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ORIGINAL PAPER

Evaluation of antioxidant and antimicrobial activities of whole flours obtained from different species of *Triticum* genus

Teresa Grande[1] · Aymen Souid[1,2] · Marco Ciardi[1] · Clara Maria Della Croce[1] · Stefania Frassinetti[1] · Emilia Bramanti[3] · Vincenzo Longo[1] · Luisa Pozzo[1]

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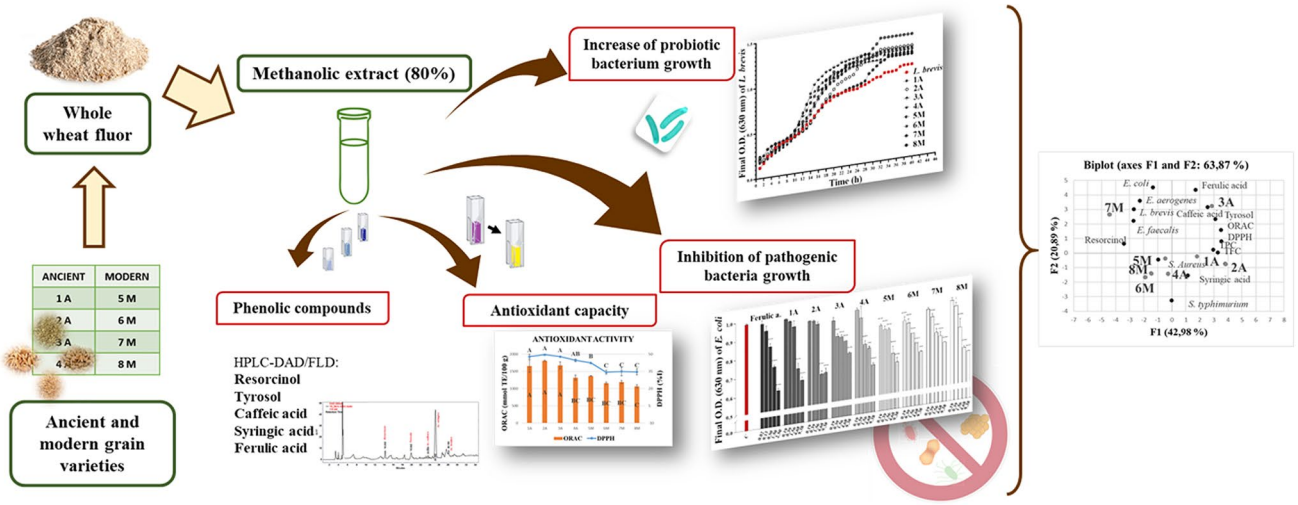
Abstract

Whole wheat is an exceptional source of phenolic compounds representing a promising phytochemical class to prevent diet-related chronic diseases thanks to its antioxidant activities. The present work reports the phenolic profile, the antioxidant capacity, the antimicrobial activity and the effect on *Lactobacillus brevis* growth of eight whole flours obtained from four ancient and modern wheat genotypes of Italian *Triticum* genus. Total phenolic content (TPC) and total flavonoid content (TFC) were quantified, and antioxidant activities were assessed using oxygen radical absorbance capacity (ORAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) in vitro tests. HPLC-DAD/FLD was used to detect the presence of phenolic compounds. Moreover, antimicrobial activity of whole flour extracts against some potentially pathogenic Gram negative and Gram positive bacteria and the effect of extracts on *Lactobacillus brevis* growth were assessed. Results showed quantitative differences ($p < 0.05$) in antioxidant activities, total phenolic content and concentrations of five phenolic acids (resorcinol, tyrosol, caffeic acid, syringic acid and ferulic acid) among the wheat genotypes. Pathogenic bacteria were significantly negatively affected by wheat extracts while the growth of *L. brevis* was stimulated. The principal component analysis (PCA) confirmed that the phenolic profile and the antioxidant activities were influenced by the genotypic characteristics of studied varieties, suggesting that the ancient Saragolla stand out for the most interesting phenolic profile. Overall, this research emphasizes how ancient and modern Italian *Triticum* spp. grains must be investigated to select the grains richer in bioactive compounds.

Graphical abstract



Keywords Wheat · Functional food · Phytochemical profile · Gram negative and gram positive bacteria · *L. brevis* growth

Extended author information available on the last page of the article

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Introduction

Cereal grains crops (wheat, corn, rice, barley oat and rye) constitute the landslide majority source of daily carbohydrates in human diet. As basis of the food pyramid, their nutritional value has a significant influence on human health. Cereals consumed in whole form show a protective effect against chronic non-communicable diseases such as diabetes, cardiovascular disease, and cancer [1]. The unprocessed kernels provide a good amount of fiber and a wide-ranging variety of phytochemicals, such as vitamin C, vitamin E (tocopherols and tocotrienols), carotenoids, phenolic acids and flavonoids. These compounds provide various biological preventive activities: antioxidant, antimicrobial, anti-inflammatory and anticancer [2, 3].

Wheat (*Triticum* ssp.) represents the most important food grain of *Poaceae* family. Thus, the scientists have focused to improve human well-being through the amelioration of whole wheat product nutritional values. The creation of different wheat varieties phytochemical profile is the main research topic to enhance functional characteristics like antioxidant capacities of *Triticum* genus. Currently, the pursuit of wheat genotypes selection was poorly led by nutritional properties. Modern wheat species are the result of intensive breeding programs with the aim of obtaining genotypes able to give products more easily processed, more productive despite crops with hight and healthier nutritional qualities [4]. This brought to rediscover and reuse old and landraces wheat cultivars whose cultivation decreased during green revolution (ca. 1940–60). Despite the ancient genotypes have a crop yield lower than modern ones [5], they response to people demand for organic natural and healthy product. They request low fertilizing thanks to a better endurance to soil nutrient deficiency and seems to have a superior nutritional profile with higher phytochemicals, especially phenolic class [6]. It's not clear yet if ancient wheat species are nutraceutical better than modern ones. It is clear and well known that bioactive compounds are the components with the highest variability in cereals. This is related to the species, the variety, the genotype and, moreover, to the interaction of genotype with environmental biotic and abiotic stresses [5, 7, 8]. All these elements are important factors that could give a considerable variation in the content of phenolic compounds. Therefore, content of bioactive compounds in wheat depends on the variety used in agriculture and this aspect must be carefully investigated to better understand their role in maintaining health status, to create foods with high nutritional value for humans, to offer consumers more correct information and to guide them in purchasing better-quality products.

This study assesses the composition and the in vitro antioxidant and antimicrobial activities of four whole "ancient" and four "modern" wheat flours, obtained from different species of the Italian *Triticum* genus, evaluating a possible correlation between the type of wheat genotype and the parameters studied.

Materials and methods

Chemicals and reagents

All chemicals and standards were of analytical grade and obtained from Fluka-Sigma-Aldrich, Inc. (St. Louis, MO, USA). Methanol was bought from VWR International PBI (Milan, Italy). The Bacterial Media Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA), were purchased from Oxoid (Basingstone, UK).

Wheat samples collection and preparation

Wheat grains samples include four ancient (A) and four modern (M) genotypes were reported in Table S1. The analysis was performed on the flour obtained from the grilling of the whole wheat caryopsis using a lab mill. Wheat grains were provided by the company Bio Val Bidente S.C.A. (Civitella di Romagna, Forlì-Cesena, Italy) and by Sant'Anna School of Advanced Studies (Pisa, Italy).

Wheat samples extraction

A double extraction with 80% ethanol water solution (v/v) was carried out. Briefly, 1 g of flour was mixed with 10 mL of 80% ethanol, and shacked for 2 h in the dark. After centrifugation at 3000 rpm for 30 min, the supernatant was recovered. The entire procedure was repeated on the pellet adding other 10 mL of 80% ethanol. The extraction was carried out in triplicate. The extracts were stored at − 20 °C until use.

Total phenolic content (TPC) and total flavonoid content (TFC) determination

Folin-Ciocalteu assay was performed to determine the total phenolic content according to the method of Singleton et al. [9] and were expressed as mg of gallic acid equivalent (GAE) per 100 g fresh weight (f.w.) of wheat flour.

Aluminium chloride method was used to calculate total flavonoid content following the procedure of Kim et al. [10] and were expressed as mg of catechin equivalent (CE) per 100 g f.w. of sample.

Determination of antioxidant activity

The capacity of the prepared extract and its fractions to scavenge the stable DPPH was monitored according to the method of of Aktumsek et al. [11]. DPPH radical was measured at 517 nm and the scavenging power of the samples was expressed as µmol of Trolox equivalent (TE) per 100 g of f.w.

ORAC assay was carried out in triplicate as described by Gabriele et al. [12]. AAPH was used as a peroxyl radicals' generator and fluorescein as the probe. Fluorescein fluorescence decay was read at $\lambda_{ex} = 485$ nm and $\lambda_{em} = 514$ nm using a VictorTM X3 Multilabel Plate Reader (MA, USA). ORAC results were expressed as µmol of Trolox equivalent (TE) per 100 g of f.w.

Quantification of phenolic compounds by HPLC-DAD/FLD

Phenolic compounds present in the extracts were analysed using a HPLC with UV/Vis Diode Array and Fluorescence Detector (DAD-FD) as previously reported by Gonzalez-Rivera et al. [13] with some modifications. An HPLC gradient pump (P4000, ThermoFinnigan) was coupled with a vacuum membrane degasser (SCM1000, ThermoFinnigan), an AS3000 autosampler (ThermoFinnigan), a UV6000 diode array detector and a FL3000 fluorescence detector (ThermoFinnigan). Phenolics separation conditions: reversed-phase HPLC column C18 Spherisorb S5 ODS2 (Waters, 250 mm × 4 mm, 5 µm) set at 40 °C and injection volume = 5 µL. Mobile phases: 5% methanol–0.1% formic acid in water (eluent A) and 95% methanol-0.1% formic acid in water (eluent B). The gradient was as follows: 0–5 min, 100% A; 5–45 min, linear gradient up to 100% B; 45–55 min 100% B; 55–57 min, linear gradient up to 100% A. Post-run time was 15 min. Elution was performed at a solvent flow rate of 0.8 mL/min. ChromQuest™ 4.2 Chromatography Data System was used to carry out HPLC–DAD/FD control, data acquisition and data analysis. Fluorescence detector was operated at $\lambda_{ex} = 280$ nm and $\lambda_{em} = 340$ nm for the quantitation of resorcinol, tyrosol, and syringic acid. Caffeic and ferulic acid were quantified by absorbance chromatograms at 324 nm [13]. The results were expressed as mg of polyphenol per 100 g f.w. of the sample.

Antimicrobial activity

Microorganism

The bacterial strains come from ATCC collection (American Type Culture Collection). *Escherichia coli* (ATCC 25,922), *Salmonella enterica* ser. *typhimurium* (ATCC 14,028), *Enterobacter aerogenes* (ATCC 13,048), *Staphylococcus aureus* (ATCC 25,923) and *Enterococcus faecalis* (ATCC 29,212) were used to evaluate the antibacterial activity against Gram negative and Gram positive aerobic bacteria. *Lactobacillus brevis* (ATCC 14,869) was applied for the microbial growth assay.

Antimicrobial activity and treatments

Antimicrobial activity was evaluated by the micro-dilution assay according to the method of Pozzo et al. [14]. One hundred µl of MHB medium, 50 µl of the bacterium strain (about 1×10^8 CFU/ml) and 50 µl of ethanol extract (0–0.19–0.39–1.56–4.68 and 9.38 mg/mL) were loaded into a 96-well microplate. Ferulic acid ethanol solution 80% (v/v) (0.015–0.125–0.5–1.5 and 2.5 mg/mL) was used as a phenolic standard for bacterial inhibition, inoculating in the well 100 µl of MHB, 50 µl of bacteria and 50 µl of phenolic solution. The plates were incubated for 24 h at 37 °C. The final optical density was measured at 630 nm in a plate reader. Data were reported as final optical densities (O.D.).

Growth rate of *Lactobacillus brevis*

Growth rate of *L. brevis* was evaluated with the same method of antimicrobial activity with some modifications. One hundred µl of MRS Broth medium, 50 µl of the bacterium strain (about 1×10^8 CFU/ml) and 50 µl of methanol extract (0.19–0.125–0.5–1.5–2.5 mg/mL) were loaded into a 96-well microplate. A positive control was performed by measuring the growth of the bacterium without extracts. The optical density of the plates was measured at regular intervals of 1 h for 40 h inside a plate reader, at 630 nm at a temperature of 37° C. For each concentration the assay was performed in triplicate. Data were reported as final optical density (OD).

Statistical analysis

Data are reported as mean ± standard deviation and the measurements for each sample was performed in triplicate. The statistically significantly differences ($p \leq 0.5$) were evaluated with analysis of variance (ANOVA) and Tukey's test performed using SPSS version 18.0. Principal Component Analysis (PCA) was applied, as the analysis of multivariate data, to characterizes and separates wheat genotypes in relationship to the variables studied. Cluster analysis was based on the Unweighted Pair Group Method with Arithmetic (UPGMA) and on euclidian distance. PCA and cluster analysis were computed using XLSTAT Version 2019 statistical software.

Results

Total polyphenol content and total flavonoid content

The total phenolic content ranged between 65.31 and 125.13 mg GAE/100 g f.w. (Table 1). The Rebelde (8 M) wheat samples showed the lowest content of phenolics followed by Primitivo (4A), Palesio (5 M), Bologna (7 M), Bolero (6 M), Saragolla (3A), and Ostro nudo (1A), while the Antigola (2A) wheat sample showed the highest content.

The total flavonoid content varied from 110.59 to 189.81 mg/100 g f.w (Table 2). The Bologna (7 M) and Primitivo (4A) wheat samples showed the lowest content of flavonoids followed by Rebelde (8 M), Ostro nudo (1A), Bolero (6 M), Palesio (5 M), and Saragolla (3A), while the Antigola (2A) wheat sample showed the highest content.

Antioxidant activity

DPPH and ORAC assay were performed in order to evaluate the antiradical activity. DPPH assay reports a radical-scavenging activities ranging from 1124.38 to 1649.28 µmol TE/100 g f.w. (Table 1). The Bologna (7 M), Bolero (6 M) and Rebelde (8 M) wheat samples showed the lowest values followed by Primitivo (4A) and Palesio (5 M), while Ostro nudo (1A), Antigola (2A) and Saragolla (3A) wheat samples showed the highest values.

Our results from ORAC assay showed that antioxidant capacity varies from 1065.34 to 1810.05 µmol TE/100 g f.w (Table 1). The Rebelde (8 M) wheat sample showed the lowest ORAC activity followed by Bolero (6 M), Bologna (7 M), Primitivo (4A) and Palesio (5 M), while Ostro nudo (1A), Saragolla (3A) and Antigola (2A) wheat samples showed the highest ORAC activity.

Table 1 Total phenolic content (TPC) (mg GAE/100 g f.w.), total flavonoid content (mg CE/100 g f.w.), DPPH (µmol TE/100 g f.w.) and ORAC (µmol TE/100 g f.w.) values of ancient (A) and modern (M) wheat genotypes

#	Genotype	TPC (mg GAE/100 g f.w.)	TFC (mg CE/100 g f.w.)	DPPH (µmol TE/100 g)	ORAC (mg TE/100 g f.w.)
1A	Ostro nudo	$96.15^{b} \pm 0.11$	$126.65^{d} \pm 6.53$	$1591.14^{a} \pm 68.84$	$1653.64^{a} \pm 177.90$
2A	Antigola	$125.13^{a} \pm 2.62$	$189.81^{a} \pm 9.38$	$1649.28^{a} \pm 0.61$	$1810.05^{a} \pm 15.99$
3A	Saragolla	$92.68^{b} \pm 0.53$	$161.63^{b} \pm 5.80$	$1592.05^{a} \pm 2.76$	$1676.92^{a} \pm 100.8$
4A	Primitivo	$72.14^{d} \pm 2.83$	$92.21^{e} \pm 6.82$	$1472.91^{ab} \pm 18.56$	$1276.83^{bc} \pm 71.64$
5 M	Palesio	$73.83^{d} \pm 0.17$	$144.30^{c} \pm 1.68$	$1404.56^{b} \pm 2.90$	$1369.18^{b} \pm 10.62$
6 M	Bolero	$87.31^{c} \pm 0.23$	$127.16^{c} \pm 8.19$	$1124.38^{c} \pm 60.46$	$1161.32^{bc} \pm 32.98$
7 M	Bologna	$74.52^{d} \pm 2.78$	$78.89^{e} \pm 1.76$	$1145.464^{c} \pm 93.35$	$1190.78^{bc} \pm 46.06$
8 M	Rebelde	$65.31^{e} \pm 0.04$	$110.59^{d} \pm 3.50$	$1133.59^{c} \pm 100.26$	$1065.34^{c} \pm 50.18$

Means ± SD of three replicates

Different letters in column show significant differences ($p < 0.05$)

Table 2 Phenolic compounds of ancient (A) and modern (M) wheat genotypes (mg/100 g f.w.) quantified by HPLC–DAD/FLD

#	Genotype	Resorcinol	Tyrosol	Caffeic acid	Syringic acid	Ferulic acid
1A	Ostro nudo	$1.82^{cd} \pm 0.15$	$4.18^{b} \pm 0.12$	$0.07^{c} \pm 0.02$	$4.24^{f} \pm 0.33$	$0.12^{b} \pm 0.02$
2A	Antigola	$1.51^{d} \pm 0.09$	$3.98^{b} \pm 0.05$	$0.07^{c} \pm 0.01$	$13.14^{a} \pm 0.14$	$0.11^{b} \pm 0.01$
3A	Saragolla	$1.99^{c} \pm 0.15$	$7.27^{a} \pm 0.01$	$0.21^{a} \pm 0.01$	$7.34^{d} \pm 0.31$	$0.30^{a} \pm 0.01$
4A	Primitivo	$2.26^{b} \pm 0.12$	Nd	$0.10^{b} \pm 0.01$	$10.99^{c} \pm 0.01$	$0.08^{cd} \pm 0.01$
5 M	Palesio	$2.54^{a} \pm 0.06$	$2.31^{c} \pm 0.02$	$0.05^{d} \pm 0.01$	$1.82^{g} \pm 0.03$	$0.08^{d} \pm 0.01$
6 M	Bolero	$2.87^{a} \pm 0.11$	Nd	$0.02^{d} \pm 0.02$	$5.41^{e} \pm 0.15$	$0.12^{b} \pm 0.02$
7 M	Bologna	$2.80^{a} \pm 0.15$	$2.32^{c} \pm 0.18$	Nd	$11.76^{b} \pm 0.17$	$0.16^{c} \pm 0.01$
8 M	Rebelde	$2.04^{b} \pm 0.21$	$1.90^{d} \pm 0.08$	$0.002^{d} \pm 0.01$	$4.34^{f} \pm 0.16$	$0.05^{e} \pm 0.01$

Means ± SD of three replicates

Different letters in column show significant differences ($p < 0.05$)

Nd not detected

Phenolic compounds identification and quantification with HPLC-DAD/FLD

The phenolic profile of the wheat "ancient" and "modern" genotypes was evaluated by HPLC-DAD/FLD (Table 2). Resorcinol, tyrosol, caffeic acid, syringic acid and ferulic acid were detected in all the wheat samples except for tyrosol, that was not detected in Primitivo (4A) and Bolero (6 M), and caffeic acid, that was not detected in Bologna (7 M). Syringic acid is the most present and abundant phenolic acid. Its content varied from 4.24 mg/100 g f.w. of Ostro nudo (1A) and 4.34 mg/100 g f.w. of Rebelde (8 M) to 13.14 mg/100 g f.w. of Antigola (2A). Rebelde showed the lower content of of tyrosol (1.90 mg/100 g f.w.), while Saragolla (3A) showed the highest content (7.27 mg/100 g f.w.). The resorcinol content ranged from 1.51 mg/100 g f.w. of Antigola (2A) and 1.82 mg/100 g f.w. of Ostro nudo (1A), 1.99 mg/100 g f.w. of Saragolla (3A), 2.04 mg/100 g f.w. of Rebelde (8 M) and 2.26 mg/100 g f.w. of Primitivo (4A) to 2.54 mg/100 g f.w. of Palesio (5 M), 2.80 mg/100 g f.w. of Bologna (7 M) and 2.87 mg/100 g f.w. of Bolero (6 M).

Caffeic acid and ferulic acid are the phenolics less represented in the analysed wheat extracts. Caffeic acid content varied from 0.002 mg/100 g f.w. of Rebelde (8 M), 0.02 mg/100 g f.w. of Bolero (6 M), 0.05 mg/100 g f.w. of Palesio (5 M) and 0.07 mg/100 g f.w. of Ostro nudo (1A) and Angola (2A) and 0.10 mg/100 g f.w. of Primitivo (4A) to 0.21 mg/100 g f.w. of Saragolla (3A).

Rebelde (5 M) showed the lowest content (0.05 mg/100 g f.w.) while Saragolla (3A) showed the highest content of ferulic acid (0.30 mg/100 g f.w.).

Antibacterial activity against gram negative and gram positive bacteria

The antibacterial activity of wheat "modern" and "ancient" genotype extract was first measured by evaluating the final growth of selected enteric bacterial strains in the presence of increasing concentrations of wheat extract (Figs. 1 and 2).

For Gram negative bacteria our results showed that, ferulic acid at the concentration of 0.5 mg/mL significantly decreased the final O.D. of *E. coli*. The Ostro Nudo (1A) and Antigola (2A) wheat samples significantly reduced the final O.D. at the concentration of 4.68 mg/ml, the Primitivo (4A) wheat sample at 1.56 mg/mL and the Saragolla (3A), Bologna (7 M) and Rebelde (8 M) wheat samples at 0.39 mg/mL, while the Palesio (5 M) and Bolero (6 M) wheat samples already significantly decreased the *E. coli* growth at the lowest concentration (0.19 mg/mL) (Fig. 1).

Ferulic acid at the concentration of 0.125 mg/mL significantly decreased the final O.D. of *S. typhimurium.* The Antigola (2A) wheat sample significantly reduced the final O.D. at the concentration of 4.68 mg/mL, the Bolero (6 M) and Rebelde (8 M) wheat samples at 1.56 mg/mL and the Primitivo (4A) wheat sample at 0.39 mg/mL, while the Ostro Nudo (1A), Saragolla (3A), Palesio (5 M) and Bologna (7 M) wheat samples already significantly decreased the final *S. typhimurium* growth at the lowest concentration (0.19 mg/mL) (Fig. 1).

Ferulic acid at the concentration of 0.125 mg/mL significantly decreased the final O.D. of *E. aerogenes*. The Antigola (2A), Bolero (6 M) and Bologna (7 M) wheat samples significantly reduced the final O.D. at the concentration of 1.56 mg/mL, while the Ostro Nudo (1A), Saragolla (3A), Primitivo (4A), Palesio (5 M) and Rebelde (8 M) wheat samples already significantly decreased the final *E. aerogenes* growth at 0.39 mg/mL (Fig. 1).

In addition, the Gram positive bacteria treatments results displayed that ferulic acid at the concentration of 0.5 mg/mL significantly decreased the final O.D. of *S. aureus*. The Ostro Nudo (1A) and Antigola (2A) wheat samples significantly reduced the final growth at the concentration of 4.68 mg/mL, the Bologna (7 M) wheat sample at 1.56 mg/mL and the Primitivo (4A) wheat sample at 0.39 mg/mL, while the Saragolla (3A), Palesio (5 M), Bolero (6 M) and Rebelde (8 M) wheat samples already significantly decreased the final O.D. of *S. aureus* at the lowest concentration (0.19 mg/ml) (Fig. 2).

Ferulic acid at the concentration of 0.13 mg/mL significantly decreased the final O.D. of *E. faecalis*. The Ostro Nudo (1A) and Antigola (2A) wheat samples significantly reduced the final growth at the concentration of 4.68 mg/mL, the Primitivo (4A) wheat sample at 1.56 mg/mL and the Bolero (6 M), Bologna (7 M) and Rebelde (8 M) wheat samples at 0.39 mg/mL, while the Saragolla (3A) and Palesio (5 M) wheat samples already significantly decreased the final O.D. of *E. faecalis* at the lowest concentration (0.19 mg/mlL) (Fig. 2).

Effect on *Lactobacillus brevis* growth

Our results showed that the growth curve of *L. brevis* incubated for 40 h with and without wheat samples at the concentration of 9.38 mg/mL of ethanol extract was increased (Fig. 3A).

In addition, the final O.D. of *L. brevis* obtained during 40 h of incubation at 37 °C with different concentrations of wheat ethanol extracts (0.19, 0.39, 1.56, 4.78, 9.38 mg/mL) (Fig. 3B).

Figure B shows that the Antigola (2A) and Primitivo (4A) wheat samples significantly increased the final O.D. of *L. brevis* at the maximum concentration (9.38 mg/mL), while the Palesio (5 M), Bolero (6 M) and Bologna (7 M) wheat varieties already significantly increased the final growth at 0.39 mg/mL concentration level. Instead, the Ostro Nudo

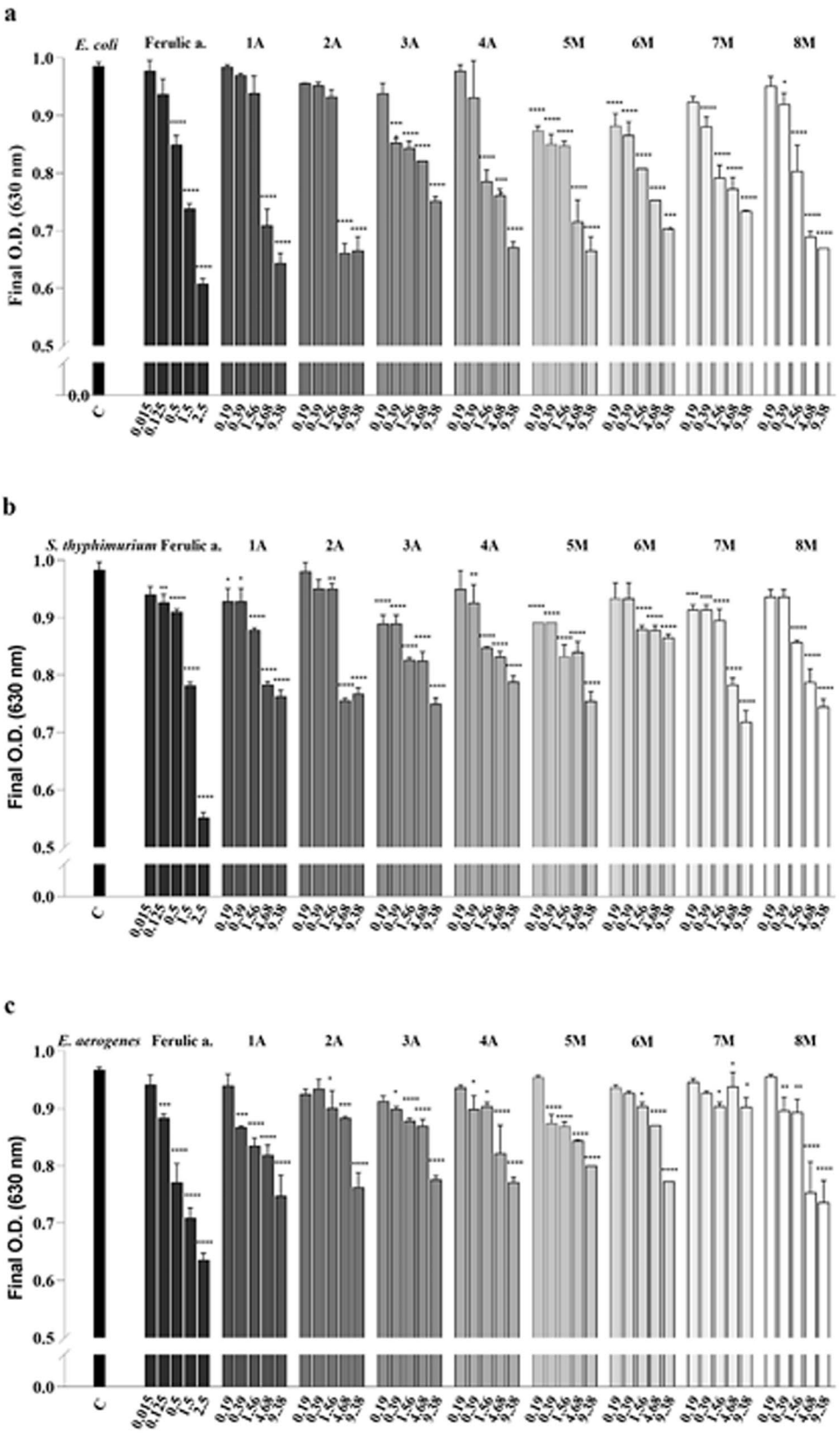

Fig. 1 Antibacterial activities against Gram negative bacteria: final O.D. (630 nm) after incubation of *E. coli* (**a**), *S. thyphimurium* (**b**), *E. aerogenes* (**c**) with different concentrations of ferulic acid (0.015, 0.125, 0.5, 1.5 and 2.5 mg/ml) and sample extracts (0.19, 0.39, 1.56, 4.68 and 9.38 mg/ml). Means ± SD of three replicates. *Indicates significant differences from control with $p < 0.0001$ (****), $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*). *C* control

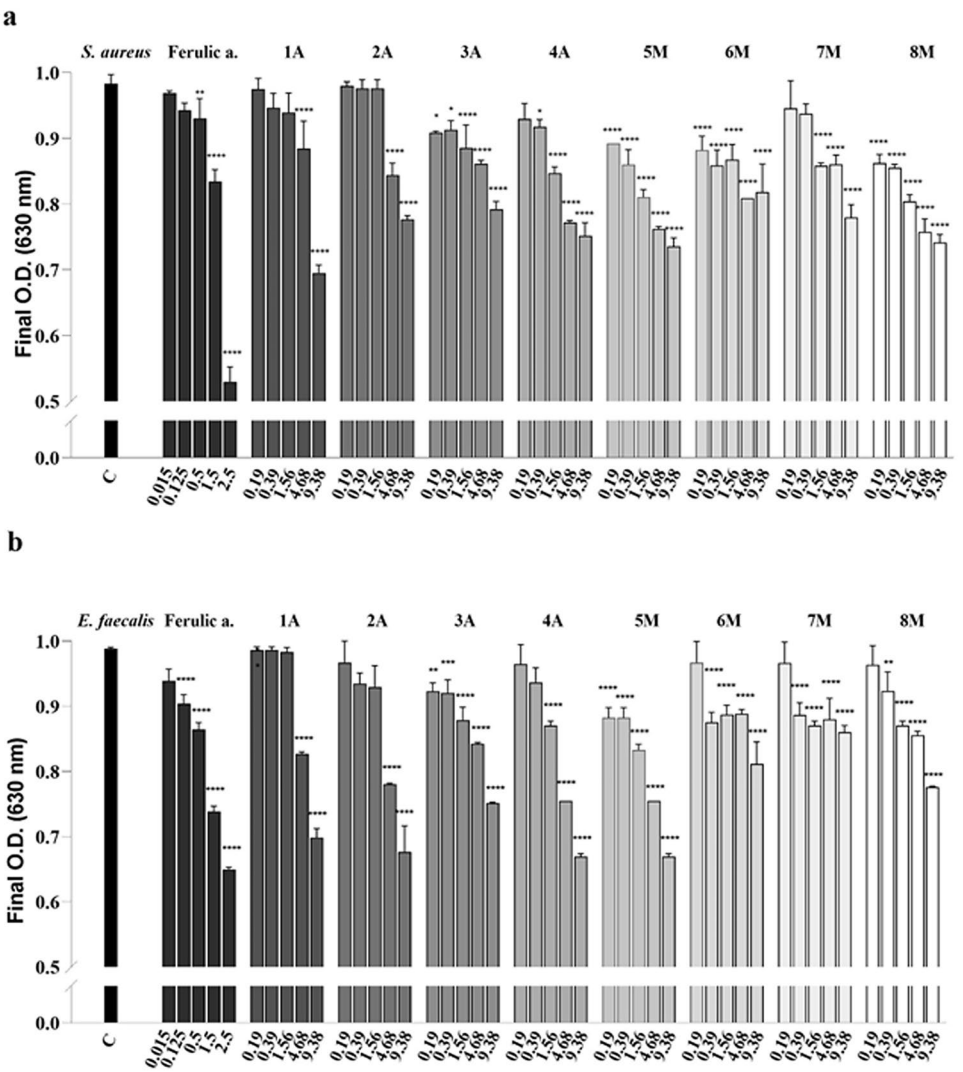

Fig. 2 Antibacterial activities against Gram positive bacteria: final O.D. (630 nm) after incubation of *S. aureus* (**a**), *E. faecalis* (**b**) with different concentrations of ferulic acid (0.015, 0.125, 0.5, 1.5 and 2.5 mg/ml) and sample extracts (0.19, 0.39, 1.56, 4.68 and 9.38 mg/ml). Means ± SD of three replicas. *Indicates significant differences from control with $p < 0.0001$ (****), $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*). *C* control

(1A), Saragolla (3A) and Rebelde (8 M) wheat samples did not show significant effects on the growth. of *L. brevis*.

Overall rate of results with PCA and cluster analysis

Principal component analysis (PCA) was performed to identify possible correlations between the different parameters of wheat extracts studied and to highlight the significant intercorrelations among the chemical and the microbiological variables (Fig. 4).

F1 and F2 explain 63.87% of the total variance. F1 explains 42.98% of the variance compared to 20.89% for the second axis F2. Resorcinol content is the main descriptor explaining F1; antibacterial activity against *E. coli* and ferulic acid content are the most discriminated variables explaining F2 (Fig. 4A). Figure S1 shows score plot of samples and Fig. 4B combines both the scores and the loading vectors in a single biplot display. Cluster analysis clustered wheat genotypes similarly to PCA results (Fig. 4C). Dendrogram shows two principal divergent groups: the first group includes the subgroup of Ostro Nudo (1A) and Saragolla (3A) and the subgroup of Antigola (2A), and the second group includes the subgroup of Primitivo (4A), Palesio (5 M) and the subgroup of Bolero (6 M), Bologna (7 M) and Rebelde (8 M).

Discussion

Cereal grains have phenolic compounds also found in fruits and vegetables [15]. Therefore wheat, as the most favored cereal in the world, represents one of the main sources of antioxidants in our daily diet when consumed in whole form [16, 17]. Wheat bran have the highest phenolic content and give to whole flour a better nutritional value [18], but phenol profile and quantities depend on the sample's genetics. The phenolic profiles, the antioxidant capacities and antimicrobial activity of four ancient and four modern genotypes were

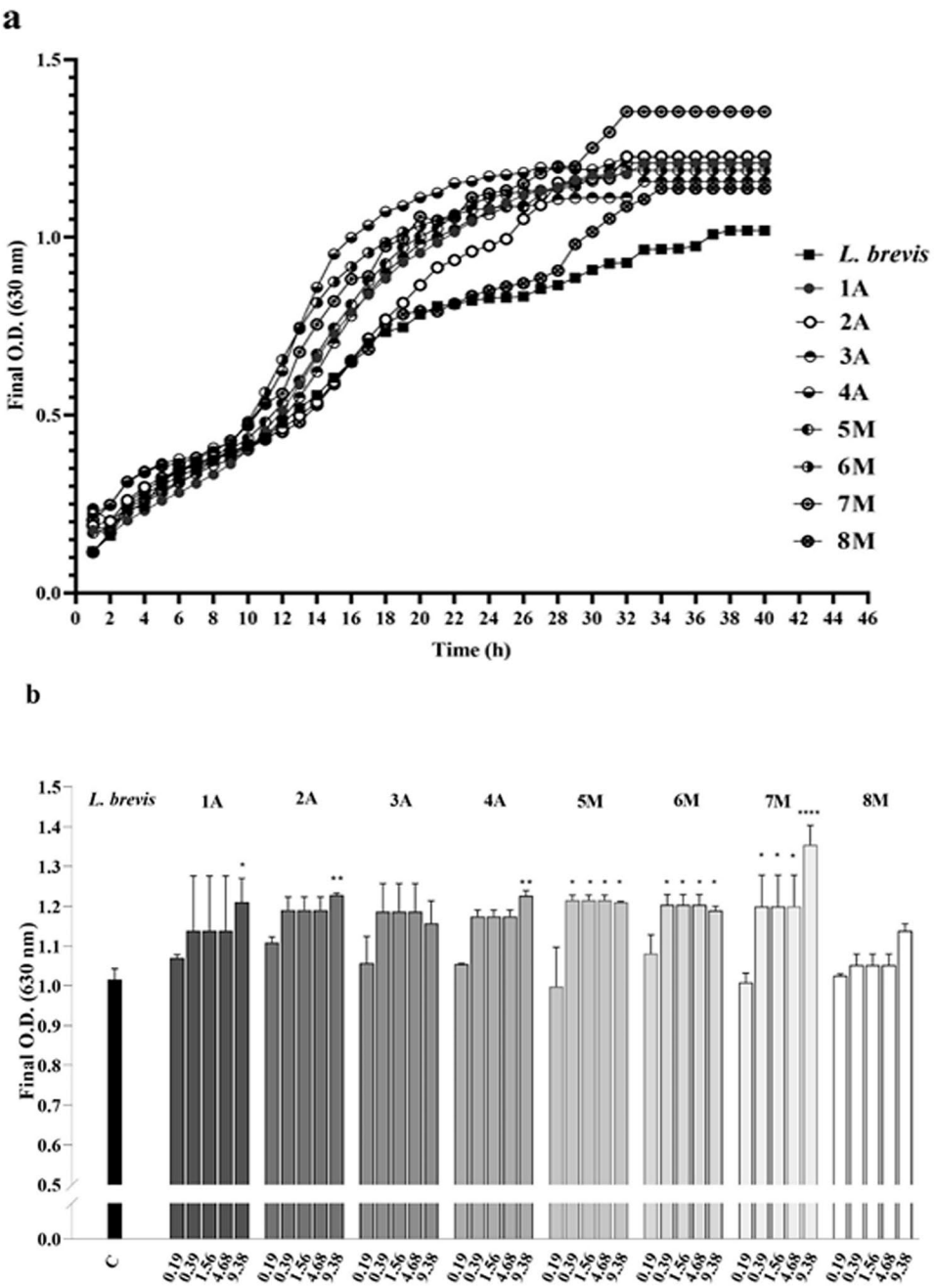

Fig. 3 Effects of wheat extract samples on *L. brevis* growth: **a** growth curves after 40 h of incubation with and without samples (9.38 mg/ml); **b** final O.D. (630 nm) after incubation of *L. brevis* with different concentrations of sample extracts (0.19, 0.39, 1.56, 4.68 and 9.38 mg/ml). Means ± SD of three replicas. *Indicates significant differences from control with $p < 0.0001$ (****), $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*). *C* control

studied to evaluate a possible difference due to the genotype influence.

TPC and TFC detected is in accordance with literature results where polyphenol extraction was carried out without preliminary hydrolysis [19–21] in order to limit the sample handling and, thus, avoiding time consuming procedures and sample contamination risk [22–24]. Our extraction is addressed to study free, soluble phenolic acids and flavonoids in better instead of insoluble bound phenolics present in wall wheat [19]. We choose this method to represent the original quali-quantities wheat profile. Furthermore, TFC were higher than TPC although the opposite would be expected. We can hypothesize that Folin-Ciocalteu assay has a limitation in detecting the full phenolic extract constituents [25] although Kaisoon et al. [26] assume that natural extracts with higher flavonoid content do not necessarily show high TPC. Overall, TPC and TFC results show significantly different values between wheat genotypes with a tendency of giving in ancient genotypes values higher then modern ones. Pasqualone et al. [27] and Valli et al. [28] found analogous differences in the study of different Italian genotype wheat flour and bread. However, Heimler et al. [19] and Dinelli

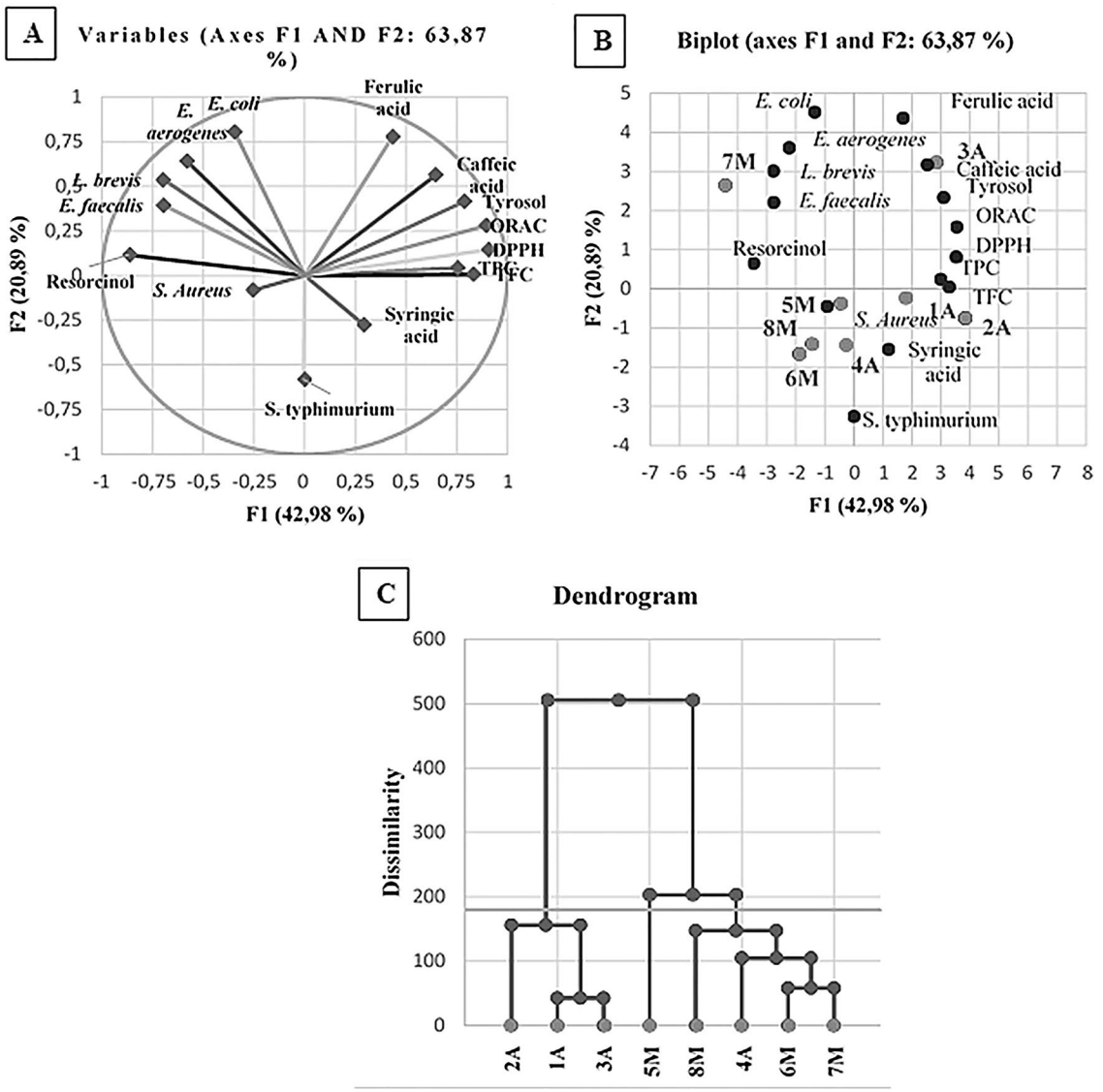

Fig. 4 Principal component analysis (PCA). **A** Variables loading plot, **B** biplot of variables and observations of PCA and **C** dendrogram from clustering of the effect of wheat genotypes on TPC, TFC, antioxidant activity (DPPH and ORAC) and antimicrobial activity (*Escherichia coli* ATCC 25,922, *Salmonella typhimurium* ATCC 14,028, *Enterobacter aerogenes* ATCC 13,048, *Staphylococcus aureus* ATCC 25,923 and *Enterococcus faecalis* ATCC 29,212) parameters. *F1* first principal component, *F2* second principal component

et al. [22] found that the moderns were characterized by higher values than the ancient ones.

According to the TPC and TFC, the DPPH and ORAC assays showed that ancient genotypes tended to have higher anti-radical activities than modern ones. Ostro nudo (1A), Antigola (2A) and Saragolla (3A) were the samples with the highest TPC and flavonoids and showed the highest antioxidant activities confirming that these parameters correlate [29]. All wheat samples showed DPPH scavenging activities higher than those reported by other authors who studied Italian wheat genotypes using similar test methods [22, 23, 28, 30]. Despite the comparison of the antioxidative capacity of the ORAC test in whole wheat flour with the literature was difficult because of the different ORAC assays applied, the values obtained were in accordance [31]. Differences between antioxidant activity of wheat genotypes were also found by Serpen et al. [32] between emmer and eikorn against two control soft grains and by Di Loreto et al. [30] between old and modern Italian *Durum* wheat varieties. Instead, Abdel-Aal et al. [33] reported few differences of antioxidant activity between ancient and modern varieties of grains belonging to different species. These results may indicate that also antioxidant activities are complex features influenced by both genotype and environmental factors [34].

The HPLC analysis revealed that syringic acid was the most represented phenolic acid in all wheat samples. Ancient wheat genotypes were characterized by a high amount of syringic acid and tyrosol. In grains belonging to the *T. aestivum* species, tyrosol was absent or detected in few amounts. *T. aestivum* group showed the highest amounts of resorcinol. The ancient genotypes also presented higher content of ferulic and caffeic acids. These data agree with the study conducted by Sanak et al. [35] who identified by HPLC syringic acid as the most abundant class of phenolic acids of some genotypes from wheat *Triticum aestivum* and *Triticum durum* grains ranging from 0.162 to 9.521 mg/100 g. Compared to other studies [36] some phenolic acids such as p-coumaric acid and vanillic acid were not detected in our samples. Phenolic acids in wheat grains may be indeed mostly bound to bran components, making them difficult to be detected with HPLC without acid hydrolysis [37]. This result was confirmed also by the low content of ferulic acid (< 1 mg/100 g). Ferulic bound form represents indeed 95% of the grain ferulic acid concentration (i.e., 95%) [35, 38].

Whole wheat phenolics, in addition to antioxidant activity, have also antibacterial properties [39]. In this study we evaluated antibacterial activities of wheat ethanol extracts containing phenolic compounds against *Gram negative* and *Gram positive* bacteria at different concentration.

It has been observed that among Gram negative the greater growth inhibition is towards *E. coli* and, among Gram positive, towards *E. faecalis*. At the best of our knowledge, few studies have been carried out on the antimicrobial activity of grains: Bursalioğlu [40] has shown that seed extract of einkorn (*T. monoccouccum)* did not have any antibacterial effect against *S. aureus* strain and *E. coli*. Saha et al. [41] have shown that *T. aestivum* variety (Pavon76) seed has antibacterial activity against *E. coli* and *S. aureus* at the concentration 450 µg/µl of its ethanol extract.

The antibacterial activity was also evaluated with respect to ferulic acid as reference phenolic. Ferulic acid showed a greater reduction of final growth against *S. aureus* and *S. typhimurium* and *E. coli*, and a minor effect on final growth against *E. aerogenes* and *E. faecalis*. The antibacterial activity of ferulic acid was also demonstrated by the study of Borges et al. [42] against *E. coli* and *S. aureus* and against other pathogenic bacteria such as *Listeria monocytogenes* with inhibitory concentrations in the range 100–1250 µg/ml.

In addition to the antibacterial activity on pathogenic bacteria, we tested the influence of wheat samples on the growth of a strain of *L. brevis*, which is a probiotic bacterium. An induction of bacterial growth was observed at the high concentration of ethanol extract tested except for Rebelde (8 M) and Primitivo (4A). This growth induction activity could be due to the TPC revealed in the extracts. Polyphenols may interact with colonic microbiota, and the beneficial microorganisms (i.e., *Lactobacillus* spp., *Bifidobacterium* spp.) could use polyphenols as substrates to grow. Polyphenols could also influence the bacteria expression of phenotypic features such as adhesion molecules [43]. Furthermore, several studies have demonstrated that lactic acid bacteria have higher tolerability to polyphenols against to pathogenic microbiota: Tabasco et al. [44] have shown that polyphenols (0.25–1.0 mg/ml) stimulate the in vitro growth of *Lactobacillus* spp. strains.; Piekarska-Radzik et al. [45] have demonstrated that cultures of *Lactobacillus brevis* (ŁOCK 0944) with the addition of purified water–ethanol and crude water–acetone extracts of polyphenols have 7 -10% higher growth than the control.

The use of different combinations of phenolic compounds as prebiotics is also a fundamental aspect. Some studies have shown that *Lactobacillus* spp. incubated with polyphenol-rich extracts or combinations of multiple polyphenols grew better than bacteria incubated with single compounds [46, 47].

The PCA and agglomeration cluster analysis presented in this work remarkably confirmed the possible correlations between the types of wheat genotypes, their bioactive compounds and related properties. The score plot (Figure S1) showed that samples were separated into four quadrants with different distances. The shorter the distance between two products in PCA score plot, the higher their degree of similarity. The nearest distances were between 2 and 1A, suggesting that they were the most similar genotypes. PCA biplots showed which parameters characterize the different wheat genotypes. Samples 1A and 2A cluster with flavonoid and syringic acid content. The 4 M, 5 M, 6 M and 8 M samples were clustered according to resorcinol content and antibacterial activity against *S. typhimurium.* The 7 M wheat extract instead clustered with antibacterial activity toward *E. coli*, *E. aerogenes* and *E. faecalis*. The Saragolla (3A) wheat sample extract was clustered the largest number of variables investigated (TPC, TFC, DPPH, ORAC and some phenolic acids content) and it was in a quadrant far from other genotypes proving to be the most interesting variety from a nutraceutical point of view among those examined. Cluster analysis showed two subgroups: one characterized by the ancient genotypes except for Primitivo (4A), which belongs to the second group characterized by genotypes belonging to *T. aestivum.*

Conclusion

This study has confirmed that wholemeal flours can be a worthy source of phenolic compounds. The Italian wheat genotypes investigated in our study showed significant differences in the radical scavenging capabilities of DPPH comparable and superior to other genotypes studied in the literature. Thus, whole flours of Italian *Triticum* spp. can be potentially

investigated to develop functional foods. Extracts from whole meal flours showed a moderate antimicrobial activity, both against Gram negative and Gram positive bacteria, and an inducing activity of *L. brevis* growth, which is a potential probiotic bacterium. PCA and cluster analysis confirmed the effect of genotypes on phenolic profile pattern and antibacterial activities underlying Saragolla as an ancient wheat interesting for better phenolic acid content. Saragolla old genotypes have shown a good TPC and antioxidant capacity. These results highlight the importance of conducting further research to screen the properties of different genotypes, and to select the most suitable species for developing new products with antioxidant and anti-bacterial potential.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements Ethical approval was not required for this research.

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Authors and Affiliations

Teresa Grande[1] · Aymen Souid[1,2] · Marco Ciardi[1] · Clara Maria Della Croce[1] · Stefania Frassinetti[1] · Emilia Bramanti[3] · Vincenzo Longo[1] · Luisa Pozzo[1]

✉ Aymen Souid
souid.ayman@gmail.com; aymen.souid@ibba.cnr.it

[1] Institute of Agricultural Biology and Biotechnology-National Research Council (IBBA-NRC), Via Moruzzi 1, 56124 Pisa, Italy

[2] Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj Cedria, BP 901, 2050 Hammam Lif, Tunisia

[3] Institute of Chemistry of Organometallic Compounds-National Research Council (ICCOM-NRC), Via Moruzzi 1, 56124 Pisa, Italy