correlated with the in vitro decrease of antioxidant activity. The study of kinetic parameters is essential to predict the quality changes that occur during thermal processing of different foods.*

Introduction

Purple corn (*Zea mays L.*) is an important source of natural food pigments due to its content in phenolic compounds. The phenolic compounds found in this source are powerful antioxidants aimed to eliminate reactive oxygen species by inhibiting free radical-producing enzymes (Atmani et al., 2011). The main interest in studying the phenolic compounds is based on the qualitative and quantitative analyses through various extraction techniques and also on the importance of their consumption benefits.The extraction techniques of polyphenolic compounds are classified as conventional and nonconventional methods. The optimal method is selected so that the extraction yield is as high as possible, does not affect or less affect the biologically active compounds and is environmentally friendly.

Material and method

Purple corn flour was supplied by a producer from Brăila city. The measured moisture content of the flour was about 12%. The flour was stored in paper bags at a maximum temperature of 18° C and a humidity of 65%.

The analyses reported on this study involved the use of the following reagents: 1N HCl solution, 70% ethanol solution, HPLC purity methanol, 5% $NaNO_2$ solution (m/v), 10% $AlCl_3$ solution (m / v), 1M NaOH solution, Folin-Ciocâlteu reagent, 20% Na_2CO_3 solution (m/v), 0.025 M KCl solution for pH = 1.0 and 0.4 M $CH_3COONa \cdot 3H_2O$ solution, pH = 4,5, DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) , KCl, 0.025M, α-glucosidase and α-amylase.

In order to extract the antioxidant compounds from the purple maize flour and for the determination of their antioxidant capacity, five experimental variants were prepared. The extraction techniques were performed following the conventional and ultrasound-assisted method using water, 70% ethanol and HCl as solvents in different proportions on different temperature conditions.

The phytochemical profile was spectrophotometrically obtained, in terms of total polyphenols content (TPC), total flavonoids content (TFC), total anthocyanins content (TAC) and antioxidant activity (AA). The specific compounds were separated and identified using liquid chromatography techniques (individual anthocyanins).

The extract was subjected to heat treatment at different temperature and time intervals, such as: 80^0C (0, 10, 20, 30, 40 min), 90^0C (0, 10, 20, 30 min), 100^0C – 110^0C (0, 5, 10, 15 min), 120^0C - 180^0C (0, 2, 5, 7 min).â

The samples were cooled using an ice water bath and the antioxidant activity and the total anthocyanins content were immediately conducted. The obtained data were fitted to a first-order degradation kinetics model.

Results and discussions

-In the first step, the extracts obtained by both conventional and ultrasound-assisted were characterised. The phytochemical profile of each variant is presented in **table 1.**

Table 1. Phytochemical characterisation of obtained extracts

Compound/ Variant	1	2	3	4	5
TAC C3G, mg/g DW	1.48±0.16	2.96±0.07	2.38±0.38	2.33±0.65	2.09±0.24
Li et al., 2008	6.8 – 82.3 mg/g FW				
TFC, mg CE/ g DW	0.77±0.03	0.67±0.10	0.66±0.05	0.58±0.05	0.51±0.06
Zilic et al., 2012	307.42 - 337.51 mg / kg DW				
TPC, mg GAE/g DW	0.93±0.06	1.48±0.02	1.12±0.07	1.22±0,07	1.22±0.11
AA, mM Trolox/g DW	3.03±0.12	5.35±0.41	4.76±0.03	4.92±0.78	4.82±0.01
Pedreschi and Cisneros-Zevallos (2006)	1.019 ± 0.05 µg TE/ µg				

-The chromatographic profile of purple maize flour extract (**figure 1**) showed the presence of two major compounds, namely cyanidin-3-glucoside and its acylated form cyanidin 3- (6 "-malonylglucoside).

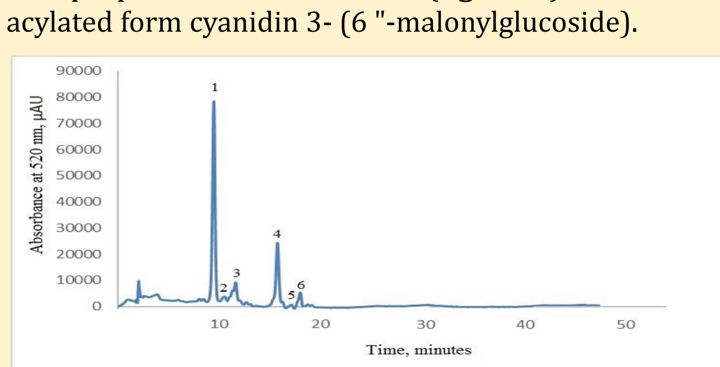

Figure 1. Chromatographic profile of the purple maize flour extract: Peak 1- cyanidin-3-O-glucoside; Peak 2-pelargonidin-3-O-glucoside; Peak 3-peonidin-3-O-glucoside; Peak 4-cyanidin-3-O-(60 0 -malonylglucoside); Peak 5—pelargonidin-3-O-(6" -malonylglucoside), and Peak 6-peonidin3-O-(6"-malonylglucoside).

-The determination of total anthocyanin content after heat treatment was performed in order to test their thermal stability. The results are shown in **table 2**.

Table 2. *Total anthocyanins content (mg G3G/G DW) from the purple maize flour extract after the heat treatment ranging from 80 to 110^0 C*

Time of maintaining (min)	Temperature (°C)			
	80	90	100	110
0	2.24±0.10	2.68±0.04	2.44±0.05	2.39±0.25
2	nd	nd	nd	nd
5	nd	nd	2.46±0.20	2.40±0.01
7	nd	nd	nd	nd
10	2.37±0.15	2.44±0.05	2.47±0.11	2.42±0.07
15			2.47±0.04	2.46±0.11
20	2.37±0.15	2.48±0.20	nd	nd
30	2.44±0.04	2.43±0.02	nd	nd
40	2.60±0.08	nd	nd	nd

The results obtained in the present study suggested that the heat treatment between 80 to 110°C does not affect the anthocyanin content of the purple maize flour.

-The obtained kinetic parameters values are presented in **table 3**, **figure 2** demonstrates that anthocyanins, which are responsible for the color and some biological properties of foods, have a higher thermostability when compared with the thermostability of spores or vegetative cells (z = 5–12 ° C).

Table 3. *Kinetic parameters for the thermal degradation of total monomeric anthocyanins in the purple maize flour extract*

Temperature (°C)	k x 10^{-2} (min^{-1})	D value (min)	$t_{1/2}$ (min)
120	2.28± 0.024*	101.01 ± 2.66	30.40 ± 1.24
130	4.99 ± 0.035	46.08 ± 3.72	13.86 ± 1.98
140	7.39 ± 0.125	31.15 ± 0.61	9.37 ± 0.97
150	14.37 ± 0.17	16.02 ± 0.39	4.82 ± 0.67
160	16.95 ± 0.23	13.58 ± 0.19	4.08 ± 0.45
170	19.29 ± 0.12	11.93 ± 0.25	3.59 ± 0.22
180	22.68 ± 0.20	10.15 ± 0.14	3.05 ± 0.12
Ea =55.75 kJ/mol ± 6.83 (0.93) z_T = 61.72 ± 2.28 (0.90)°C			

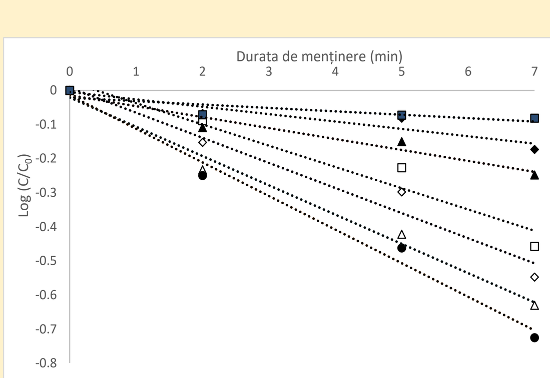

Figure 2*. First-order kinetic model for the thermal degradation of total monomeric anhocyanin content in the purple maize flour extract in acidified water at 4.5 pH at different temperature (■ 120°C, ◆130°C, ▲140°C, □150°C, ◊160°C, Δ170°C,●180°C)*

-Antioxidant activity of purple corn is represented by all polyphenolic compounds (anthocyanins, phenolic acids, nonanthocyanic flavonoids), which are thermolabile. This was also highlighted by determining the antioxidant activity after heat treatment (**table 4** and **figure 3**).

Table 4*. Kinetic parameters for the thermal degradation of antioxidant activity of the purple maize flour extract*

Temperature (°C)	*k* x 10^{-2} (min^{-1})	*D* value (min)	$t_{1/2}$ (min)
80	0.16 ± 0.01*	1428.57 ± 25.67	429.96 ± 13.45
90	0.34 ± 0.02	666.66 ± 17.59	200.65 ± 11.26
100	0.57± 0.10	400 ± 19.35	120.39 ± 16.78
110	0.64 ± 0.09	357.14 ± 8.89	107.49 ± 11.27
120	1.42± 0.28	161.29 ± 7.66	48.54 ± 2.24
130	1.70 ± 0.26	135.13 ± 3.82	40.67 ± 2.87
140	1.93 ± 0.25	119.04 ± 4.61	35.83 ± 2.98
150	2.18 ± 0.27	105.26 ± 2.39	31.68 ± 1.97
160	2.23 ± 0.13	103.09 ± 2.19	31.02 ± 2.67
170	3.91 ± 0.18	58.82 ± 1.12	17.70 ± 1.43
180	4.85 ± 0.29	47.39 ± 1.14	14.26 ± 1.82
Ea =41.12 kJ/mol ± 3.00 (0.95)		z_T = 75.75 ± 2.87 (0.93)°C	

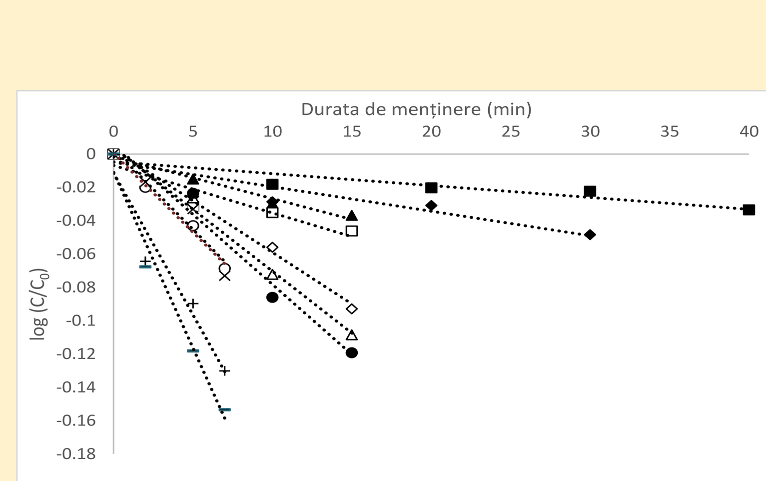

Figure 3*. First-order kinetic model for the thermal degradation of antioxidant activity content in the purple maize flour extract in acidified water at 4.5 pH at different temperature (■ 80°C, ⍰90°C, ▲100°C, □110°C, ■ 120°C, ◆130°C, ▲140°C, □150°C, ◊160°C, Δ170°C,●180°C)*

Throughout the temperature range , the antioxidant activity presented a slight decrease, suggesting that other compounds with different thermostability could be involved on this phenomena.

As expected, the z-value indicates that the thermal resistance of the compounds responsible for antioxidant activity (75.75 ± 2.87 ° C) is higher than for spores or vegetative cells, suggesting that the rates of thermal destruction of the biologically active compounds from the purple corn extract are less dependent on temperature.

-The unheated anthocyanic extracts (equivalent to 10 mg C3G/mL) were digested sequentially in the gastric and intestinal simulated juices. During in simulated gastric digestion, the untreated anthocyanins showed no changes in the first 60 min of reaction, with a significant decrease of approximately 21% after 120 min (**Figure 4.a**).

After simulated intestinal digestion, the anthocyanins were slowly decreased to a maximum of 12% for the unheated extracts, whereas an 83% decrease was found after preliminary heating at 180^0C (**Figure 4.b**).

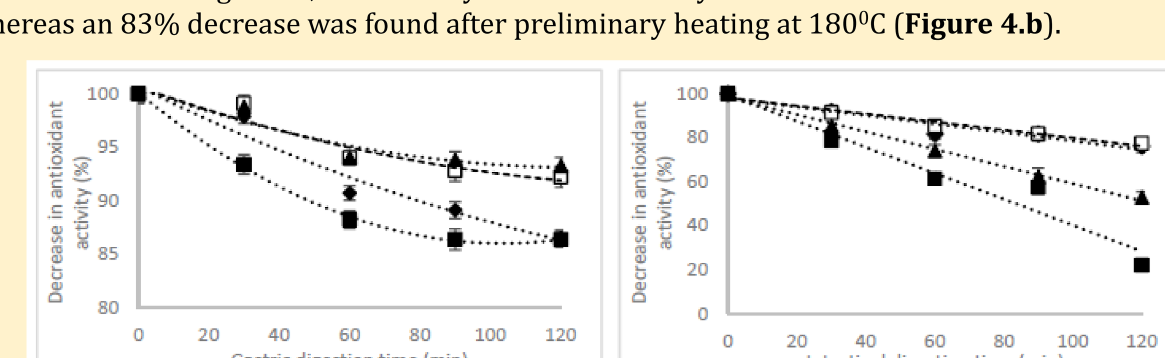

Figure 4. The decrease in total monomeric anthocyanins in the purple maize flour extract in gastric (**a** and intestinal (**b**) simulated in vitro digestion as affected by thermal treatment. Temperature: ◆ 25^0C, □80^0C, ▲120^0C, ■ 180^0C.

McGhie and Walton (2007), suggested that the acidic conditions of the simulated gastric fluid contributed to the stability of the anthocyanin content. Regarding the mechanism of ANC metabolism, Stevens and Maier (2016) reported that the process involved the opening of the intramolecular heterocyclic flavylium ring under alkaline conditions in the intestinal fraction. It is well known that anthocyanins are typically stable at an acidic pH, but unstable at an alkaline pH.

Conclusions

Purple corn extract showed a significant concentration of anthocyanins and an important value of antioxidant acitvity.

After chromatographic identification and separation of the compounds from purple corn extract, two major anthocyanins namely cyanidin-3-glucoside and its acylated form cyanidin 3- (6 "- malonylglucoside) were highlighted. The compound with the lowest concentration was the acylated form of pelargonidin-3-glucoside.

The application of kinetic studies of thermal degradation in the temperature range 80 to 180°C showed a high thermostability of anthocyanins in the temperature range 80-120°C. It can be noted that the thermal degradation parameters related to antioxidant activity were significantly lower compared to those estimated for the thermal degradation of anthocyanins, which indicates a different thermostability of biologically active compounds in the purple maize extract.

The thermal treatment affected the anthocyanin stability during the in vitro digestion. Different patterns in anthocyanins and antioxidant activity were found, in gastric and intestinal digestion, highlighting a good stability when preliminarily heated up to 120^0C.

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