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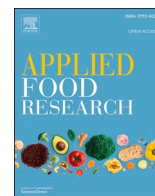
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## Improvement of solubility and functional properties of wheat bran fibers by ozone treatment

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### ABSTRACT

Wheat Bran (WB) components have multiple beneficial properties for human health. Physical, Chemical and biological treatments are used to improve WB physicochemical properties or extract WB bioactive components, but they can have drawbacks (cost, conditions, scalability). In this study an attempt was made to investigate the effect of ozone treatment on the physicochemical and functional properties of WB to further improve its nutritional and health benefits. Ozone treatment was carried out on a semi-industrial pilot scale with different O<sub>3</sub> consumption levels ranging from 3 to 24 g/kg of WB. WB structural characterization was carried out using spectroscopy (FT-IR) and microscopy (SEM) analyses. WB fiber molecular effect was studied using fractionation analysis. Composition and functional properties (antioxidant power, binding capacities, inhibitory activities) were also assessed. As expected, ozone treatment did not impact WB structure. However, ozonation significantly increased the soluble dietary fiber content in WB by 32–67 % indicating transformation of insoluble hemicelluloses. This was likely due to the ability of ozone to oxidize phenolic acids and to cleave β-glucans glycosidic bond. This reduces WB fiber molecular weight. Ozone shows major positive effects on WB functional properties by improving cholesterol adsorption capacity or α-amylase and α-glucosidase inhibitory activities. As conclusion, ozone could be a good candidate for the optimization of WB physicochemical and functional properties without altering its macromolecular structure. Furthermore, it is a green process that offers greater scalability for industrial applications compared to other treatments, enabling its incorporation in a wide range of food products.

### 1. Introduction

Wheat bran (WB), the most important by-product of wheat flour processing, generally represents 14 to 19 % of the total grain weight. It mainly contains the outer layers of the seed (pericarp, testa and hyaline). Its histological and chemical composition is heterogeneous and largely depends on the industrial process. WB typically contains, on a dry matter basis, 43 to 60 % non-starch polysaccharides (NSP), 11–24 % starch, 14–20 % protein, 3–4 % lipids, and 3–8 % minerals (Hell et al., 2014). WB is rich in bioactive and nutritional molecules, particularly dietary fibers (DF) including arabinoxylans and β-glucans, polyphenols, alkyl-resorcinols, lignans, and vitamins (Prückler et al., 2014). These components may help to improve intestinal microbiota (Aoe et al., 2020; Jefferson & Adolphus, 2019), reduce blood sugar levels (Mao et al., 2021; Pino et al., 2021; Shen et al., 2016) and reduce risk of metabolic syndrome (Ortiz de Erive et al., 2020; Stephen et al., 2017). Despite the proposed and established health benefits associated with the bioactive

compounds present in WB as well as substantial economic growth in animal feed, this by-product is little used in human food. Its incorporation in food product formulation has a significant negative impact on technological qualities such as rheological or textural properties, and deteriorates the organoleptic and sensory characteristics, leading to poor consumer acceptance and rejection of the bran-enriched foods (Hemdane et al., 2016; Steglich et al., 2015). In the last decades, technologies based on mechanical (milling and superfine grinding), biological (enzymatic hydrolysis and fermentation), physical (autoclaving, dry heat, extrusion and steam explosion) and chemical (hydrolysis, hydroxypropylation, and acylating) treatments of WB have been adopted and tested to overcome these drawbacks (Sztupecki et al., 2023). Some of these approaches were efficient for the production of functional WB with enhanced features (increased SDF content, improved antioxidant activity, flavor profile and shelf life) (Kong et al., 2020; Onipe et al., 2021). However, some treatments may present drawbacks, such as issues related to sustainability, environmental impact, cost or unwanted

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alterations of the matrix (Sztupecki et al., 2023). Finally, not all of these treatments are scalable to an industrial level.

In this context, ozone (O<sub>3</sub>), which is 'generally recognized as safe' (GRAS) for use in the food industry in the United States (Food and Drug Administration, Department of Health and Human Services), could be an interesting alternative chemical technology for WB optimization. Whilst it is being used primarily to reduce microbial charge during food storage (Sarron et al., 2021), including for whole wheat grain treatment (Gozé et al., 2016, 2017). We found in this previous work that ozonation of wheat grains has led to significant modifications of their molecular properties, which strongly affected bread making quality.

Studies on wheat grain have shown that ozone treatment can induce changes such as introduction of carbonyl/carboxyl groups in starch, creation of disulfide bound in proteins, change in protein secondary structure, lipid oxidation and reduction of polyphenols content (Gozé et al., 2017; Lemeune et al., 2004). Nevertheless, some authors reported enhanced rheological properties after wheat grain O<sub>3</sub> treatment (F. Zhu, 2018). Ozone also seems to have interesting effects on NSP. An increase of SDF content and a reduction of  $\beta$ -glucans molecular weight (Mw) have been reported (Gozé, 2016; C. Li et al., 2021). We hypothesize that ozone oxidative power will have an interesting potential for cleaving covalent bonds in NSP and changing WB physicochemical properties, leading to increased functional potential. Considering its positive effects on WB and relatively adverse effects on starch, it would be more appropriate to investigate the effect of ozone on isolated bran. Only one study has investigated the effect of isolated WB ozonation for the extraction of xylooligosaccharides, but the associated functional properties for WB valorization in human food have not been studied (Murlidhar Sonkar et al., 2021).

The main objective of this research is to analyze the effect of ozone treatment on physicochemical, functional and structural properties of WB. The ozonation process is widely studied for decontamination purposes; therefore, using ozonation as a method to improve the nutritional quality of bran in the food industry is a highly innovative topic. This investigation would contribute valuable insights into the application of ozone treatment to produce healthier and highly functional WB. This semi-industrial-scale work also aims to promote the widespread use of WB in the agri-food industry. Furthermore, these findings will form a significant foundation for new research projects aiming at getting desirable effects on nutritional, physiological and functional properties of WB and bran-based food.

## 2. Material and methods

### 2.1. Materials

Soft wheat (*Triticum aestivum* L.) bran with 90 % of the particle size comprise between 0.2 mm and 1.25 mm was obtained from Paulic Meunerie S.A. (Pontivy, France).  $\alpha$ -amylase porcine pancreatic 75 000 U/g (E-PANAA-12 G) and kits for  $\beta$ -glucans (K-BGLU 01/21), starch (K-TSTA-100A 11/20) and total fiber (K-TDFR-200A 04/17) quantification were purchased from Megazyme, Bray, Ireland. All chemicals, methanol (34,885), Hydrochloric acid (320,331), DMSO (34,869), Folin-Ciocalteu (F9252), 2,2-diphenyl-1-picrylhydrazyl (DPPH, D9132), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ, T1253), FeCl<sub>3</sub> (372,870), sodium carbonate (223,530), acetic acid (695,092), acetonitrile (34,851), trolox (238,813), wheat starch (S5127),  $\alpha$ -glucosidase (G5003), cholesterol assay kit (MAK043), sodium cholate (C1254), furfural (185,914),  $\beta$ -glucan from barley (G6513), sodium azide (S2002), 4-nitrophenyl  $\alpha$ -D-glucopyranoside (N1377), polyphenols standards: 2,5-dihydroxybenzoic (149,357), 3-hydroxybenzoic (H20008), 4-hydroxybenzoic (240,141), chlorogenic (C3878), vanillic (94,770), caffeic (C0625), syringic (S6881), *p*-coumaric (C9008), ferulic (128,708), sinapic (D7927), *trans*-cinnamic (C80857), gallic (27,645) acids, epicatechin (E1753), myricetin (M6760), quercetin (Q451), naringenin (N5893), kaempferol (60,010) were purchased from Sigma-Aldrich, Saint-

Quentin-Fallavier, France. The water used was ultrapure water purified at the laboratory with Milli-Q Synergy® UV (Merck KGaA, Darmstadt, Germany).

### 2.2. Wheat bran ozonation

Experiments were conducted in UniLaSalle, Beauvais, France on a semi-industrial pilot reactor. The twin shaft paddle reactor was fed with 40 kg batches of WB. WB was humidified at 4 % (w/w) by pressurized nozzle injection. Then, ozone was injected into the airtight reactor onto the bran in rotation for various times according to the experimental design (Table 1). O<sub>3</sub> is generated from oxygen 99.5 % (Messer, Suresnes, France) with CFS-14 generator (Ozonia, Saint-Maurice, France). The oxygen flowrate and O<sub>3</sub> concentration at the entrance of the reactor were maintained at 5.05 m<sup>3</sup>/h and 110 g/m<sup>3</sup>, respectively. Inlet and outlet O<sub>3</sub> concentrations were continuously measured and recorded with Ozone Analyzer BMT 964 (BMT Messtechnik GMBH, Stahnsdorf, Germany). The ozone off-gas was destroyed at 350 °C by a thermal destruction unit. Freshly treated-WB were aliquoted in 5 kg bags under air-vacuumed conditions and stored (-20 °C) until required for use. pH was used as a control of ozonation process. It was measured immediately after treatment. 1 ± 0.1 g WB fresh weight (FW) was soaked in 20 mL distilled water, stirred for 30 s on a vortex mixer and then measured with a pH-meter. Water activity (Aw) was measured with LabSwift-aw (Novasina AG, Lachen, Switzerland).

### 2.3. Composition characterization

The ash, moisture, total lipids, total proteins and starch contents of WB were determined by the AACC methods 08-01, AACC 44-15A, AACC 30-25, AACC 56-10 and AACC 76-13.01. SDF, Insoluble (IDF) and total fiber (TDF) contents were determined using Megazyme kit (K-TDFR 04/17) according to AOAC 991.43 and AACC 32-07.01 official methods.  $\beta$ -glucans content was analyzed using Megazyme kit (K-BGLU 01/21) according to AACC method 32-23.01. Free polyphenols content was determined using the Folin-Ciocalteu method with modifications (Tikent et al., 2024). Briefly, 100  $\mu$ L of extracts obtained from 0.8 g of WB diluted in 10 mL of 80 % ethanol (v/v in H<sub>2</sub>O) were mixed with 900  $\mu$ L of 80 % ethanol. Then, 7.5 mL of ultrapure water and 500  $\mu$ L of Folin-Ciocalteu reagent were added. The samples were incubated for 20 min at 40 °C. Next, 1.5 mL of 20 % sodium carbonate solution were added. After cooling the tubes in ice, the absorbance was measured at 755 nm using a gallic acid standard curve. Free polyphenols characterization has been done on HPLC on WB ethanol extracts filtered with PTFE filter 0.45  $\mu$ m (Marcherey-Nagel, Hoerd, France). Polyphenols were analyzed on Thermo Scientific™ Ultimate 3000 system (Thermo Electron Corporation, Courtabœuf, France) using a Prodigy 5 u, ODS 3, 100A (250 × 4.6 mm) column (Phenomenex, le Pecq, France) serially connected with an identical phase pre-column. The mobile phase consisted of 2 solvents (A) ultrapure water with 1 % acetic acid (v/v) and (B) acetonitrile with 1 % acetic acid. A flow rate of 0.38 mL/min was used, applying the following elution binary gradient: 0–20 min, linear from 97 to 92 % solvent A; 20–30 min, linear from 92 to 88 % A; 30–60 min, linear from 88 to 20 % A; 60–65 min, linear from 20 to 0 % A, and 65–70, isocratic 100 % A. The injection volume was 5  $\mu$ L. Channel settings on

**Table 1**  
Ozone reaction parameters.

WB sample	Ozonation reaction time (min)	Ozone consumed (g O <sub>3</sub> / kg WB)
Control	0.0	0.0
3G	13.5	3.21
6G	27.0	6.26
9G	40.0	9.04
12G	53.0	11.88
24G	106.0	23.42

DAD detector were 220, 280, 320 and 354 nm. Polyphenol standards ranging from 5 to 400 µg/mL were used for external calibration.

## 2.4. Molecular and structural characterization of WB and DF

### 2.4.1. The microstructural features of WB

Microstructure was observed with Scanning Electron Microscopy (SEM) with TM-1000 tabletop microscope (Hitachi High-Tech Corporation, Tokyo, Japan). The WB sample was fixed on the SEM holder with double-sided tape; the morphology was observed between 40x and 200x magnification.

### 2.4.2. Fourier transform-infrared spectroscopy

WB sample were preliminary dried in an oven at 40 °C for 24 h, milled and analyzed in triplicate by FTIR spectroscopy with attenuated total reflectance (ATR) spectrometer (Nicolet iZ10 module, Thermo Fisher scientific, Courtabœuf, France). The spectral acquisition ranged between 600 and 3700 cm<sup>-1</sup>, the resolution was 8 cm<sup>-1</sup>, and the number of scans was 64 times.

### 2.4.3. Molecular features of soluble fibers

SDF for AFFFF (Asymmetrical Flow Field-Flow Fractionation) analysis were extracted as follows: 1 g of WB FW was mixed in triplicate with 10 mL ultrapure water, agitated (50 rpm, 30 min), stirred and incubated (1 h, 60 °C) in a water bath before centrifugation (13 000 x g, 10 min, 22 °C). The supernatant was collected, and SDF were precipitated with 4 vol of 95 % ethanol (v/v H<sub>2</sub>O) for 1 h at room temperature before centrifugation (9 000 x g, 5 min, 22 °C). Ethanol was discarded and SDF residue was solubilized in 10 mL 0.05 % (w/v) sodium azide aqueous solution and 100 µL NaOH 1 M at 80 °C for 2 h with recurrent stirring. Then samples were filtered through 0.45 µm regenerated cellulose (RC) filter (Macherey-Nagel, Hoerd, France) before injection into the AFFFF system.

β-glucans for AFFFF analysis were extracted according to Megazyme kit (K-BGLU 01/21). All samples were lyophilized, resuspended in 1 mL 0.05 % sodium azide aqueous solution and filtered through 0.45 µm RC filter before injection (100 µL). β-glucan from barley was used as standard.

SDF and β-glucans Mw distribution was determined using AFFFF / Multi-Angle Laser Light Scattering - Quasi-Elastic Light Scattering (MALLS-QELS) system consisted of an Eclipse3 F System (Wyatt Technologies Corporation, Europe) serially connected to a UV detector (Agilent 1200, Agilent Technologies, Germany), a MALLS-QELS detector (Dawn multi-angle Heleos TM, Wyatt Technologies Corporation, Europe) and an interference refractometer (Optilab rEX, Wyatt Technology Corporation, Europe). Fractograms were analyzed with ASTRA® 7.1.2 software (Wyatt Technology Corporation, Europe). Fractionation method and calculation parameters were previously described (Goze et al., 2016).

## 2.5. Antioxidant activity

### 2.5.1. Determination of radical scavenging antioxidant capacity with DPPH test

To determine radical scavenging activity, DPPH stock solution was prepared by mixing 0.067 g in 320 mL absolute methanol (Yen & Duh, 1994). A part of this solution was diluted with methanol to obtain Abs<sub>517nm</sub> = 0.9. WB ethanol extracts from free polyphenols analysis were diluted (0, 50, 75, 100, 200, 300 and 400 µL / mL) with 80 % ethanol (v/v H<sub>2</sub>O). 1 mL of methanolic DPPH solution was added to each sample tube then mixed and incubated 1 h in the dark at room temperature to allow the color to develop. Finally, the absorbance was read at 517 nm with methanol as blank. Antioxidant capacity was expressed as the half maximal inhibitory concentration (IC50) which was calculated from the regression equation of sample linear curve that has been established between the percentage inhibition of the DPPH radical and polyphenol

contents.

### 2.5.2. Ferric reducing antioxidant power (FRAP) determination

FRAP solution was prepared by mixing 300 mM acetate buffer (pH: 3.6), 10 mM TPTZ solution and FeCl<sub>3</sub> solution (3.25 mg/mL) (10/1/1; v/v). To find sample optimal dilution, 50 µL of a range of WB ethanol extracts dilutions in 80 % ethanol (v/v H<sub>2</sub>O) were mixed with 950 µL FRAP solution, incubated 5 min in the dark and absorbance was read at 593 nm. Absorbance should be between 0.3 and 0.7. Then, the diluted sample was mixed with FRAP solution and incubated (30 min, 37 °C) before absorbance reading. The Trolox Equivalent Antioxidant Capacity (TEAC) of the sample was calculated using the linear regression line of a trolox standard prepared at 0.254 mg/mL followed by serial dilutions.

## 2.6. Binding properties of WB

### 2.6.1. Water binding capacity (WBC)

For each sample 1 ± 0.009 g was weighed in triplicate and water was added drop by drop until saturation (when water forms a film on WB but does not drip when the tube is inverted). WB was let absorbing water 1 night (room temperature) before centrifugation (500 × g, 5 min, 22 °C). Excess water was discarded with micropipette and the saturated WB weight (W1) was recorded before drying overnight (80 °C) and finally the WB DW (W) was recorded. WBC was calculated according to the equation (a).

$$\text{WBC (g water / g WB DW)} = \frac{W1 - W}{W} \quad (1)$$

### 2.6.2. Oil holding capacity (OHC)

OHC was determined based on (Wang et al., 2012) with modifications, 1 g WB FW (W1) was dried at 60 °C for 24 h to avoid any residual water preventing the oil from being absorbed. 10 mL of sunflower oil was added to WB DW (W) and the samples were left to soak 1 night at room temperature. Unbound oil was discarded with pipette after centrifugation (5000 × g, 5 min, 22 °C). WB soaked in oil weight was recorded (W0) Finally, OHC was calculated as follows:

$$\text{OHC (g bound oil / g WB DW)} = \frac{W0 - W1}{W} \quad (2)$$

### 2.6.3. Cholesterol adsorption capacity (CAC)

CAC was measured in triplicate according to Luo et al. (2018); Wang et al. (2021) with modifications. Samples (0.1 g) were mixed with 2.5 mL diluted yolk solution (a fresh egg yolk diluted with nine volumes of distilled water and homogenized) at pH 2.0 and 7.0 corresponding to stomach and intestinal pH respectively. The blanks are fresh egg yolk without sample. Sample are incubated (2 h, 37 °C, 80 rpm), before centrifuged (550 x g, 15 min, 22 °C) and the cholesterol content of the supernatant was measured using a cholesterol assay kit. CAC was calculated as follows:

$$\text{CAC (\% Cholesterol absorbed)} = \frac{(C_c - C_s) \times 100}{C_c} \quad (3)$$

Where C<sub>c</sub> and C<sub>s</sub> are the concentrations of control sample and sample respectively (µg cholesterol / mg WB) obtained with the regression curve of the kit standard.

### 2.6.4. Sodium cholate adsorption capacity (SCAC)

SCAC was determined according to Hua et al. (2019); L. Wang et al. (2021). Samples (1.0 g) were mixed with 30 mL of sodium cholate (SC) solution (1 mg/mL), shaken for 2 h, and centrifuged (2 200 x g, 15 min, 22 °C). The supernatant of the sample (1 mL) and 1 mL of standard solution were mixed with 6 mL of HCl solution (45 %, v/v H<sub>2</sub>O) and 1 mL of furfural solution (0.3 %, v/v water) and incubated 30 min at 65 °C in water bath. The mixture was cooled to room temperature and the absorbance read at 620 nm. SCAC was calculated as follows:

$$\text{SCAC (mg/g WB DW)} = \frac{(C_{sc} - C_s) \times V}{W} \quad (4)$$

Where  $C_{sc}$  and  $C_s$  are SC concentrations before (1 mg/mL) and after adsorption calculated with the standard curve equation from  $\text{Abs}_{620\text{nm}}$  value;  $V$  is the extraction volume (30 mL), and  $W$  is WB DW (g).

## 2.7. Enzymes inhibitory activities

### 2.7.1. $\alpha$ -amylase inhibitory activity (AAIA)

AAIA was measured according to Wang et al. (2021); Yan et al. (2019) with modifications. 200 mg of WB FW in triplicate were mixed with 10 mL of water (1h30 min, 40 rpm). 500  $\mu\text{L}$  of sample were mixed with 500  $\mu\text{L}$  of  $\alpha$ -amylase (440 U/mL) and samples were incubated in water bath (10 min, 37 °C). 500  $\mu\text{L}$  of wheat starch solution (1 % w/v water) were added and incubated (30 min, 37 °C). Sample were centrifuged (400  $\times$  g, 15 min, 22 °C) and glucose content was determined with GOPOD test using a glucose standard curve. AAIA (%) was calculated as follows:

$$\text{AAIA (\%)} = \frac{C_s - C_c}{C_c} \times 100 \quad (5)$$

with  $C_s$  and  $C_c$ , the glucose concentrations of the sample and control sample, respectively.

### 2.7.2. $\alpha$ -glucosidase inhibitory activity (AGIA)

The AGIA was performed from Etsassala et al. (2020) with some modifications. For each sample, 1 g WB was diluted with 10 mL ethanol 80 % (v/v  $\text{H}_2\text{O}$ ) overnight (40 rpm, room temperature). Tubes were centrifuged (10,000  $\times$  g, 5 min), 300  $\mu\text{L}$  of supernatant was used for quantification of free polyphenols using the Folin-Ciocalteu method and 3 mL of each sample was evaporated under nitrogen and taken up in DMSO 22 % (v/v  $\text{H}_2\text{O}$ ) to achieve an equivalent concentration of total polyphenols at 1.6 mg/mL.

In a 96-well plate, 50  $\mu\text{L}$  phosphate buffer (pH 7.1 at 16.8 °C), 10  $\mu\text{L}$   $\alpha$ -glucosidase (1U/mL), and 20  $\mu\text{L}$  sample are incubated (37 °C, 15 min), then 20  $\mu\text{L}$  4-nitrophenyl  $\alpha$ -D-glucopyranoside (5 mM) were added and incubated (37 °C, 20 min). The reaction was stopped by adding 50  $\mu\text{L}$  of sodium carbonate (0.1 M). The release of p-nitrophenol from the reaction mix, which relates to the enzymatic activity of  $\alpha$ -glucosidase, was measured at 405 nm with a spectrophotometer and expressed as a percentage of inhibitory activity compared with the control sample, which represents 100 %.

## 2.8. Statistical analysis

The statistical significance of the data was analyzed by variance analysis (ANOVA) using XLSTAT software (Lumivero, Denver, USA) Significant differences of means were determined by Tukey test ( $p < 0.05$ ).

## 3. Results and discussion

### 3.1. Effect of ozone treatment on WB composition

Ozone consumption (based on inlet and outlet  $\text{O}_3$  concentrations – Table 1) were measured as markers of a successful ozonation process. Ozone electrochemical potential is  $-2.07$  Volts which makes this molecule a strong oxidizing agent (Varga & Szigeti, 2016), hence pH acidification indicates a successful interaction between  $\text{O}_3$  and the matrix. A linear decrease in pH value ( $-1.4$  point) was reported in Table 2 showing dose-dependent acidification of the WB matrix.

Aw is another parameter impacting ozone treatment efficiency (Gozó, 2016). A significant reduction of Aw (Table 2) was observed due to the WB air-drying, influenced by increasing contact time with gaseous  $\text{O}_3$  which is an ultradry gas (dew point:  $-70$  °C). A drop in Aw

**Table 2**  
Wheat bran general chemical composition.

Parameter	WB sample					
	Control	3G	6G	9G	12G	24G
pH	6.97 <sup>a</sup> ± 0.05	6.65 <sup>b</sup> ± 0.06	6.24 <sup>c</sup> ± 0.12	6.04 <sup>d</sup> ± 0.03	5.90 <sup>e</sup> ± 0.15	5.48 <sup>f</sup> ± 0.17
Aw	0.798 <sup>a</sup> ± 0.004	0.801 <sup>a</sup> ± 0.004	0.761 <sup>ab</sup> ± 0.007	0.753 <sup>bc</sup> ± 0.008	0.748 <sup>bc</sup> ± 0.003	0.731 <sup>c</sup> ± 0.018
Protein (%)	15.5 <sup>a</sup> ± 0.3	15.6 <sup>a</sup> ± 0.5	15.3 <sup>a</sup> ± 0.6	15.6 <sup>a</sup> ± 0.5	15.6 <sup>a</sup> ± 0.1	15.8 <sup>a</sup> ± 0.2
Starch (%)	23.6 <sup>a</sup> ± 3.3	20.6 <sup>a</sup> ± 0.7	22.7 <sup>a</sup> ± 0.6	20.9 <sup>a</sup> ± 0.6	19.0 <sup>a</sup> ± 0.5	19.3 <sup>a</sup> ± 1.3
Ash (%)	5.47 <sup>a</sup> ± 0.38	5.30 <sup>a</sup> ± 0.25	5.42 <sup>a</sup> ± 0.22	5.36 <sup>a</sup> ± 0.32	5.60 <sup>a</sup> ± 0.08	5.42 <sup>a</sup> ± 0.02
TDF (%)	46.1 <sup>a</sup> ± 2.6	44.5 <sup>a</sup> ± 1.5	43.5 <sup>a</sup> ± 0.9	45.0 <sup>a</sup> ± 0.9	45.2 <sup>a</sup> ± 1.1	44.6 <sup>a</sup> ± 0.7
SDF (g/100 g TDF)	8.0 <sup>a</sup> ± 0.8	7.7 <sup>a</sup> ± 0.4	8.9 <sup>ab</sup> ± 0.4	9.1 <sup>ab</sup> ± 1.4	10.6 <sup>b</sup> ± 0.5	13.4 <sup>c</sup> ± 1.1
$\beta$ -glucans (%)	2.2 <sup>a</sup> ± 0.2	2.3 <sup>a</sup> ± 0.2	2.0 <sup>a</sup> ± 0.2	2.1 <sup>a</sup> ± 0.3	2.1 <sup>a</sup> ± 0.0	2.0 <sup>a</sup> ± 0.0
Lipids (%)	3.7 <sup>a</sup> ± 0.5	4.2 <sup>a</sup> ± 0.6	4.0 <sup>a</sup> ± 0.5	3.2 <sup>a</sup> ± 0.5	4.3 <sup>a</sup> ± 0.6	3.8 <sup>a</sup> ± 0.5

Results are expressed in g for 100 g WB DW except for SDF content which is expressed in g SDF for 100 g TDF and pH. Different letters in row indicate a significant difference:  $p \leq 0.05$  (Tukey).

may thus reduce ozone transfer rate as it has been reported that  $\text{O}_3$  needs water to be efficient (Sarron et al., 2021).

WB is constituted of TDF  $46 \pm 0.6$  g/100 g, starch  $18.5 \pm 0.3$  g/100 g, proteins  $15.2 \pm 0.4$  g/100 g, minerals  $5.08 \pm 0.61$  g/100 g, lipids  $3.8 \pm 0.5$  g/100 g, and  $\beta$ -glucans  $1.9 \pm 0.1$  g/100 g (Table 2). WB ozonation did not significantly change these parameters. However, it significantly increased content of SDF, which is composed of  $\beta$ -glucans, water extractable arabinoxylans, and oligosaccharides. SDF content increased from 7.7 to 13.4 g/100 g for control and 24 G samples respectively, representing an increase of 67.5 %. Similar results were obtained using sodium carbonate (Deng et al., 2024) and steam explosion-assisted superfine grinding (Kong et al., 2020). A 2.5 fold increase in SDF content (from 3.1 % to 7.6 %) was achieved with extrusion (R. Li et al., 2023). The increase of SDF content could be attributed to the conversion and breakdown of IDF mainly composed of cellulose, hemicellulose and lignin. This could be consequent to molecular cleavage or changes in spatial conformation and substitution degree. It is highly likely that oxidation of hemicellulose led to the release of the more water-soluble arabinoxylans (Qin et al., 2025). It can be concluded that WB ozonation promoted the conversion of IDF, mainly insoluble arabinoxylans, into SDF. This result is comparable to other treatments (Y. Li et al., 2022; Ye et al., 2021; Zhao et al., 2017) but lower than acid-alkaline combined treatment (Bader Ul Ain et al., 2019)

Total free polyphenols content was significantly reduced ( $p < 0.0001$ ) by 27 % after ozonation from 2.1 mg GAE/g WB DW for control

**Table 3**  
Free polyphenol content of wheat bran using Folin-Ciocalteu method.

WB sample	Free Polyphenols (mg GAE/g)
Control	2.1 <sup>a</sup> ± 0.4
3G	1.9 <sup>ab</sup> ± 0.3
6G	1.8 <sup>abc</sup> ± 0.3
9G	1.7 <sup>bc</sup> ± 0.4
12G	1.6 <sup>bc</sup> ± 0.3
24G	1.5 <sup>c</sup> ± 0.3

Means of polyphenols content expressed in mg / g WB DW. Different letters in column indicate a significant difference:  $p \leq 0.05$  (Tukey).

sample to 1.5 mg GAE/g WB DW for 24 G ozonated sample (Table 3). This result showed that  $O_3$  could strongly oxidize all sensitive molecules including polyphenols. This result is different from most biological treatments, which enhance polyphenols release (Liao et al., 2022; Lu et al., 2022; Ye et al., 2021). But it is coherent with literature data, which reports a dose-dependent 37 % reduction of phenol with 4 % ozone treatment (Murlidhar Sonkar et al., 2021). This could reduce WB anti-oxidant properties.

### 3.2. Effect of ozone on WB molecular structure

#### 3.2.1. Characterization of WB molecular structure

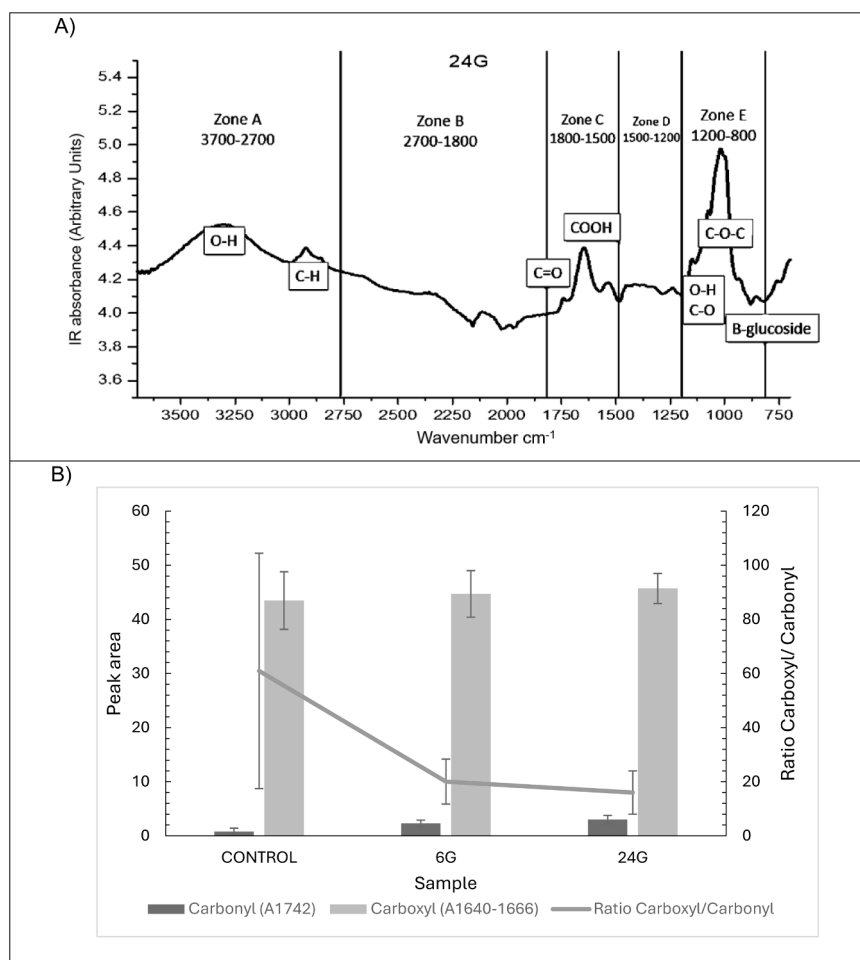
In contrast to physical treatments that can be applied to this type of fibrous material (Xu et al., 2025), ozonation of WB in rotating screw reactor did not significantly impact particle size distribution. The granular microstructure exhibiting rough surface with sharp protrusions was also not affected (Fig. S1).

FTIR spectroscopy was done to determine the impact of ozone treatment on the structural properties of the WB samples (Fig. 1A). All samples showed similar FTIR profile indicating that ozonation did not lead to the creation and loss of functional groups. There were no changes in  $800\text{--}1200\text{ cm}^{-1}$  and  $2700\text{--}3700\text{ cm}^{-1}$  regions which were assigned for C—O—C stretching of the glycosidic linkages and O—H and C—H bonds, respectively. In contrast, analysis revealed significant changes in  $1500\text{--}1800\text{ cm}^{-1}$  region corresponding to carbonyl and  $1640\text{--}1666\text{ cm}^{-1}$  region relating to carboxyl group (Amir et al., 2013). The intensity of the  $1742\text{ cm}^{-1}$  band increased after ozone treatment, indicating a

production of carbonyl (C = O) groups (Fig. 1B). In addition, ozonation also increased carboxyl (-COOH) groups, explaining the reduction in pH. Production of carboxyl groups (-COOH) could be explained in part by transformation of C = O bonds characterizing hemicellulose, lignin and pectin. As a result, the carboxyl/carbonyl ratio decreased, suggesting that the production of C = O bonds was accompanied by its oxidation into -COOH groups. These results could explain the conversion of IDF into SDF via functionalization and cleavage of bounds. This would enhance the interaction between these generated groups and water molecules, improving the water solubility and hydrophilicity of the fiber molecules. It can be concluded that ozone treatment brings additional functional groups on WB compounds.

#### 3.2.2. Fiber molecular weight distribution

**3.2.2.1. Soluble fiber mw determination.** The average Mw, hydrodynamic radius (Rh), gyration radius (Rg), dispersity (Mw/Mn), mass fraction and conformation (Rg/Rh) are parameters for the investigation of ozone effect on Mw distribution (Table 4). As can be seen, ozonated samples showed a reduction of SDF Mw that is significant from 6 G up. The average reduction was about 78 %. Mass fraction of these SDF significantly decreased, revealing a shift of this SDF major fraction to SDF of low Mw. The reduction of SDF Mw could only be due to the cleavage of polysaccharides initially present in SDF and those released from IDF. This result confirms the ability of ozone to break down glycosidic linkages as has been demonstrated for IDF (i.e. cellulose) (Devarajan et al., 2022; Lemeune et al., 2004; L. Zhang et al., 2024).



**Fig. 1.** FTIR characterization of wheat bran: A) FTIR spectra example of 24 G sample, 2 mm particle-size based fraction divided in zones in which stretching type is related to the wavenumber B) Peak areas and ratio of area  $A_{1640+1666} / A_{1742}$  for CONTROL, 6 G and 24 G samples.

**Table 4**

Molecular features of high Mw soluble fibers from ozonated and control wheat bran.

WB Sample	Mw ( $\times 10^4$ kDa)	Dispersity (Mw/Mn)	Rh (nm)	Rg (nm)	Rg/Rh	Mass fraction (%)
Control	7.87 <sup>a</sup> $\pm$ 1.9	15.4 <sup>ab</sup> $\pm$ 1.3	144 <sup>a</sup> $\pm$ 16	110 <sup>a</sup> $\pm$ 26	0.78 <sup>a</sup> $\pm$ 0.27	9.0 <sup>a</sup> $\pm$ 0.1
3G	7.3 <sup>a</sup> $\pm$ 4.1	18.6 <sup>a</sup> $\pm$ 0.3	171 <sup>ab</sup> $\pm$ 30	86 <sup>a</sup> $\pm$ 27	0.54 <sup>a</sup> $\pm$ 0.28	8.8 <sup>a</sup> $\pm$ 1.2
6G	1.85 <sup>b</sup> $\pm$ 0.1	8.5 <sup>c</sup> $\pm$ 1.0	184 <sup>abc</sup> $\pm$ 14	87 <sup>a</sup> $\pm$ 6	0.47 <sup>a</sup> $\pm$ 0.04	10.1 <sup>a</sup> $\pm$ 1.4
9G	1.56 <sup>b</sup> $\pm$ 0.8	8.1 <sup>c</sup> $\pm$ 1.7	238 <sup>bc</sup> $\pm$ 3	78 <sup>a</sup> $\pm$ 16	0.35 <sup>a</sup> $\pm$ 0.07	4.8 <sup>b</sup> $\pm$ 0.3
12G	2.02 <sup>ab</sup> $\pm$ 0.9	7.3 <sup>c</sup> $\pm$ 0.0	259 <sup>c</sup> $\pm$ 42	92 <sup>a</sup> $\pm$ 34	0.37 <sup>a</sup> $\pm$ 0.19	5.3 <sup>b</sup> $\pm$ 0.5
24G	1.79 <sup>b</sup> $\pm$ 0.5	12.8 <sup>b</sup> $\pm$ 0.8	ND	109 <sup>a</sup> $\pm$ 55	ND	2.4 <sup>c</sup> $\pm$ 0.3
Tends	\	\	\	\	\	\
<i>p</i>	0.005	<0.0001	0.010	0.832	0.285	<0.0001

ND: Not Determined.

3 G ozonation slightly increased the dispersity in comparison to the control sample. This could be explained by the onset of polymer degradation, increasing their heterogeneity. Then, as oxidation of WB by ozone increased, the polymers generated were smaller in mass and more homogeneous. However, a high dose of ozone increased this parameter, explained by a sharp drop in molar mass in number (Mn). This coincided with the increase in SDF, suggesting a release of large polymers without, however, fully offsetting the decrease of Mw. Rh was determined using QELS measurements and was calculated by means of the diffusion coefficient. Rh increased with ozone treatment suggesting variations in diffusion coefficient which could be affected by intermolecular interactions. Samples (9 G and 12 G) exhibited significantly larger Rh than WB control. Therefore, ozonated polymers are likely associated with a more open structure and lower density.

Rg did not show significant changes and were smaller in comparison with the Rh values. This resulted in Rg/Rh about 0.7 which indicated the sphericity of SDF polymers in WB control (L. Zhu et al., 2021). It also revealed the presence of a large water sheath around the polymers. Ozonated samples showed important changes in conformation. The lower ratios of SDF clearly indicated an open conformation with lower density and higher solvation. However, very low  $R_g/R_h$  values suggested the formation of a dense impenetrable core (Sheveleva et al., 2024).

**3.2.2.2.  $\beta$ -glucans molecular weight characterization.**  $\beta$ -glucan LS chromatograms showed two peaks for the standard, the first one around 10 min which corresponds to low Mw  $\beta$ -glucans ( $10^3$  kDa) and the second one at 20 min corresponds to higher Mw ( $10^4$  to  $10^5$  kDa) (Fig. S2). 24 G ozonated sample showed a significant reduction in Mw of high Mw  $\beta$ -glucan leading to a significant decrease of its dispersity (Table 5). The 24 G sample exhibited also a significant increase of Mw for low Mw  $\beta$ -glucan ( $1.5 \times 10^4 \pm 0.3 \times 10^4$ ) while other samples up to 12 G presented a stable 15-fold lower Mw for their low Mw  $\beta$ -glucan. This could be explained by ozone breaking down high-Mw  $\beta$ -glucans into smaller fragments, which is coherent with the previous result showing ozone's ability to cleave the glycosidic bonds of SDF. This result is confirmed by a drastic 94 % decrease in dispersity. The other parameters were stable for all samples: the mass fraction corresponds to  $39 \pm 7$  %; Rg was increased showing a reduction in the size of  $\beta$ -glucan, but  $R_g/R_h$  ratio remained unchanged, suggesting that the molecules conformation was not impacted (Table 5).

We can conclude that ozonation reduced SDF Mw, but  $\beta$ -glucans were impacted only at high ozone concentration.

**Table 5**Molecular weight of  $\beta$ -glucans from standard (STD), ozonated and control wheat bran.

WB Sample	Mw ( $\times 10^4$ kDa)	Rh (nm)	Rg (nm)	Rg/Rh	Dispersity (Mw/Mn)	Mass fraction (%)
STD*	3.7 $\pm$ 0.1	201 $\pm$ 19	145 $\pm$ 2	0.7 $\pm$ 0.1	4.5 $\pm$ 0.3	3.1 $\pm$ 0.9
Control	10.9 <sup>b</sup> $\pm$ 0.6	79 <sup>a</sup> $\pm$ 9	98 <sup>b</sup> $\pm$ 19	1.3 <sup>a</sup> $\pm$ 0.4	22.0 <sup>a</sup> $\pm$ 9.3	34 <sup>a</sup> $\pm$ 12
3G	13.4 <sup>b</sup> $\pm$ 2.2	100 <sup>a</sup> $\pm$ 42	119 <sup>ab</sup> $\pm$ 19	1.3 <sup>a</sup> $\pm$ 0.3	21.1 <sup>a</sup> $\pm$ 5.8	44 <sup>a</sup> $\pm$ 6
6G	23.8 <sup>a</sup> $\pm$ 3.0	117 <sup>a</sup> $\pm$ 0	148 <sup>a</sup> $\pm$ 11	1.3 <sup>a</sup> $\pm$ 0.1	19.2 <sup>a</sup> $\pm$ 2.6	34 <sup>a</sup> $\pm$ 5
9G	9.4 <sup>b</sup> $\pm$ 0.4	111 <sup>a</sup> $\pm$ 3	127 <sup>ab</sup> $\pm$ 3	1.1 <sup>a</sup> $\pm$ 0.0	11.6 <sup>ab</sup> $\pm$ 3.0	42 <sup>a</sup> $\pm$ 3
12G	13.6 <sup>bc</sup> $\pm$ 0.6	74 <sup>a</sup> $\pm$ 16	107 <sup>ab</sup> $\pm$ 15	1.5 <sup>a</sup> $\pm$ 0.1	12.4 <sup>ab</sup> $\pm$ 0.0	40 <sup>a</sup> $\pm$ 5
24G	6.1 <sup>c</sup> $\pm$ 0.9	143 <sup>a</sup> $\pm$ 59	145 <sup>a</sup> $\pm$ 3	1.1 <sup>a</sup> $\pm$ 0.4	1.3 <sup>b</sup> $\pm$ 0.1	39 <sup>a</sup> $\pm$ 1
Tendency	\	\	\	=	\	=
<i>p</i>	<0.0001	0.085	0.012	0.542	0.003	0.425

\*Barley  $\beta$ -glucan.

### 3.3. Effect of wheat bran ozonation on its functional properties

#### 3.3.1. Antioxidant activity

Polyphenols are highly bioactive molecules that contribute to WB antioxidant activity. Therefore, the loss of polyphenols could consequently modify this property. Our findings indicate that IC50 of DPPH test significantly decreased in a dose-dependent manner after ozonation (Fig. 2A) which meant that WB radical scavenging activity was increased. Other authors found an increase in DPPH antioxidant capacity after ozone treatment which could be related to lignin solubilization (Murlidhar Sonkar et al., 2021). Here we noticed an increase in vanillic acid content from 9.2 (CONTROL) to 36.4 (12 G sample)  $\mu$ g/g and the apparition of a molecule at 29.9 min retention time (Table 6). This can result from degradation of polyphenolic oligomers or lignin (Y. Wang et al., 2018). So, the generation of electron-donating molecules and functionalization of substances as shown by FTIR analysis could explain the increase of antioxidant capacity. This property is interesting to protect cells from the damage (Losada-Barreiro et al., 2022).

However, the FRAP test showed a reduction of TEAC with ozone consumption (Fig. 2B), which meant lower ferric reducing capacity. The FRAP test measures the capacity of the sample to reduce metal ions. Wheat ferulic and *p*-coumaric acids are known to chelate Fe(II) (Truong et al., 2020). Our observation probably results from the transformation of free ferulic acids into their hydroxybenzoic form (vanillic acid) that are known to have lower values for FRAP antioxidant capacity (Skroza et al., 2022) and could be linked to the oxidation of molecules as demonstrated by the Folin-Ciocalteu test. Some authors suggest that complexification of ferric iron by polyphenols can have antinutritional effect because it reduce  $Fe^{3+}$  transformation into its bio-disponible form  $Fe^{2+}$  (Xu et al., 2021).

It is important to note that we studied antioxidant activity of free polyphenols extracts, but polyphenols in WB can also exist in bound or conjugated forms, which also exhibit antioxidant activity. The bound form is the most abundant, and in our study, we obtained  $12.3 \pm 0.5$  mg / g WB, with no significative differences correlated with ozone consumption. We can therefore assume that the antioxidant properties of bound and conjugated polyphenols should not be affected by ozonation, and that this would help preserve their beneficial effects on the colonic microbiota during DF fermentation.

#### 3.3.2. Binding capacities

The WB WBC was between 3.7 and 4.1 g/g (Fig. 3A) which corresponds to the range of values mentioned in literature (Sztupecki et al., 2023), while other treatments such as fermentations increase the WBC

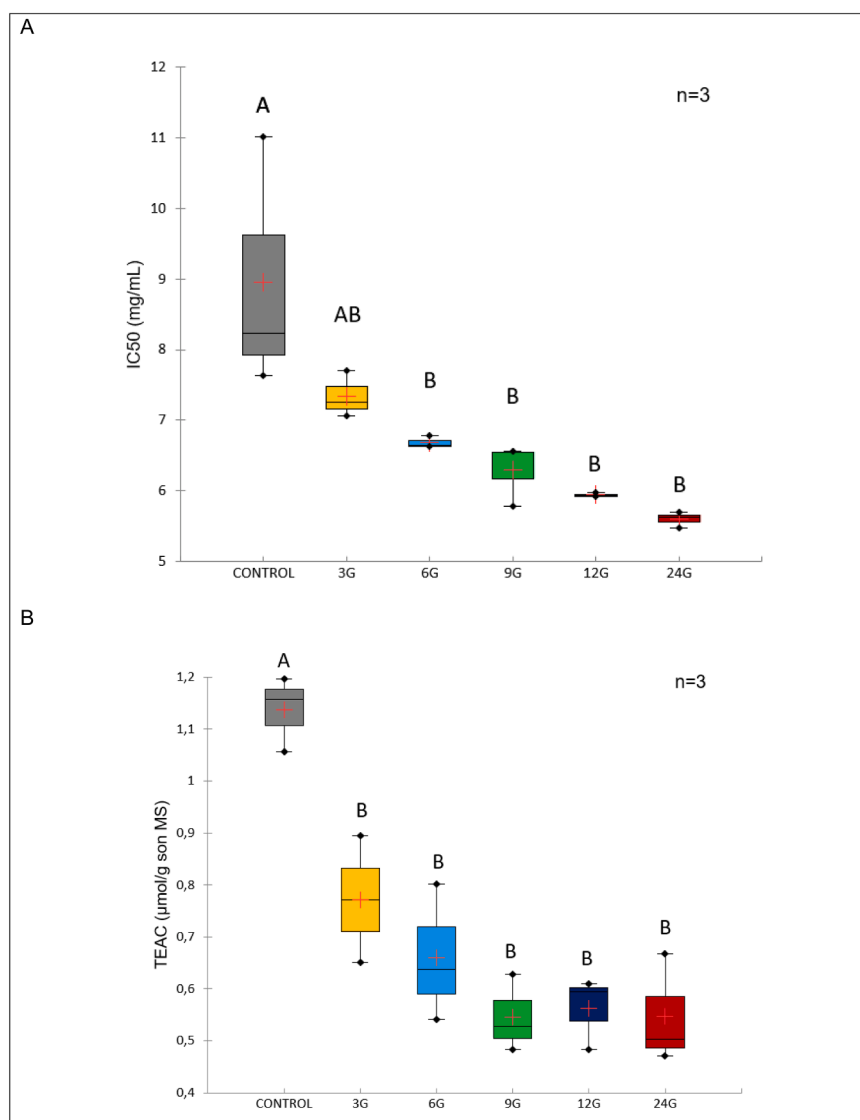


Fig. 2. Antioxidant capacities of ethanolic WB extracts with A) DPPH and B) FRAP tests. DPPH is represented as IC50. FRAP test results represented as TEAC means ( $\mu\text{mol/g}$  WB DW) per sample. *p*-values: 0.002 and <0.0001 respectively.

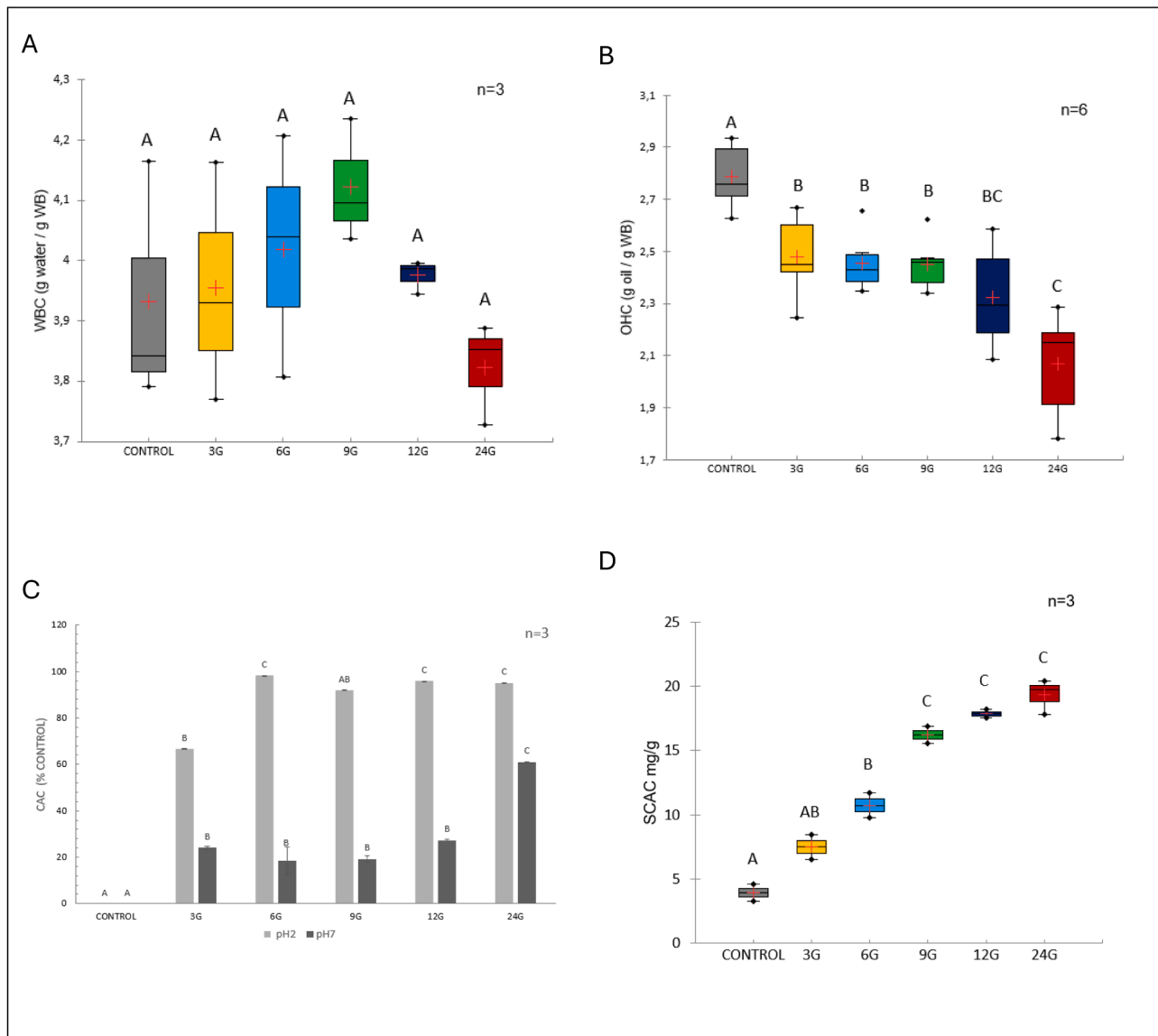
Table 6

WB ethanolic extracts free polyphenols quantification in  $\mu\text{g} / \text{g}$ .

Phenolic compound	WB ethanolic extract sample						<i>P</i>
	Control	3G	6G	9G	12G	24G	
RT 11.5	8755.3 <sup>a</sup> ± 650.5	6129.4 <sup>b</sup> ± 135.8	1095.3 <sup>c</sup> ± 4.6	809.9 <sup>c</sup> ± 17.3	603.7 <sup>c</sup> ± 45.6	405.9 <sup>c</sup> ± 30.6	<0.0001
Vanillic acid	9.2 <sup>c</sup> ± 1.8	15.8 <sup>d</sup> ± 0.4	27.0 <sup>c</sup> ± 0.9	32.9 <sup>b</sup> ± 0.5	36.4 <sup>a</sup> ± 1.8	29.6 <sup>bc</sup> ± 1.1	<0.0001
Syringic acid	2.3 <sup>d</sup> ± 0.4	4.5 <sup>d</sup> ± 0.1	9.2 <sup>c</sup> ± 1.4	12.7 <sup>b</sup> ± 1.4	15.9 <sup>a</sup> ± 0.9	5.4 <sup>d</sup> ± 1.2	<0.0001
RT 29.9	0.0 <sup>d</sup> ± 0.0	37.8 <sup>c</sup> ± 0.8	51.9 <sup>ab</sup> ± 0.9	46.9 <sup>bc</sup> ± 5.3	46.8 <sup>bc</sup> ± 7.4	60.1 <sup>a</sup> ± 4.8	<0.0001
RT 34.1	3.7 <sup>a</sup> ± 0.3	3.8 <sup>a</sup> ± 0.2	3.8 <sup>a</sup> ± 0.1	2.8 <sup>b</sup> ± 0.1	2.9 <sup>b</sup> ± 0.2	2.5 <sup>b</sup> ± 0.6	0.000
<i>p</i> -coumaric acid	0.0	0.0	0.0	0.0	0.0	0.0	Nd
RT 36.5	13.6 <sup>a</sup> ± 1.6	15.6 <sup>a</sup> ± 0.1	16.9 <sup>a</sup> ± 1.1	15.2 <sup>a</sup> ± 1.5	15.0 <sup>a</sup> ± 1.3	14.2 <sup>a</sup> ± 1.6	0.118
RT 37.2	4.9 <sup>a</sup> ± 0.4	5.4 <sup>a</sup> ± 0.1	5.6 <sup>a</sup> ± 0.3	5.8 <sup>a</sup> ± 0.4	5.7 <sup>a</sup> ± 0.3	5.2 <sup>a</sup> ± 0.5	0.101
RT 37.4	17.8 <sup>a</sup> ± 2.5	21.1 <sup>a</sup> ± 0.1	22.9 <sup>a</sup> ± 1.6	21.1 <sup>a</sup> ± 2.2	21.2 <sup>a</sup> ± 2.2	20.4 <sup>a</sup> ± 2.5	0.145
Ferulic acid	12.3 <sup>a</sup> ± 1.2	10.8 <sup>a</sup> ± 0.2	8.1 <sup>b</sup> ± 0.3	5.4 <sup>c</sup> ± 0.5	4.7 <sup>c</sup> ± 0.4	4.1 <sup>c</sup> ± 0.5	<0.0001
RT 39.5	2.4 <sup>a</sup> ± 0.3	2.0 <sup>ab</sup> ± 0.3	2.5 <sup>a</sup> ± 0.3	1.6 <sup>b</sup> ± 0.2	1.6 <sup>b</sup> ± 0.3	1.3 <sup>b</sup> ± 0.4	0.001
RT 39.7	6.7 <sup>a</sup> ± 0.6	5.6 <sup>a</sup> ± 0.2	7.2 <sup>a</sup> ± 0.8	5.8 <sup>a</sup> ± 0.7	6.1 <sup>a</sup> ± 0.8	6.1 <sup>a</sup> ± 0.1	0.077
RT 40.0	0.7 <sup>a</sup> ± 0.5	0.4 <sup>a</sup> ± 0.2	0.9 <sup>a</sup> ± 0.2	0.2 <sup>a</sup> ± 0.0	0.4 <sup>a</sup> ± 0.0	0.5 <sup>a</sup> ± 0.3	0.154
RT 61.9	20.3 <sup>a</sup> ± 2.9	0.0 <sup>b</sup> ± 0.0	0.0 <sup>b</sup> ± 0.0	0.0 <sup>b</sup> ± 0.0	0.0 <sup>b</sup> ± 0.0	0.0 <sup>b</sup> ± 0.0	<0.0001

Nd: Not determined.

Unidentified peaks are labelled with their retention time (RT) in minutes.



**Fig. 3.** Binding properties of control and ozonated WB with A) WBC,  $p$ -value = 0.337; B) OHC,  $p$ -value < 0.0001; C) CAC at pH 7 and pH 2 ( $p$ -value < 0.0001 for both); D) SCAC,  $p$ -values < 0.001; 0.001 and 0.022 respectively for 1, 2 and 3 g SC/L.

(Lu et al., 2022; Ye et al., 2021), as can be seen here, no significant differences were observed. However, from 0 to 9 g of ozone consumption the WBC increased linearly, indicating dose-dependent production of hydrophilic sites. When the amount of ozone increased from 9 to 24 g, the WBC decreased linearly, indicating losses of hydrophilic sites which were due to the reduction of IDF. This trend showed that ozonation may impact water binding sites. There is no doubt that WBC largely depends on WB structure. It has been shown that particle size modification strongly influences WBC. Indeed, physical treatments that reduce particle size such as milling or extrusion can reduce the WBC (Aktas-Akyildiz et al., 2020; Jacobs et al., 2015; Lin et al., 2021). WBC is also particularly related to IDF composition and content. It has been demonstrated that bacterial treatment of WB IDF increase their WBC (Liao et al., 2022). Indeed, water is chemically linked to hydrogens bound of polysaccharide's hydrophilic groups (Jacobs et al., 2015). In our case, changes could be related to the reduction of IDF content and / or its functionalization.

OHC significantly decreased by 25 % between control sample and 24 G (Fig. 3B). OHC is impacted by fiber physicochemical structure. An increase of porosity and capillarity attraction can enhance physical

entrapment of oil (Wang et al., 2012). This parameter can also be impacted with particle size; however, we did not observe differences in granularity and microstructure. Another explanation of OHC reduction could be related to a decrease of lipophilic sites and/or increase of hydrophilic sites of IDF due to the partial depolymerization of DF (Huang & Ma, 2016). Since hydration properties do not appear to be significantly altered, we could hypothesize that ozonation could have an impact on lipophilic sites such as lignin, phenolic acid benzenic rings and lipids. Polyphenol oxidation by ozone could open benzoic rings leading to a decrease in lipophilic sites and free polyphenol content.

Regarding CAC results, it significantly increased after ozonation for both stomach (pH 2) and intestinal (pH 7) conditions (Fig. 3C). At pH 2, the maximum (100 %) absorption was reached only after 6 G ozone consumption while the maximum absorption of about 60 % was obtained for 24 G. The increase of CAC may be linked to the observed increase in SDF thus we know that there is a positive correlation between SDF content and CAC (Chen et al., 2014). In fact, we observed a correlation between the percentage of SDF increase and CAC at pH 7 ( $R^2 = 0,79$ ) but not at pH 2 ( $R^2 = 0,28$ ). WB CAC can be increased with other treatments that increase SDF content such as combination of grinding

and fermentation with fungus (Dong et al., 2025) or snail enzymes (Liu et al., 2021). But acidic chemical treatment can alter this property (L. Wang et al., 2021).

WB capacity to adsorb sodium cholate increased significantly together with ozone consumption (Fig. 3D). Similar to CAC, SCAC is related to SDF content and its capacity to form gel (Wang et al., 2015). It was reported that SDF had stronger adsorption of SC than IDF and TDF (S. Zhang et al., 2023). Sodium cholate is the binding form of bile acids, increased SCAC thus plays a role in lowering blood lipids because cholesterol will be converted into SC to regulate the loss (Hua et al., 2019).

Although WB OHC was reduced after ozone treatment, making lipids more available to enter the blood stream during digestion, the increase of CAC and SCAC after ozonation counterbalance this result. Therefore, CAC and SCAC would help reduce blood lipids and prevent cardiovascular diseases.

These results concerning binding capacities are similar to other treatments used to increase SDF content of WB but ozonation shows a better optimization of CAC and SCAC which can decrease with particle size reduction (Wu et al., 2018) and high acidic conditions.

### 3.3.3. Enzymes inhibition

All ozonated samples exhibited a significantly higher AAIA than control (Fig. 4A). The highest inhibitory activity (about 60 %) was

obtained for 24 G sample. We noticed significant and linear correlation with ozone consumption indicating concentration-dependent effect. Inhibition of  $\alpha$ -amylase can postpone glucose release from starch during digestion and therefore limit glucose entering blood circulation, leading to reduced glycemia. Ozone effects on AAIA could be due to modification of carbonyl / carboxyl groups making sugars less recognized by  $\alpha$ -amylase, physical blockage or inhibition of the enzyme by polyphenols and other oxidized molecules (Aleixandre et al., 2022). This last could be a result of DF partial depolymerization producing free polysaccharides with inhibitory activity (Lv et al., 2021) and  $\alpha$ -amylase inhibitors (Meziani et al., 2021). Polyphenols molecular modifications can be involved in enzyme inhibitory activity (Aleixandre et al., 2022). Indeed, we found correlation between AAIA increase and reduction of phenolic compound at RT 11.5 and ferulic acid ( $R^2 = 0.89$  and  $0.91$ ). AAIA is also correlated with the increase of vanillic acids production ( $R^2 = 0.83$ ). Also DF are able to encapsulate digestive enzymes and SDF of low Mw can highly inhibit  $\alpha$ -amylase according to Yan et al. (2019).

Like  $\alpha$ -amylase,  $\alpha$ -glucosidase is a saccharide-digesting enzyme which acts as a second step to hydrolyze oligo- and disaccharides resulting from  $\alpha$ -amylase digestion, into glucose units. The inhibition of this enzyme slows down carbohydrate digestion. AIAG significantly ( $p = 0.012$ ) doubled at 3 G revealing production and / or release of inhibitory molecules (Fig. 4B), then decreased linearly as a function of ozonation to reach control value at 12 G. 3 G and 6 G exhibited significantly higher

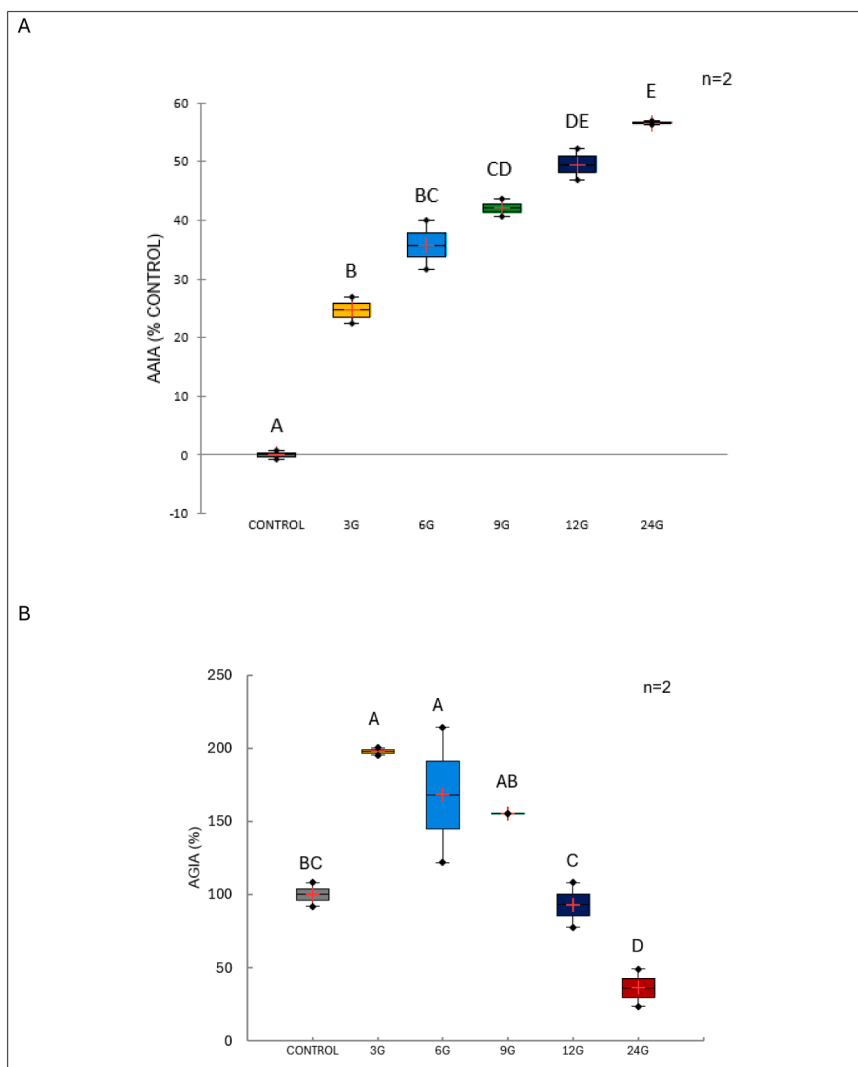


Fig. 4. Enzyme activity of control and ozonated WB with A)  $\alpha$ -amylase inhibitory activity,  $p$ -value  $< 0.0001$ ; B)  $\alpha$ -glucosidase inhibitory activity,  $p$ -value  $< 0.0001$ .

AGIA that control while 24 G sample showed 64 % lower inhibitory activity than the control. The mechanism of AIAG could be due to the formation of hydrogen bonds and hydrophobic interactions between molecules such as polyphenols/polysaccharides and specific amino acid residues of active site in  $\alpha$ -glucosidase. Our results showed that consuming ozonated bran could delay starch digestion and potentially limit the absorption of glucose.

#### 4. Conclusion

This study showed that ozone treatment was able to positively modify the physicochemical and functional properties of WB. Our results indicated a significant increase in SDF content (+ 67 %). This increase is due to the release of molecules from IDF fraction, most likely arabinoxylan, via partial depolymerization phenomena. In addition, O<sub>3</sub> also cleaved glycosidic bonds of soluble polysaccharides which led to a reduction in SDF molar mass. Indeed, the oxidation caused by ozone increased carbonyl and carboxyl groups, led to matrix acidification and reduced total free polyphenol content (-28 %) explaining the reduction of FRAP antioxidant activity. However, DPPH activity significantly increased probably due to functionalization of molecules as demonstrated by FTIR profiles. Additionally, WB ozonation positively changed several functional properties. Ozone treatment increased significantly lipid binding capacities (CAC, SCAC). It also significantly increased  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities which could reduce the level of blood sugar. In comparison to other treatments traditionally used to improve WB physico-chemical properties, O<sub>3</sub> is more sustainable. First, it is formed from O<sub>2</sub> which is a renewable source. O<sub>3</sub> is easily degraded back into oxygen and does not leave residues on the material. Also, its gaseous form is interesting to use less water, no solvent and requires less processing time than biological or chemical treatments. Another advantage is its ability to scale up. Ozone therefore seems to be a good candidate for the optimization of WB physicochemical and functional characteristics without altering the matrix macrostructure. It is important to understand that physicochemical and functional modifications will play a role on both nutritional characteristics as well as rheological and technological properties. For such a staple food, it is essential to keep in mind the balance of health benefits together with technological parameters. Further studies are underway to investigate digestibility and mechanistic impact of ozonated WB in cellular models, but also technological properties of bread baked using those products and their physiological impact on the consumer.

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#### CRediT authorship contribution statement

**Wendy Sztupecki:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Flore Depeint:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Conceptualization. **Thierry Aussenac:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Conceptualization. **Larbi Rhazi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.afres.2025.101400](https://doi.org/10.1016/j.afres.2025.101400).

#### Data availability

Data will be made available on request.

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