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EXTRACTION AND EVALUATION OF TOTAL PHENOLICS CONTENT FROM RED CORN BRAN

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Abstract

Red and purple corn varieties were well studied in terms of their profile of biological active compounds, trying to explore the great potential of agricultural resources in different advanced applications, in food, pharmaceutic or medical fields. In this research paper, Bloody Butcher red corn variety was studied related to its total phenolics content and antioxidant activity. The research was conducted with milled corn and sifted milled corn, analyzing the use of several solvents for the extraction experiments and different methods (magnetic stirring, ultrasound, microwaves). Water-ethanol (1/1) was found to be the optimal extraction solvent mixture and ultrasound treatment was chosen as the best method to obtain extracts with high polyphenolic content (290 mg GAE/100 g DM). Microwave assisted extraction lead to similar results at 700W power level and 20 seconds exposure. Also, the highest antioxidant activity resulted for sifted milled corn after 60 minutes of magnetic stirring extraction with aqueous-ethanol solvent (451.71 mM TE/100 g DM).

***Key words**: antioxidant, magnetic stirring, phenolics, purple corn, ultrasound*

INTRODUCTION

Important efforts are made all over the world to harness agricultural biomass (cereals and waste) in a progressive manner, by obtaining extracts of biological interest (Ursu et al., 2023; Romano et al., 2023; Francavilla and Joye, 2020). In this context, pigmented grains (corn, rice, barley, wheat, millet, in different colours as black, purple, blue, red, etc.) are well known for their great content of phytochemicals as phenolics, being explored in different ways to obtain active molecules for advanced application, in food, nutraceutical or biomedical fields (Francavilla and Joye, 2020; Bangar et al., 2023; Ed Nignpense et al., 2022).

Due to its worldwide availability and its easy cultivation, from an economic point of view and the necessary climatic conditions, the use of corn and its numerous by-products were considered in different research studies (Francavilla and Joye, 2020; Kim et al., 2023).

Natural dyes attract great interest among different industries and the recovery of pigments from cereals and agricultural waste is one of the best solutions for a sustainable economy. Recently, biorefining methods of dry purple corn cobs have been proposed, in order to recover anthocyanins and obtain pigments used in dyeing natural fibers (De Nisi et al., 2021). Lau et al. (2019) investigated the composition of sweet corn cobs, identifying high contents of minerals, proteins, and phenolic compounds in their waste. Phenolics play different roles in promoting human health, such as detoxification, inhibition of enzymes involved in inflammatory processes, antibiotic action against pathogen (fungi or bacteria) (Drăgan et al., 2022). In addition, natural compounds that possess antioxidant and anti-inflammatory (through phenolic compounds) properties are deeply involved in promoting healthy aging and combating senescence (Miu et al., 2023). Of all the coloured varieties of corn, purple corn attracts attention, and several studies were focused on the extraction of anthocyanin pigments (Kim et al., 2023). However, because there are different varieties of purple corn, adapted to cultivation in different climatic zones, this natural resource is still not fully exploited.

Conventional and non-conventional methods were studied for the extraction of phenolics from different materials, including purple corn. The energy consumption, considerable volumes of solvents and the risk of degrading those compounds of interest (some of them sensitive under certain conditions) are the main drawbacks related to the conventional extractive methods, such as Soxhlet / solvent extraction, maceration and other methods currently used (Ivanovic et al., 2014; Cristianini and Guillén Sánchez, 2020). In the context of the global concern regarding energy consumption and a clear requirement for sustainable processes, non-conventional extraction technologies were considered. In this context microwave assisted extraction is one of the simplest and most efficient method for obtaining extracts rich in bioactive molecules; in addition, this process does not affect the integrity and quality of the targeted compounds (Cristianini and Guillén Sánchez, 2020; Piyapanrungrueang et al., 2016).

This paper focuses on the extraction of phenolic compounds from Bloody Butcher red corn variety cultivated in Romania. The grinded purple corn was subjected to extraction, either in the form of bran or sifted bran. Different conventional and non-conventional methods were used: extraction under magnetic stirring, ultrasonic-assisted extraction, or microwave-assisted extraction. In order to select the best method for extracting phenolics, several extraction parameters were tested such as: types of solvent or extraction time period. The total polyphenolic content (TPC) and total antioxidant activity (AA) were evaluated for the studied corn extracts.

MATERIALS AND METHODS

Samples

The raw material, Bloody Butcher red corn variety, was cultivated and harvested in 2022 from a region in Brăila county, Romania. The dried corn kernels were processed by grinding, the obtained product (milled corn, MC) being further subjected to different extraction methods tested in the study. Also, the MC product was sieved (1 mm sieve mesh size.), and the resulting sieved material (sifted milled corn, SMC) was subjected to the same extraction methods.

Materials

Several analytical grade solvents were used either for extraction or for different analysis: ethanol (96% purity, Sigma Aldrich), hydrochloric acid (36.5-38.0%, Sigma Aldrich), distilled water (Milli-Q), 2,2′-Azino-bis (3-ethylbenzo-thiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid (GA), sodium carbonate, sodium acetate, potassium persulfate (Sigma-Aldrich), Folin-Ciocâlteu reagent (Merck).

Phenolics extraction

Purple corn extracts were obtained either starting from the milled corn (MC) or from the sifted milled corn (SMC). Based on literature review (Cristianini and Guillén Sánchez, 2020; Lao and Giusti, 2018; Muangrat et al., 2018; Magni et al., 2018), different solvents were selected for this research, as shown in Table 1. Aqueous and hydro-alcoholic extracts were obtained, using distilled water (DW) and distilled water-ethanol mixtures (DW-EtOH), with or without acid addition (1% (v/v) addition of 1N HCl solution,), in a ratio of MC or SMC vegetal matter/solvent of 1/10.

The samples were subjected to two different types of treatment such as: magnetic stirring (1000 rpm) or ultrasound (30W power) for 60 minutes (Table 1).

In the second phase of the experiment, the different parameters were tested such as extraction time (1-45 minutes) and treatments (magnetic stirring or ultrasound), in order to select the optimal extraction method based on the total amount of phenolics obtained related to minimal energy consumption (economic aspects). In this stage of the research 28 extraction experiments were performed, 14 for the MC material and 14 for SMC material (stirring/ultrasounds), all of them with the optimal solvent.

The third experimental setup involved microwaves as the extraction technique for the phenolic compounds from the vegetable raw material. The microwave oven had 6 power levels and a maximum of 700 W, mechanical control (Hansa, Germany). Thus, 4 extraction times were tested (5, 10, 15 and 20 seconds), on 2 power levels (350 and 700 W) for the optimal solvent (based on E1-E8 experiment according to Table 1), for both MC and SMC products, resulting in a total number of 16 extraction experiments.

Table 1. Phenolics extraction experiments from purple corn

Experiment	Solvent	Solvent composition %	Extraction parameters
E1	DW	100	MC and SMC materials ***magnetic stirring*** 60 minutes | RT, 1000 RPM
E2	DW/ HCl	99/1 (v/v)	
E3	DW-EtOH	50/50 (v/v)	
E4	DW-EtOH/HCl	99/1 (v/v)	
E5	DW	100	MC and SMC materials ***ultrasounds treatment*** 60 minutes | RT, 30W power
E6	DW/HCl	99/1 (v/v)	
E7	DW-EtOH	50/50 (v/v)	
E8	DW-EtOH/HCl	99/1 (v/v)	

All the obtained extracts were filtered (Whatman filter paper, Grade 1) and the recovered filtrates were further subjected to different analysis. Each experimental phase was performed in triplicate and the reported results represent the average value of the three determinations.

The dry matter was calculated using freeze drying method. The samples were frozen and ground with dry ice and kept in a Freeze Dryers, D-37520 (Osterode am Harz, Germany) for 24 h. The samples were weighed in duplicate.

Quantification of the total polyphenolic content

The obtained purple corn extracts were evaluated in terms of the total phenolic content (TPC) using the well-known Folin-Ciocâlteu method (Munteanu and Apetrei, 2021; Ramos-Escudero et al., 2012). Briefly, 0.1 mL extract, 0.5 mL Folin-Ciocâlteu reagent and 1.5 mL of distilled water were mixed and maintained for 10 minutes at RT in a dark place. Then, 1.5 mL Na_2CO_3 solution (20%) was added, and the resulting samples are kept under the same conditions to reach a total incubation time of 2 hours, to complete the reaction and to generate the blue complex. The absorbance was measured at 750 nm for each studied extract using a Helios Beta UV-Vis spectrophotometer with Vision software (Thermo Electron Corporation, Waltham, MA, United States). Aqueous solutions of gallic acid (GA) with known concentration were employed for the calibration curve further used to assess the phenol content of the purple corn studied extracts (Ballus et al., 2015; Tociu et al., 2019; Odumose et al., 2015). TPC was expressed as mg of gallic acid equivalent (GAE) per 100g dry matter (DM).

Assessment of antioxidant capacity

To evaluate the total antioxidant capacity of the studied extracts from purple corn, TEAC method (Trolox equivalent antioxidant capacity) was considered (Lungu et al., 2016; Kim et al., 2022). Briefly, the ABTS•+ radical solution was obtained by mixing appropriate volumes of ABTS and $K_2S_2O_8$ in order to achieve final concentrations of 7 mM and 2.45 mM respect-tively, due to the rapid reaction of the antioxi-dant compounds within the tested samples and the blue-green ABTS•+ radical cation. The mixture was kept at room temperature, protected from the light, for 16 h. Before use, the ABTS•+ stock solution was diluted with ethanol to a final absorbance of 0.70 ± 0.02 at 734 nm. To a volume of 990 μL of this diluted solution were added 10 μL of sample or Trolox solution prepared in ethanol. The absorbance was recorded exactly after 1 minute. An appropriate solvent blank was run for each assay.

The results were calculated using a calibration curve made with Trolox solutions with concentrations ranging from 0.5 mM to 2.5 mM. The percentage of inhibition of absorbance at 734 nm was calculated for each Trolox solution and plotted as a function of concentration. Also, the percentage of inhibition of absorbance was calculated for each sample ($y = 34.8x + 12.944$, where y = percentage of inhibition, x = concentration of standard) and R2 = 0.9946). Antioxidant activity values of extracts were expressed as mmol/L Trolox equivalents, and the final results were expressed as Trolox equivalents/ 100g dry matter (mM TE/ 100g DM). All determinations were performed in triplicate.

RESULTS AND DISCUSSION

Evaluation of the extraction solvents based on TPC assays

Each selected solvent was involved in the extraction experiments for MC and SMC raw materials, by employing magnetic stirring and ultrasound treatment (Table 1). The freshly obtained extracts were evaluated in terms of the TPC through Folin-Ciocâlteu spectrophotometric method, using a gallic acid (GA) calibration curve. The absorption of the samples prepared as described in the Methods section were measured at 750 nm against a blank sample, the obtained results being - presented in Table 2.

Table 2. TPC of the purple corn extracts in different solvents (extraction time, 60 minutes)

Extraction solvent	Magnetic stirring TPC (mg GAE/100 g DM)		Ultrasound treatment TPC (mg GAE/100 g DM)	
	MC	SMC	MC	SMC
DW	194.98 ± 19.00	153.53 ± 30.46	152.80 ± 28.84	171.71 ± 26.25
DW/HCl	143.35 ± 20.16	146.98 ± 33.18	98.98 ± 22.05	202.25 ± 41.36
DW-EtOH	202.98 ± 27.08	216.07 ± 38.85	290.25 ± 25.74	275.71 ± 49.01
DW-EtOH/HCl	115.71 ± 22.75	96.80 ± 29.41	291.71 ± 32.72	237.16 ± 33.86

According to the data presented in Table 2, TPC of the studied extracts ranges in a large interval for the different extraction solvents. The lowest values related to the phenolic content (TPC, as mg GAE/100 g dry matter) was registered for those extracts in acidified hydro-alcoholic medium, for both MC and SMC vegetal products, when magnetic stirring was employed, since the greater content of phenolics were obtained for the same solvent mixture, free of acid (DW-EtOH). The high extraction capacity of the phenolic compounds, associated with the DW-EtOH mixture is also validated when using ultrasounds (for MC and SMC also), while the HCl-aqueous media seems to have the lowest potential in this case, slightly higher results were observed for SMC under ultrasound treatment. From these experimental results the first conclusion that may be drawn is that the mixture of DW-EtOH (1:1 v/v) is the optimal extraction medium for the purple corn variety explored in this study.

The extraction method influences the solvent extraction capacity, ultrasound treatment being more efficient, conducing to the higher values of the calculated TPC. Also, ultrasound treatment seems to influence positively the phenolics extractive processes for MC vegetal material. Probably, in this case a much better contact of the solvent with the corn bran is achieved and, which mostly represents the exterior layers, the hull of the corn kernels, responsible also for the purple colour and known for the great content of the phenolic compounds (Razgonova et al., 2022; Li et al., 2017).

The samples obtained after 60 minutes of aqueous extraction were frozen (-18°C) and then lyophilized. For the two MC and SMC samples, the obtained dry matter for the extracts was, as follows: 3.3% (MC, magnetic stirring), 2.3% (MC, ultrasounds), 2.7% (SMC, magnetic stirring), 2.5% (SMC, ultrasounds).

The assessment of the optimal extraction time based on TPC

Once established the optimal extraction solvent, the experimental procedure passes in the second step, identifying the optimal extraction time, with respect to low energy consumption. For this, TPC was determined for the purple corn extracts, obtained in DW-EtOH, for both MC and SMC products, employing the two magnetic stirring and ultrasound treatment methods, at different extraction times, according to Table 3.

Table 3. TPC of the purple corn extracts at different extraction time (solvent, DW-EtOH)

Extraction time (min.)	Magnetic stirring TPC (mg GAE/100 g DM)		Ultrasound treatment TPC (mg GAE/100 g DM)	
	MC	SMC	MC	SMC
1	23.93 ± 4.77	31.06 ± 6.26	37.17 ± 10.51	43.79 ± 7.12
5	53.97 ± 10.32	58.56 ± 12.24	70.78 ± 12.27	70.78 ± 7.87
10	76.38 ± 17.57	85.04 ± 17.47	104.89 ± 9.11	98.27 ± 10.42
15	98.78 ± 23.28	108.97 ± 15.39	119.66 ± 21.74	143.08 ± 14.19
20	116.10 ± 18.36	129.34 ± 20.22	176.18 ± 31.14	204.19 ± 26.35
30	128.32 ± 22.28	169.05 ± 19.75	208.26 ± 14.05	220.48 ± 22.06
45	144.61 ± 26.88	178.22 ± 20.29	230.66 ± 19.73	238.81 ± 24.17
60	202.98 ± 27.08	216.07 ± 38.85	290.25 ± 25.74	275.71 ± 49.01

In accordance with the TPC values calculated for the obtained purple corn extracts, it may be assumed that 20 minutes represents the optimal time for an efficient extraction process of the phenolics from the purple corn. The phenolics content ranges from almost 416% (for SMC, magnetic stirring) to 485% (for MC with magnetic stirring), while for the products extracted by ultrasounds the increase of the TPC is 466% (for SMC) and 474% (for MC), all comparisons being made against the TPC values at 1 min of extraction. The results show that after 20 minutes of extraction (either with magnetic stirring or by ultrasound treatment), the increase of the TPC values is lower, probably correlated with the instability of these compounds exposed to oxygen and light. Usually, longer extraction times increase the extraction yield, but stability of the phenolic compounds has to be considered too because they are responsible for the biological effects.
In this experimental phase, the registered TPC values were greater when sifted corn brans were used as starting material. Probably the elimination of the fine part (as flour, which mainly comes from grinding of the corn kernels pulp) leads to a very good contact of the solvent with the brans, rich in phenolic derivatives (Razgonova et al., 2022). Also, ultrasound treatment resulted in higher amount of TPC, magnetic-stirring treatment being an adequate alternative to obtain corn extracts with high content of phenolics.

The microwave-assisted extraction

A non-conventional method was tested in the current study to extract the phenolic compounds from the selected corn variety, for both MC and SMC raw materials. Thus, microwave assisted extraction was performed, using two different power levels: 350W and 700W respectively, the process being performed at different extraction time (5, 10, 15 and 20 seconds) of contact of the samples with the optimal solvent DW-EtOH. The obtained purple corn extracts were evaluated regarding their TPC (mg GAE/ 100g DM), experimental results being reported in the graphs below (Figure 1).

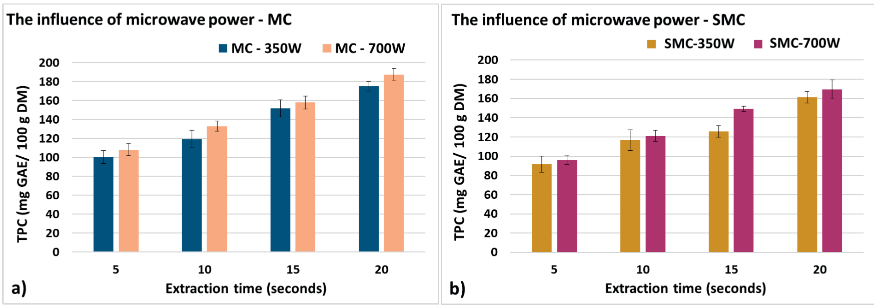

Figure 1. TPC of the corn extracts obtained using microwave assisted extraction: a) MC extract, b) SMC extract

According to the obtained results, the mean TPC values (mg GAE/100 g DM) increase with the increase of the extraction time, the higher phenolics level being noted for those MC and SMC extract resulted after 20 seconds of microwaves exposure. The experiment revealed that higher power level of the microwaves (700W) leads to an increase in the content of phenolic compounds in the MC and SMC extracts, at each extraction time (5-20 seconds). When comparing the MC and SMC from which the extracts were obtained, it may be assumed that the microwave treatment is more effective for the unsifted purple corn bran. Like ultrasound treatment, the microwave mediates good contact of the solvent with the samples, leading to extracts richer in phenolics, which are probably extracted both from the outer shell and from the ground core of the corn kernels. Additionally, probably higher temperature developed for short period during the process contributes to higher extraction yield due to the increased solubility and diffusion coefficient.
Comparing the tested methods, with respect to solvent and time optimized parameters for the extraction of the phenolic compounds from the MC and SMC products, it is obvious that ultrasound assisted extraction and microwave assisted extraction conducted to higher content of the phenolic moieties, while magnetic stirring

is associated with the lowest levels for the studied extracts (Figure 2).

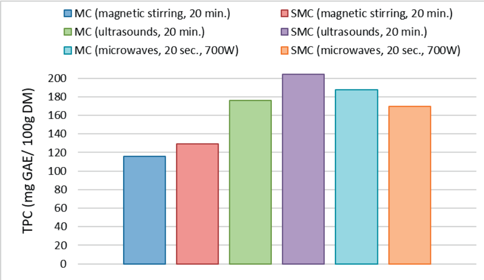

Figure 2. Comparison of the TPC levels for MC or SMC extracts through different extraction methods

Also, low energy consumption method using microwaves conducted to great TPC values, especially when the extraction was performed for the MC material, while for SMC material the highest extraction yield was obtained when performing ultrasound procedure.

Antioxidant activity

The total antioxidant activity was assessed for some MC or SMC selected extracts, obtained through the different extraction methods, using different experimental parameters, as shown in Table 4.

Table 4. Total antioxidant activity evaluated for selected corn extracts

Total antioxidant capacity (mM TE/100 g DM)											
Magnetic stirring				**Ultrasound treatment**				**Microwaves**			
MC	**SMC**	**MC**	**SMC**	**MC**	**SMC**	**MC**	**SMC**	**MC**	**SMC**	**MC**	**SMC**
20 min	20 min	60 min	60 min	20 min	20 min	60 min	60 min	350W	350W	700W	700 W
150.45	214.61	339.29	451.71	296.29	345.11	402.73	430.71	275.00	289.85	385.34	396.67

The total antioxidant activity of the studied corn extract (mM TE/ 100g DM) is well correlated with the total polyphenolic content discussed before, for low extraction period (20 min). The highest antioxidant activity resulted for SMC after 60 minutes of magnetic stirring extraction (451.71 mM TE/100 g DM). For the samples subjected to microwave treatment, the antioxidant activity is higher, particularly when SMC material is used to obtain the extracts, with values around 290 mM TE/100 g DM (350W power level) and 397 mM TE/100 g DM (700W power level). The modified ranking of the effectiveness of the extraction methods may be explained by taking into consideration that the antioxidant potential of the phenolic compounds is correlated with their molecular structure, associated with the total number of -OH groups and also to the position of this functionalities on the chemical structure (Heim et al., 2002; Zheng et al., 2019). Probably longer exposer to ultrasounds (60 min) and higher temperature developed during the process, may affect this chemical structure resulting lower antioxidant capacity for the extracted samples comparing with magnetic stirring ones. When microwaves are used, it is possible to obtain corn extract rich in bioactive compounds with high antioxidant capacity, such as anthocyanins and phenolic acids (Razgonova et al., 2022; Heim et al., 2002). Also, the aqueous ethanol solvent used for the extractive procedures may promote the extraction of the cyanidins, with strong antioxidant potential due to their -OH groups position within the molecule (Hao et al., 2022; Tan et al., 2019). On the other hand, obtaining the best results when magnetic steering was applied may suggest that other factors than the total amount of polyphenols in the extracts could play a significant role in determination of the antioxidant capacity.

CONCLUSIONS

In this study, several extraction experiments were carried out in order to establish the best parameters for extracting phenolics from Bloody Butcher red corn variety cultivated in Romania. Therefore, the water-ethanol (1/1) solvent lead to higher total phenolics content after 60 minutes at room temperature, using ultrasound treatment. Good results were also obtained with only 20 minutes of extraction with both magnetic stirring and ultrasound treatments, for both sifted and un-sifted milled corn brans. Regarding microwave assisted extraction, higher power, and longer exposure lead to higher total phenolics contents. Thus, 700 W power level and 20 seconds extraction time lead to similar results such as ultrasound assisted extractions.

The total antioxidant activity lead to better results for sifted milled corn for either type of extraction, the highest being obtained after 60 minutes of magnetic stirring extraction (451.71 mM TE/100 g DM).

Based on the obtained experimental results, it was concluded that this variety of purple corn can be considered in future experiments for the isolation of phenolic compounds. Also, the profile of phenolic compounds deserves to be studied and these extracts to be tested in food, pharmaceutical or cosmetic applications, as potential additives with antioxidant activity. This may be considered due to the selected extraction parameters, such as cell-friendly solvents (water and ethanol), an aspect closely related to sustainability considerations.

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