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# Wheat and Corn By-Products as New Sources of Functional Ingredients

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## Abstract

In the last years many efforts have been made to recycle agricultural byproducts. Today, cereals are considered one of the main sources of byproducts and the possibility to recover from them healthy components increases day by day. In particular, the research focused on the recovery of the polyphenolic fraction, known to possess many biological activities.

The aim of the present work was to optimize the recovery of polyphenols from corn cob (CC) and wheat byproducts (WBP) using a green method. A Design of Experiment approach was used to optimize the solvent to solid ratio (SSR), the organic solvent percentage (EtOH%), temperature (T), and time of extraction (t). The extracts obtained by applying the best extraction conditions (88.06°C, 42.8 mL/g, 62.4% EtOH, 5 min for CC; 40°C, 24.5 mL/g SSR, 23.8% EtOH, and 5 min for WBP) were investigated for their potential antiglycative and antimicrobial activities. Different model systems were used to monitor the efficacy of the extracts in the three steps of the protein glycation reaction. CC generally had a higher activity then WBP; in fact, it was able to inhibit the fructosamine formation and the Advanced Glycation End products (AGEs) production with activity values higher than 75%. Conversely, WBP was able to better trap the reaction intermediates methylglyoxal and glyoxal than CC. As regards the antimicrobial activity, different clinical isolates, gram positive and gram negative bacteria and yeasts, have been tested. CC was active against methicillin-resistant *Staphylococcus aureus* (MRSA), differently from WBP which was active against *Escherichia coli* (at concentration equal or higher than 60 mg/mL). CC and WBP extracts were active against *Candida albicans* when tested at 40 mg/mL and 60 mg/mL, respectively.

Our results supported the possibility to recycle cereal byproducts to produce new ingredients possessing antiglycative and antimicrobial activities, potentially useful in the production of food supplements and/or fortified foods.

***Keywords:*** *Agrifood Byproducts; Corn Cob; Wheat Byproducts; Polyphenols; Antiglycative Activity; Antimicrobial Activity*

## Abbreviations

CC: Corn Cob; WBP: Wheat Byproducts; MRSA: Methicillin-Resistant *Staphylococcus aureus*; BHIB: Brain Heart Infusion Broth; MGO: Methylglyoxal; GO: Glyoxal; AG: Aminoguanidine; NBT: Nitrotetrazolium Blue Chloride; GLU: Glucose; BSA: Bovine Serum Albumin; OPD: *o*-Phenylenediamine; DPPH: 2,2-Diphenil-1-Picryzil-Hydrazyl; ABTS: 2,2'-Azino-Bis(3-Ehtylbenzothiazoline-6-Sulphonic Acid) Diammonium Salt; MAE: Microwave Assisted Extraction; DOE: Design of Experiments; TMC: Total Metabolite Content; SSR: Solvent to Solid Ratio; AGEs: Advanced Glycation End Products

## Introduction

In the last years, many efforts have been made to recycle agricultural byproducts. Among cereals, corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) are the most world's cultivated crops and therefore the main organic byproducts sources [1]. Such wastes contain different healthy compounds which have a significant impact on human health [2,3], such as micronutrients (vitamins and minerals), fiber and phytochemicals (phytosterols and polyphenols). Bran and husk are the main wheat byproducts obtained from milling and flour production and from the cereal dehulling process, respectively [4]. Differently, corn cob represents the central core of the corn ear. Such byproducts are known to contain polyphenols such as phenolic acids, both hydroxycinnamic (ferulic, *p*-coumaric, and caffeic acids) and hydroxybenzoic derivatives (gallic, synergic, and vanillic acids), and flavonoids [5-7]. As regards corn cob, in literature the composition in polyphenols has been mainly investigated in pigmented varieties [8].

It is also well known that polyphenols are involved in the reduction of oxidative stress and of all related chronic-degenerative pathologies [9,10]. Recently, different action mechanisms have been proposed for polyphenols acting as natural antiglycative agents due to their antioxidant and carbonyl compounds reduction activities, thus preventing advanced glycation end products (AGEs)-related disorders [11]. In particular, hydroxycinnamic acid and its derivatives were active in different glycation model systems, as previously reported for rice and rice husk extracts [12-15].

In addition, many investigations pointed out the antibacterial activity of such secondary metabolites [16-18] and in the last years the search for new antimicrobial agents to treat pathogenic infections increased due to the need of effective therapeutic strategies against antibiotic resistance. In fact, resistance to antimicrobial drugs represents an increasingly large European and global health problem, which limits or makes therapeutic options less effective, causing a worsening of the quality of life. This has serious economic consequences in terms of increased healthcare costs and lost productivity. It is estimated that, in the European Union, every year, more than 30,000 deaths are caused by infections due to bacteria antibiotic resistant and that globally, in the next thirty years, the number of deaths caused by infections due to bacteria resistant to antimicrobial agents will increase higher than that of cancer deaths [19,20]. A microorganism is defined as resistant to antibiotic when it is able to survive in the presence of concentrations of the drug that are inhibitory for most strains of the same species. Antibiotic resistance is present both in many gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and streptococci, and in a large part of gram-negative bacteria, in particular the *Enterobacteriaceae* such as *Klebsiella pneumoniae carbapenemase* (KPC) and *Pseudomonadaceae*.

Paradoxically, considering the high costs for the research and synthesis of new antibacterial molecules, associated with the rapid expansion of drug resistance phenomena, in recent years there has been a slowdown in the research and development of new drugs by the pharmaceutical industry also due to the reduction of economic incentives and stringent regulatory requirements [21]. For this reason, the use of substances of natural origin, capable of replacing or interacting with the action of existing antimicrobial drugs, seems to be a good resource.

In this study we investigated corn cob (CC) and wheat byproducts (WBP) polyphenolic extracts for their antiglycative capacity and tested the possible use of such extracts as a potential source of antimicrobial substance or adjuvants to be associated with antibiotics already present but for which the phenomenon of drug resistance occurs. For these purposes, *in vitro* model systems mimicking the different phases of the glycation reaction and a selected group of clinical isolates, gram positive and gram negative bacteria and yeasts, were used.

## Materials and Methods

### Chemical and reagents

Brain Heart Infusion Broth (BHIB) was purchased from Difco Laboratories (Detroit, Michigan). Methylglyoxal (MGO, 40% aqueous solution), glyoxal (GO, 40% aqueous solution), aminoguanidine hydrochloride (AG, purity grade ≥ 98%), nitrotetrazolium blue chloride (NBT, purity grade ≥ 90%), bovine serum albumin (BSA, purity grade ≥ 98%), D-(+)-glucose (GLU, grade purity ≥ 99.5%), 5-methylquinoxaline (5-MQ, purity grade ≥ 98%), *o*-phenylenediamine (OPD, purity grade ≥ 98%), sodium dihydrogen phosphate monohydrate (purity grade ≥ 98%), disodium hydrogen phosphate dodecahydrate (purity grade ≥ 99%), sodium azide (purity grade ≥ 99.5%), 6-hydoxy-2,5,7,8-tetramethylchroman-2-carboxilic acid (Trolox C), 2,2-diphenil-1-picryzil-hydrazyl (DPPH), 2,2'-azino-bis(3-ehtylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), sodium hydroxide pellets, potassium hydrogen phosphate, and potassium persulfate were from Merck KGaA (Darmstadt, Germany). Ethanol (EtOH) 96% and methanol were provided by Carlo Erba (Milan, Italy).

### Sample materials

Wheat byproducts (WBP) and corn cob (CC) were kindly provided from Lombard Italian Region organic farm (Italy) and dried overnight (final residual moisture of about 1%). Corn cob was minced into powder as previously reported by Frosi., *et al*. [14] (2023). The same sample treatment was also applied to obtain wheat powder. Sample powders were stored at 4 - 8°C in amber glass bottles before their use.

### Extract preparation by experimental design

A microwave assisted extraction (MAE) was applied to byproducts according to Frosi., *et al*. [14] (2023) procedure to obtain the polyphenolic extracts. A microwave apparatus equipped with a teflon vessel system (Ethos LEAN, Sorisole, Italy) was used and experimental designs (Statgraphics Centurion 19 software was used - Statgraphics Technologies, Inc. The Plains, VA, USA) defined the operating conditions for the screening phase which were for CC as follows: solvent to solid ratio (SSR) in the range 15-35 mL/g, temperature (T) in the range 40 - 70°C, hydro-alcoholic mixtures in the range 30-70% EtOH, and irradiation time in the range 5 - 30 minutes. Differently, for WBP the experimental conditions were 20 - 35 mL/g SSR, 40 - 80°C T, 40-80% EtOH, and 30 - 90 minutes of irradiation time. All the obtained extracts were dried and stored at 4°C before HPLC-DAD analysis. A Box Benken Design approach was used to optimize the polyphenols extraction and the complete designs, including the predicted and observed extraction yields, are reported in table 1 and 2 for CC and WBP, respectively. The new experimental ranges considered in the optimization phase were: SSR of 25 - 45 mL/g for CC and 20 - 50 mL/g for WBP; T of 60 - 90°C for CC and 40 - 90°C for WBP; 50 - 80% EtOH for CC and 10 - 90% for WBP; extraction time was fixed at 5 minutes. The polyphenols yield was considered as the response variable and was expressed as the total area of all the peaks recorded in RP-HPLC-DAD chromatograms at 320 and 280 nm (selected as wavelength with a higher intensity signal for CC and WBP, respectively) and indicated as total metabolite content (TMC). A second-order polynomial equation for the response surface was derived and its validation was obtained performing three additional experiments under the optimal predicted conditions for each byproduct.

| Run No. | EtOH (%) | T (°C) | SSR (mL/g) | TMC (mAU*s) (at 320 nm) |
|---|---|---|---|---|
| 1 | 65 | 60 | 25 | 190067.8 |
| 2 | 65 | 60 | 45 | 249988.1 |
| 3 | 65 | 90 | 25 | 243994.5 |
| 4 | 65 | 90 | 45 | 282002.1 |
| 5 | 50 | 75 | 25 | 234220.0 |
| 6 | 50 | 75 | 45 | 261448.2 |
| 7 | 80 | 75 | 25 | 179224.3 |
| 8 | 80 | 75 | 45 | 237021.7 |
| 9 | 50 | 60 | 35 | 232585.5 |

| | | | | |
|---|---|---|---|---|
| 10 | 50 | 90 | 35 | 265836.0 |
| 11 | 80 | 60 | 35 | 186870.1 |
| 12 | 80 | 90 | 35 | 265623.5 |
| 13 | 65 | 75 | 35 | 258155.4 |
| 14 | 65 | 75 | 35 | 254921.5 |
| 15 | 65 | 75 | 35 | 258442.0 |

***Table 1:*** *Box-Behnken design for microwave assisted extraction of polyphenols from CC. Actual variables.*

| Run No. | EtOH (%) | T (°C) | SSR (mL/g) | TMC (mAU*s) (at 280 nm) |
|---|---|---|---|---|
| 1 | 10 | 40 | 35 | 25365.1 |
| 2 | 90 | 40 | 35 | 9212.1 |
| 3 | 10 | 90 | 35 | 20704.7 |
| 4 | 90 | 90 | 35 | 10155.0 |
| 5 | 10 | 65 | 20 | 21538.6 |
| 6 | 90 | 65 | 20 | 10459.0 |
| 7 | 10 | 65 | 50 | 19515.4 |
| 8 | 90 | 65 | 50 | 11059.0 |
| 9 | 50 | 40 | 20 | 19392.9 |
| 10 | 50 | 90 | 20 | 20215.0 |
| 11 | 50 | 40 | 50 | 21560.2 |
| 12 | 50 | 90 | 50 | 26072.3 |
| 13 | 50 | 65 | 35 | 22740.0 |
| 14 | 50 | 65 | 35 | 21590.9 |
| 15 | 50 | 65 | 35 | 21204.3 |

***Table 2:*** *Box-Behnken design for microwave assisted extraction of polyphenols from WBP. Actual variables.*

### RP-HPLC-DAD analyses

For HPLC analyses an Agilent 1200 instruments (Waldbronn, Germany) was used. It was coupled with a diode array detector, an online degasser, and a quaternary pump. A Gemini® C18 analytical column (150 × 2.0 mm, i.d., 5 *μm*, Phenomenex, Torrance, CA, USA) was used and the separation of polyphenols was performed as reported by Frosi., *et al*. [14]. Chromatograms were recorded at 280 and 320 nm. The ChemStation software was used for data acquisition and processing Statistical analysis.

### Antiglycative assays

The capacity of CC and WBP extracts to inhibit the formation of Amadori products measured as fructosamine generated by GLU was evaluated according to Zhang., *et al*. [22] and Frosi., *et al*. [14], and expressed according to Frosi., *et al*. [14].

The capacity of CC and WBP extracts to inhibit the AGEs formation generated in BSA-GLU and BSA-MGO systems was evaluated and expressed according to Frosi., *et al*. [14]. Aminoguanidine (AG) was used in these assays as positive control.

The capacity of CC and WBP to directly trap MGO and GO was quantified using the chromatographic method proposed by Mesia., *et al*. [23] and subsequently modified by Maietta., *et al*. [24], and calculated as reported by Frosi., *et al*. [14].

### Antiradical activity

The antiradical activity was spectrophotometrically evaluated as the capacity of CC and WBP extracts to inhibit the formation of both the stable-colored DPPH free radical and the ABTS cation radical [14,25] and calculated as reported by Frosi., *et al*. [14].

### Microbial strains used and growth conditions

All bacterial and fungal strains were grown in BHIB and in Brain Heart Infusion agar (BHIB with addition of 12 g/L of agar) and incubated at 37°C for 24h for the bacteria and 48h for *Candida albicans* fungus.

In this study, clinical strains collected during routine diagnostics at the Microbiology and Virology Unit of the Verona University Hospital were used. The microorganisms selected are listed below: MRSA, *Staphylococcus epidermidis* (Gram positive bacteria), *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Escherichia coli* (Gram negative bacteria), and *Candida albicans*. Permission to store and further analyze the isolates is implicitly included in the patient-hospital agreement. Therefore, no ethical committee nor study-specific informed consent should be needed. Strains used were stored in Microbank (Pro-Lab Diagnostics, Neston, UK) at -80°C.

### Antimicrobial tests

The antimicrobial activity of CC and WBP extracts was evaluated using a modified Kirby Bauer diffusion method assay. The different microorganisms were plated on Brain Heart Infusion (BHI) agar and grown at 37°C for 24h. A single colony was inoculated into BHI medium and grown for 16/18 hours at 37°C under shaking. Absorbance was measured at 600 nm and microbial cells were diluted to 1 x $10^6$ CFU/mL. 0.1 mL of the microbial suspension was evenly spread onto BHI agar plates. Next, one drop (10 uL) of each concentration (20X, 40X, 60X, 80X respect to initial concentration, i.e. 20, 40, 60, and 80 mg/mL) of CC or WBP extracts was placed on the surface of the BHI agar plates. After incubation for 24h at 37°C under aerobic conditions (48h for *C. albicans*) the halo inhibition zone was measured [26].

### Statistical analysis

All experiments were repeated three times, and statistical values are expressed as the mean ± standard deviation (SD). Differences were considered significant at $p < 0.05$ and $p < 0.01$. Statistical analysis was carried out using Microsoft Office 365.

## Results and Discussion

The first part of the research was focused on the optimization of the polyphenol extraction conditions. The used design of experiments (DOE) approach planned three work phases: screening, optimization, and validation. The results obtained in the screening phase led to the identification of the significant factors affecting the quality process which were the composition of the extraction solvent, specifically the EtOH percentage, SSR value, and T. All the parameters positively affected the extraction yield. Effectively, the use of high percentage of the organic solvent in the extraction mixture provides higher polyphenols recovery due to EtOH dielectric properties [27]. In addition, despite the thermolabile nature of polyphenolic compounds, the use of MAE increased the polyphenols stability even when high T were applied, as already reported in literature [28,29]. The second step consisted in the use of the response surface methodology to set the best experimental conditions. A Box Benken Design approach was used to optimize the experimental conditions and the derived-second order polynomial equations representing the response surface were:

Y= 381282.0 - 29696.1*SSR + 4033.25*T + 3652.02*EtOH + 561.612*SSR2 + 52.5278*SSR*T + 441.724*SSR*EtOH - 63.3073*T2 + 50.5599*T*EtOH - 73.2862*EtOH2 + 1.16415E-10*SSR3- 4.34403*SSR2*T - 5.5825*SSR2*EtOH + 1.43356*SSR*T2 - 2.61934E - 10*SSR*T*EtOH - 2.03727E -10*SSR*EtOH2 + 1.45519E-10*T2*EtOH - 1.01863E- 10*EtOH3

and

Y = 29834,8 + 90,3499*EtOH - 218,909*T - 42,6863*SSR - 3,64154*EtOH2 + 1,40082*EtOH*T + 1,093*EtOH*SSR + 0,545007*T2 + 2,46*T*SSR - 1,66931*SSR2

for CC and WBP, respectively.

ANOVA results obtained for CC and WBP are reported in table 3 and 4, respectively, and highlighted that EtOH significantly ($p < 0.05$) affected the yield both for CC and WBP. As regard SSR and T, these parameters were significant only for CC even if they displayed a positive impact for WBP. In addition, the $R^2$ and $R^2_{adj}$ values were 99.94% and 90.61% for CC and 93.43% and 81.59% for WBP suggesting a close agreement between experimental and predictive results.

| Source | Sum of squares | df | Mean square | F ratio | *P* value |
|---|---|---|---|---|---|
| A: SSR | 1.80E9 | 1 | 1.80E9 | 473.03 | 0.0021 |
| B: T | 3.14E9 | 1 | 3.14E9 | 820.82 | 0.0012 |
| C: EtOH | 5.27E8 | 1 | 5.27E8 | 138.02 | 0.0072 |
| AA | 5.96E8 | 1 | 5.96E8 | 115.99 | 0.0063 |
| AB | 1.20E8 | 1 | 1.20E8 | 31.42 | 0.0304 |
| AC | 2.34E8 | 1 | 2.34E8 | 61.15 | 0.0160 |
| BB | 3.22E7 | 1 | 3.22E7 | 8.44 | 0.1009 |
| BC | 5.17E8 | 1 | 5.17E8 | 135.48 | 0.0073 |
| CC | 1.00E9 | 1 | 1.00E9 | 262.75 | 0.0038 |
| AAB | 8.49E7 | 1 | 8.49E7 | 22.22 | 0.0422 |
| AAC | 1.40E8 | 1 | 1.40E8 | 36.70 | 0.0262 |
| ABB | 2.08E7 | 1 | 2.08E7 | 5.44 | 0.1448 |
| PRESS | 1.72E7 | | | | |

***Table 3:*** *The analysis of variance of the Box-Behnken model for CC polyphenolic compounds extraction.*

| Source | Sum of squares | df | Mean square | F ratio | *P* value |
|---|---|---|---|---|---|
| A:EtOH | 2.67E8 | 1 | 2.67E8 | 45.93 | 0.0011 |
| B:T | 326715 | 1 | 326715 | 0.06 | 0.8221 |
| C:SSR | 5.45E6 | 1 | 5.45E6 | 0.94 | 0.3777 |
| AA | 1.25E8 | 1 | 1.25E8 | 21.54 | 0.0056 |
| AB | 7.85E6 | 1 | 7.85E6 | 1.35 | 0.2979 |
| AC | 1.72E6 | 1 | 1.72E6 | 0.3 | 0.6100 |
| BB | 428412 | 1 | 428412 | 0.07 | 0.7970 |
| BC | 3.40E6 | 1 | 3.40E6 | 0.58 | 0.4789 |
| CC | 520882 | 1 | 520882 | 0.09 | 0.7768 |
| Total error | 2.91E7 | 5 | 2.91E7 | | |

***Table 4:*** *The analysis of variance of the BBD model for WBP polyphenolic compounds extraction yield.*

Figure 1 and 2 reported the surface plot obtained for CC total metabolite content as a function of T and SSR and for WBP as a function of EtOH and T. As regards CC extract, the optimal conditions predicted by the software were 88.06 °C, 42.8 mL/g, and 62.4% EtOH with a total peaks area of 282495.0 mAu*s with an inferior and superior 95% confidence level for the mean value of 276718.0 mAu*s and 288272.0 mAu*s, respectively. Differently, for WBP the optimal conditions were 23.8% EtOH, 40°C, and 24.5 mL/g SSR. This configuration yielded a calculated total peak area of 24372.0 mAu*s and the 95% confidence interval for the mean extraction yield value ranged from 19373.6 mAu*s to 29370.4 mAu*s.

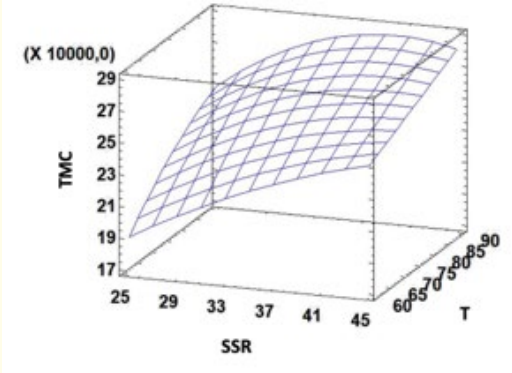


***Figure 1:*** *Response surface plot showing the effect of temperature (T) and solvent to solid ratio (SSR) on CC polyphenolic yield, expressed as total metabolite content (TMC).*

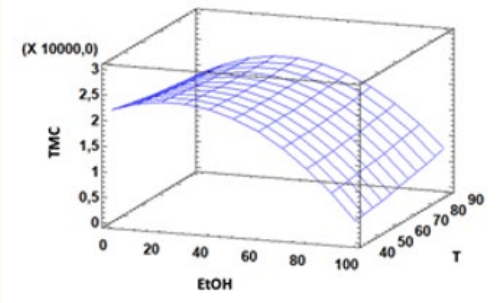


***Figure 2:*** *Response surface plot showing the effect of EtOH content and temperature (T) on WBP polyphenolic yield, expressed as total metabolite content (TMC).*

The validation of the second-order polynomial equation models was achieved in the last step of the work by performing an additional set of three experiments under the optimal predicted conditions. The results indicated that the mean total area of peaks fell within the predicted interval both for CC and for WBP (285345.0 ± 3737.67 mAu*s and 22129.3 ± 394.9 mAu*s for CC and WBP, respectively).

The optimized extracts were tested in the next research phase. Three different concentrations, i.e. 0.5, 1, and 2 mg dry matter/mL, have been tested for their potential ability to mitigate the non-enzymatic protein glycation using different model systems according to the stage of the protein glycation reaction.

The impact of the extract on Amadori products formation (initial stage) was evaluated by the quantification of fructosamine which is generated from the reaction between sugar carbonyl and protein amino groups, followed by a molecular rearrangement [30]. The model system consisted of GLU and BSA and NBT assay was used to monitor fructosamine formation. A dose-activity relationship was registered (Figure 3a and 3b) for both the extracts and CC extract better inhibited fructosamine formation even when tested at the lowest concentration, reaching an activity value higher than 70% when tested at 2.5 mg/mL.

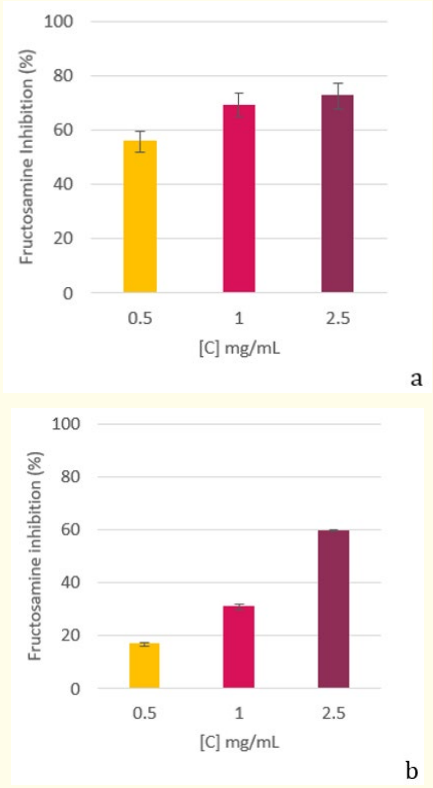


***Figure 3:*** *Inhibition of the fructosamine formation by CC (a) and WBP (b) extracts tested at different concentrations (0.5, 1, 2.5 mg dry matter/mL) in NBT assay.*

In the intermediate stage of the protein glycation reaction, dicarbonyl compounds, such MGO and GO, are generated. These oxoaldehydes are considered precursor compounds of AGEs whose toxicity is well known [11]. A BSA-MGO model system was used to investigate the capacity of CC and WBP extracts to inhibit the pentosidine- and argpyrimidine-like AGEs formation over 1, 4, and 7 days. In figure 4a, 4b, 5a and 5b the obtained results are reported. CC extract displayed the highest activity also when tested at 0.5 mg/mL. In addition, the activity was always similar or higher than that registered. For aminoguanidine (AG, 0.5 mg/mL), a well-known antiglycative compound, not yet recommended in therapy due to its many side effects, but generally used as a positive control. Finally, when tested at 2.5 mg/mL it was able to completely inhibit pentosidine-like AGEs during the entire monitoring period as well as previously reported for rice husk and purple corn cob extracts [14,31]. WBP extract reached an activity value of about 20 - 30% after 7 days of incubation when tested at the highest concentration.

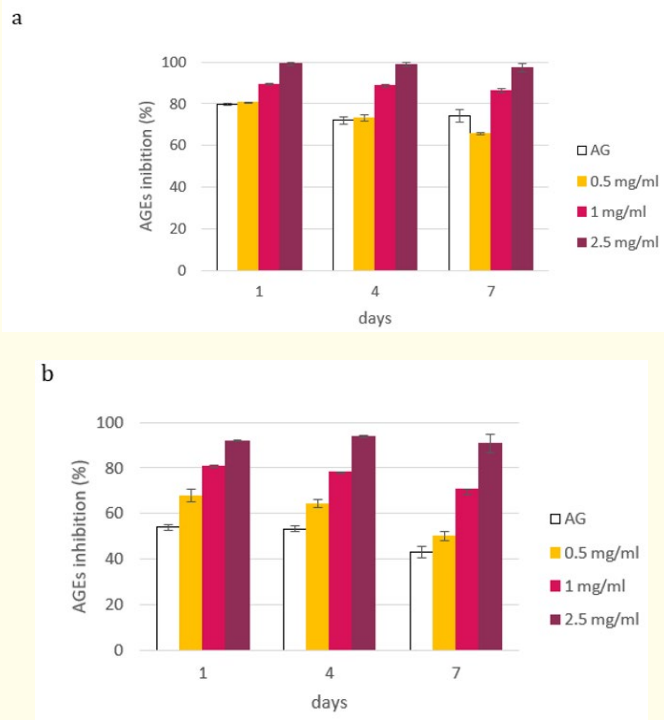


***Figure 4:*** *Inhibition of the pentosidine-like AGEs (a) and of argpyrimidine-like AGEs (b) by CC extract.*

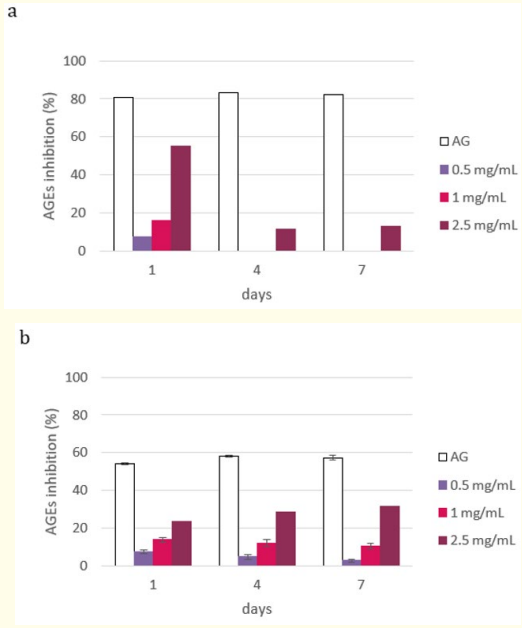


***Figure 5:*** *Inhibition of the pentosidine-like AGEs (a) and of argpyrimidine-like AGEs (b) by WBP extract.*

Considering that in literature different mechanisms of action have been reported for polyphenols antiglycative capacity, including the dicarbonyl compounds trapping activity [32], a model system consisting in the direct incubation of extracts with MGO or GO for different times (1, 24, and 48h) was set up. CC extract had the lowest trapping activity against MGO (Figure 6) and no activity against GO, differently from WBP extract which was able to trap almost total MGO after 48h at 2.5 mg/mL (Figure 7a and 7b). The higher trapping capacity against MGO than GO could be due to the easy polarization of GO in aqueous medium which limits its capture and separation from the solution [33].

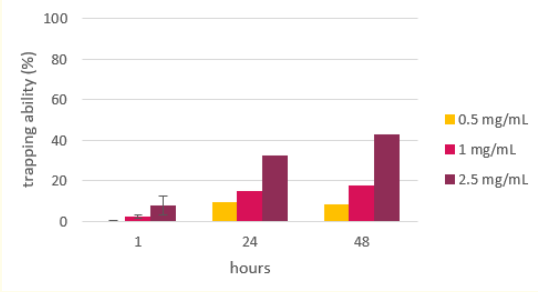


***Figure 6:*** *Trapping ability of CC extract against MGO.*

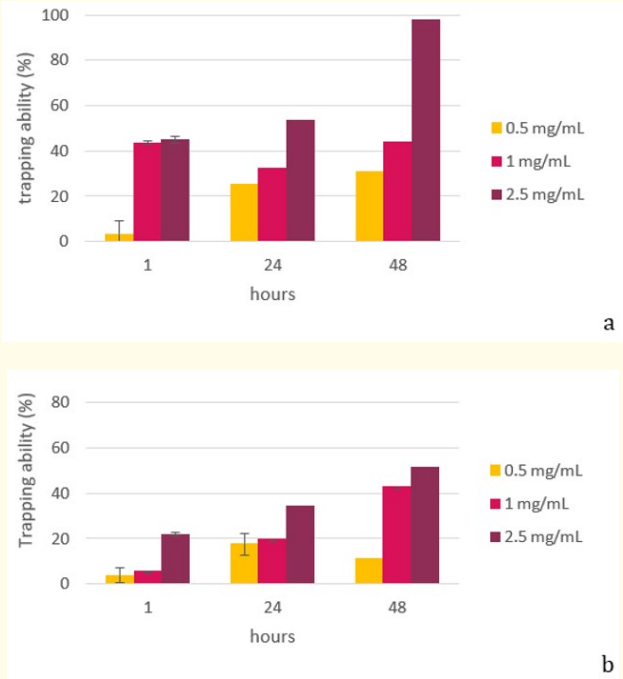


***Figure 7:*** *Trapping ability of WBP extract against MGO (a) and GO (b).*

Finally, the end stage of the glycation reaction was simulated using a BSA-GLU system generating argpyrimidine-like AGEs which have been monitored at 7, 14, and 21 days [24]. All CC extract concentrations had the capacity to inhibit about 80% of AGEs after 21 days (Figure 8), thus showing a better activity than that of other byproduct extracts reported in literature, such as rice husk extract [14]. Differently, WBP activity reached only approximately 30% at the highest tested concentration by the end of the monitoring period (Figure 9).

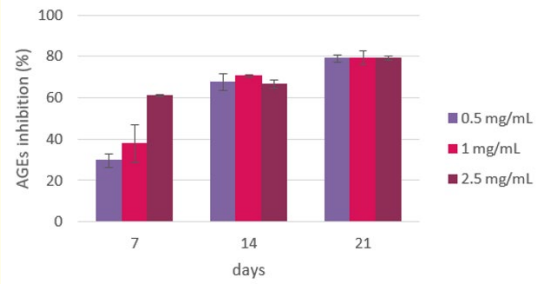


***Figure 8:*** *Inhibition of the AGEs formation in BSA-GLU system by CC extract at different concentrations (0,5, 1, 2.5 mg/mL dry matter).*

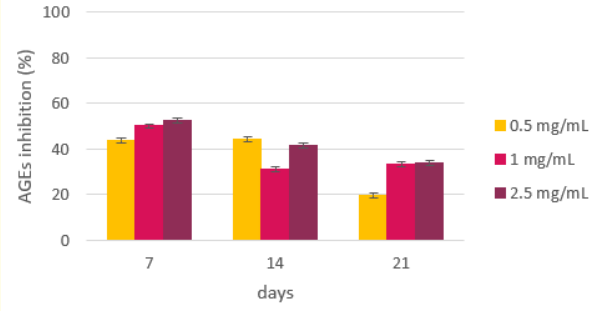


***Figure 9:*** *Inhibition of the AGEs formation in BSA-GLU system by WBP extract at different concentrations (0,5, 1, 2.5 mg/mL dry matter).*

The results obtained from the tested cereal wastes are generally in accordance with those reported in literature also for rice bran [34], sorghum buckwheat hulls [35], and triticale bran [36]. Generally, the research about such activities for white cereal is poor and mainly focused on pigmented varieties [37,38]; however, our results obtained for CC are in line with those published for colored corn. The different activity registered for the two extracts should be due to the different qualitative and quantitative composition in polyphenols and, even if we considered byproducts, also to the cereal growth environment [39]. In fact, according to previous researches flavones, for example, could be able to stronger inhibit AGEs formation than other flavonoids, such as flavanones, flavonols, and isoflavones, but the flavonoids' activity is generally lower than that of hydroxycinnamic acid derivatives [40,41].

Considering that in the middle step of the glycation reaction reactive oxygen and carbonyl species are generated, we investigated the potential antioxidant activity of the extracts using two spectrophotometric antiradical assays. Figure 10 and 11 reported CC and WBP scavenging activity against the colored stable DPPH radical and the ABTS cation radical. A dose-activity relation was registered with significantly higher values for CC ($p < 0.01$) in the $ABTS^{+}$ radical assay; only a moderate capacity to scavenge DPPH radicals was registered for CC and, differently, WBP had quite no activity.

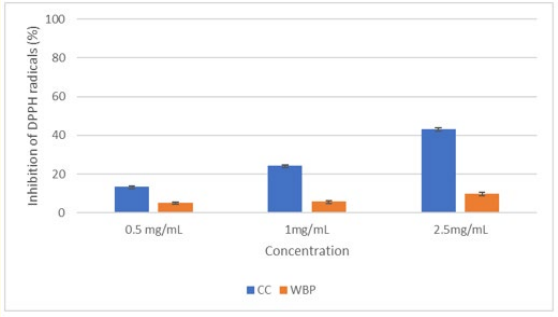


***Figure 10:*** *Inhibition percentage of DPPH radical formation by CC and WBP extracts at different concentrations.*

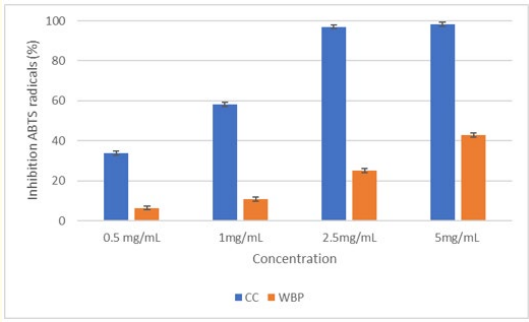


***Figure 11:*** *Inhibition percentage of the ABTS•+ radical formation by CC and WBP extracts at different concentrations.*

To evaluate the correlation between the antiglycative capacity observed at the end of the monitoring period in various assays and the antiradical activities, the Pearson correlation coefficient was calculated (Table 5) and it was generally higher than 0.9 with the exception of the value related to correlation between the antiglycative action in the system BSA-GLU and the antiradical capacities.

| | ASSAY | DPPH$^{\bullet}$ ($R^2$) | ABTS$^{\bullet+}$ ($R^2$) |
|---|---|---|---|
| CC | NBT | 0.9441 | 0.9512 |
| | MGO | 0.8812 | 0.8916 |
| | GLU | 0.5299 | 0.5486 |
| WBP | NBT | 0.9835 | 0.9993 |
| | MGO | 0.9704 | 0.9951 |
| | GLU | 0.6095 | 0.7180 |

***Table 5:*** *The Pearson's correlation coefficients ($R^2$) were calculated to assess the relationship between the antiglycative activities and the antioxidant activities of CC and WBP extracts at the various concentrations tested.*

In order to extend the knowledge about the possibility of considering CC and WBP extract as ingredients for functional food and/or food supplements, the potential antimicrobial activity against the selected clinical strains was investigated. Different concentrations were tested (20, 40, 60, and 80 mg/mL) and CC was able to inhibit the growth of most of the tested bacteria, with exception of *E. coli*,

at concentrations higher than 40 mg/mL (Table 6). WBP extract was only active again *C. albicans* and *E. coli* whose growth have been inhibited at concentration higher than 60 mg/mL. Differently, no growth inhibitory activity was registered for the other bacteria selected for this study (Table 7) These results partially agree with those reported by Saha., *et al*. [42] (2018) and subsequently by Kaviya., *et al*. [43] (2022), highlighting the growth-inhibitory action against multi-resistant bacteria of wheat seeds when tested at high concentrations. In particular, the ethanolic extract of *T. aestivum* grass showed good potential inhibitory action against *Bacillus* species confirming similar results obtained by Kim., *et al*. [44] (2010), who demonstrated that wheat germ has a good protective role against *B. cereus*. Furthermore, in a work by Linard de Carvalho., *et al*. [45] (2019) the obtained results indicated that *Zea mays* contains bioactive compounds with both antibacterial and antibiotic modulation properties, determining a synergistic effect with aminoglycoside antibiotics against *S. aureus* and *P. aeruginosa*.

| Microorganisms | 20 mg/mL | 40 mg/mL | 60 mg/mL | 80 mg/mL |
|---|---|---|---|---|
| *S. aureus* MRSA | n.i. | n.i | Active | Active |
| *S. epidermidis* | n.i | n.i | Active | Active |
| *E. coli* | n.i | n.i | n.i | n.i |
| *K. pneumoniae* | n.i | n.i | n.i | Active |
| *P. aeruginosa* | n.i | n.i | n.i | Active |
| *C. albicans* | n.i | Active | Active | Active |

***Table 6****: Evaluation of CC extract on grown inhibition microorganisms.*

*n.i: No Inhibitory Activity.*

| Microorganisms | 20 mg/mL | 40 mg/mL | 60 mg/mL | 80 mg/mL |
|---|---|---|---|---|
| *S. aureus* MRSA | n.i. | n.i. | n.i. | n.i. |
| *S. epidermidis* | n.i | n.i | n.i | n.i |
| *E. coli* | n.i | n.i | active | active |
| *K. pneumoniae* | n.i | n.i | n.i. | n.i. |
| *P. aeruginosa* | n.i | n.i | n.i | n.i |
| *C. albicans* | n.i | n.i | Active | Active |

***Table 7:*** *Evaluation of WBP extract on grown inhibition microorganisms.*

*n.i.: No Inhibitory Activity.*

Although further investigations are necessary, CC and WBP extracts can be considered promising agents useful to counteract the bacterial growth of some microorganisms. In particular, it could be useful to test various associations with different types of antibiotics to verify a possible synergistic effect. This would lead to the reuse of old antibiotics already present and for which the phenomenon of antibiotic resistance is currently observed.

## Conclusion

The results obtained in this research highlighted the possibility to obtain a polyphenolic extract from corn cob and wheat byproducts potentially useful in preventing the antiglycative reaction with different action mechanisms. It was also possible to optimize the recovery of these secondary metabolites by applying the DOE approach, using a green extraction method based on the hydro-alcoholic (ethanol) microwave-assisted extraction. In particular, corn cob extract had the best activity in the first and middle stages of the protein glycation,

differently from wheat byproducts extract which was better able to directly trap dicarbonyl compounds (glyoxal and methylglyoxal). An antiradical capacity was also registered for both the extracts which very well correlates with the antiglycative activity.

In addition, a good antibacterial activity against different microorganisms was registered for concentrations equal to or higher than 60 mg/mL. The registered activity was different for the tested byproduct extracts and was related to the bacteria considered. It is noteworthy that both extracts are active against *Candida albicans*.

Generally, the different activities registered for the considered byproducts could be ascribed to the different composition in polyphenols of the extracts which is under investigation to fully chemically characterize the extracts.

In conclusion, this research supports the recycle of agrifood byproducts to obtain new ingredients for food supplements or functional foods production. The research is going on also to investigate the extracts' stability. Finally, considering their potential use in food sector, it will be also necessary to evaluate the bioaccessibility of their components in order to obtain a product really possessing antiglycative and/or antimicrobial activities.

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## Conflict of Interest

The Authors declare that no financial interest or any conflict of interest exists.

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