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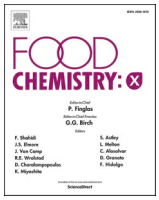

Insights into the effects of extractable phenolic compounds and Maillard reaction products on the antioxidant activity of roasted wheat flours with different maturities

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Trolox (Pubchem CID40634)
Vanillic acid (Pubchem CID8468)
Protocatechuic acid (Pubchem CID72)
P-Coumaric acid (Pubchem CID637542)
Gallic acid (Pubchem CID370)
4-Hydroxybenzoic acid (Pubchem CID135)
Caffeic acid (Pubchem CID689043)
Syringic acid (Pubchem CID10742)
Ferulic acid (Pubchem CID445858)

ABSTRACT

Experiments were performed to determine the effect of roasting whole wheat flours at 80 °C, 100 °C and 120 °C for 30 min on four forms of phenolics, Maillard reaction products (MRPs), and the DPPH scavenging activity (DSA) at 15, 30 and 45 days after flowering (15-DAF, 30-DAF, and 45-DAF). Roasting increased the phenolic content and antioxidant activity of the wheat flours, which were the dominant contributions to the formation of Maillard reaction products. The highest total phenolic content (TPC) and total phenolic DSA (TDSA) were determined in the DAF-15 flours at 120 °C/30 min. The DAF-15 flours exhibited the highest browning index and fluorescence of free intermediate compounds and advanced MRPs, suggesting that a substantial quantity of MRPs were formed. Four forms of phenolic compounds were detected with significantly different DSAs in the roasted wheat flours. The insoluble-bound phenolic compounds exhibited the highest DSA, followed by the glycosylated phenolic compounds.

Introduction

Immature wheat has been traditionally consumed in China, North Africa and the Middle East, under names such as “Nianzhuan and Firiks” (Zhang et al., 2022; Özkaya et al., 2018). Immature wheat is becoming an increasingly popular food throughout the world. A considerable literature has recently grown up around the nutritional compounds in immature wheat. Some researchers have reported that immature wheat contains more phytochemicals with human health benefits than mature wheat, such as minerals, dietary fiber (arabinoxylan, β-glucan and fructan), essential amino acids, phenolic compounds (Iametti et al., 2006; Kapoor and Heiner, 1982; Verspreet et al., 2013; Yang et al., 2012, Kim and Kim, 2016). Immature wheat grains have also attracted the attention of researchers and are being applied to an increasing number of functional foods (Zhang et al., 2022; Kim and Kim, 2017).

Roasting is an important approach for processing immature wheat flours from raw into edible foods: in particular, roasting is used to produce bakery items and changes the composition of phenolic compounds in grains. Roasting grains results in kernel puffing, generates numerous cracks on the grain surface, and increases the antioxidant activity of soluble phenolic compounds (Tian et al., 2021). Bai et al. (2021) found that roasting highland barley increased the antioxidant activity because of the formation of Maillard reaction products and a reduction in the total phenolic content. The antioxidant action of MRPs hinders the formation of radical chains by quenching scavenging free radicals (Feng et al. 2022; Nooshkam et al. 2019). Maillard reaction products (MRPs) are ubiquitous in heat-treated food, including reducing sugars and amino elements. The roasting temperature influences the

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formation of MRPs, such as melanoidins, the final products of the Maillard reaction.

Phenolic compounds are one of the most important components in plans and classified into soluble forms (free, esterified, and glycosylated) and insoluble-bound forms (Pihlava et al., 2015). Four forms of phenolic compounds can be extracted by alkali and acid methods (Xu et al., 2007; Pihlava et al., 2015). Recently, an increasing number of studies have suggested that the forms of phenolic compounds have different antioxidant properties (Wu et al., 2021; Deng et al., 2021; Zhu et al., 2019).

Although research has been conducted on immature wheat flours used as food ingredients, there have been few reports on the specific phenolics and antioxidant properties of roasted immature wheat. MRPs contribute to the antioxidant properties and nutritive value of food products. The effect of different forms of phenolic compounds and MRPs produced by roasting wheat flours of different maturities on the antioxidant activity was investigated in this study.

Materials and methods

Sample collection and wheat flour preparation

Wheat (*Triticum aestivum* L.) seeds of Zhen Mai 366 were planted in an experimental field situated in Zhoukou, Henan, China (33.37 N, 114.65 E). The seeds with three maturity degrees (MD) were collected from the 15th day to the 45th day after flowering, and grains were collected at 15-day intervals (DAF 15: 15 days after flowering; DAF 30: 30 days after flowering; DAF 45: 45 days after flowering). The growth cycle of wheat is about 45 days, so the wheat harvested 45 days after flowering is the fully maturity time. The collected samples were dried in a drying oven and ground in a cyclone sample mill (CT 293 Cyclotec™, FOSS, Hilleroed, Denmark) installed with a 1-mm sieve. All samples were stored at 4 °C until further analysis.

Roasting treatment

Wheat flour samples were separated into three 15-gram portions. The samples were placed on a metal tray, spread out evenly and roasted in a heated oven (Model Bulepard ® DHG-9013A, Yiheng Co., ltd., Shanghai, China) for 30-min periods at 80 °C, 100 °C and 120 °C. Each roasting treatment was repeated three times.

Chemicals

2,2-Diphenyl-1-picryhydrazyl (DPPH); 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic (Trolox); vanillic acid; protocatechuic acid; *p*-coumaric acid; gallic acid; 4-hydroxybenzoic acid; caffeic acid; syringic acid; and ferulic acid were supplied by Sigma–Aldrich, Missouri, USA (HPLC, ≥98 %). Folin-Ciocalteu phenol reagent was purchased from Aladdin Industrial Co. (Shanghai, China). HPLC grade methanol and formic acid were purchased from Merck KGaA (Darmstadt, Germany). All other chemicals used were analytical grade.

Methods

Dry matter content

The protein content of the wheat flours was analyzed according to the Association of Official Analytical Chemists (AOAC) Method 925.10 (AOAC, 2002), as shown in Table S1.

Ethanol extraction of wheat flours

The roasted and unroasted wheat flours (0.3 g) were extracted with 3 mL of 80 % (V/V) ethanol. The resulting solutions were homogenized at 400 rpm and 25 °C for 20 min and centrifuged (12000 rpm for 10 min at 25 °C). The supernatant from each solution was reserved, and the extraction was repeated with 3 mL of 80 % ethanol. The final volume of the extract was adjusted to 8 mL using the extraction solvent.

Extraction of four forms of phenolic compounds

Fig. S1 shows an overview of the extraction processes. Four forms of phenolic compounds (free, esterified, glycosylated and insoluble-bound) in the extracts were performed following the method of Xu et al. (2007) with minor modifications. The roasted wheat flours were extracted with 80 % ethanol using the method described in Section 2.4.1. The 8-mL combined supernatants were subjected to a sequence analysis of the free, esterified and glycosylated compounds. Eight-milliliter aliquots of the combined extracts were placed in a nitrogen evaporator (Model EFSFRC-DC24-RT, ANPEL Laboratory Technologies Inc., Shanghai, China) to remove ethanol at room temperature.

1) Free phenolic compounds

Next, a solution of ethyl acetate and the extract (1:1, v:v) were thoroughly mixed, and this process was repeated three times. The ethyl acetate portions were reserved and evaporated in a nitrogen evaporator at room temperature, and the recovered dry fractions were dissolved in methanol to determine the free phenolic compounds.

2) Esterified phenolic compounds

Then, 4 mL of 4 M NaOH were added to the aqueous phase, and the mixture was stirred for 1 h under nitrogen at ambient temperature. The mixture was adjusted to pH 2 using 6 M HCl and extracted three times with 4 mL of ethyl acetate at room temperature. The ethyl acetate layers were collected and evaporated in a nitrogen evaporator at room temperature; the resulting dry fractions were dissolved in methanol to determine the esterified phenolic compounds.

3) Glycosylated phenolic compounds

Finally, 4 mL of 6 M HCl were added to the aqueous phase, and the resulting mixture was hydrolyzed at 72 °C for 30 min. The hydrolyzed mixture was cooled to room temperature, and 4 mL of ethyl acetate were used to extract the glycosylated phenolic compounds. Ethyl acetate was evaporated under nitrogen, and the dry residue was reconstituted with absolute methanol to determine the glycosylated phenolic compounds.

4) Insoluble-bound phenolic compounds

The insoluble-bound phenolic compounds were extracted from the precipitated wheat flours using 80 % ethanol. The precipitate was hydrolyzed with 4 mL of 4 M NaOH for 1 h under nitrogen, homogenized at 400 rpm for 5 min and shaken at 25 °C. The solution pH was adjusted to 2.0 with 6 M HCl, and the bound phenolics were extracted three times with ethyl acetate. The ethyl acetate layer was collected, evaporated to dryness using a nitrogen evaporator, and redissolved in methanol for analysis.

Determination of the phenolic content and analysis of phenolic acids

Phenolic content of the ethanol extract. The phenolic content of the ethanol extract (EEPC) was determined using the method of Xiang et al. (2019) with minor modifications. Sixty microliters of the ethanol extract of the whole wheat flours were added to 200 μL of Folin-Ciocalteu phenol reagent, the mixture was allowed to react for 5 min, and 740 μL of a sodium hydroxide solution were added to the resulting mixture. The solution mixture was incubated for 1 h at ambient temperature in the dark. The absorbance was read at 765 nm using a microplate absorbance reader (iMark™680, Bio-Rad Laboratories, Inc., Hercules, CA, USA). A standard curve was generated using gallic acid. The results were expressed as gallic acid equivalents (GAE) mg per 100 g of dry weight (DW).

Contents of four forms of phenolic compounds. The four forms of extracted phenolic compounds were determined as described in Section 2.4.4.1. The free phenolic content (FPC), esterified phenolic content (EPC), glycosylated phenolic content (GPC), insoluble-bound phenolic content (IBPC) and total phenolic content (TPC, the sum of the FPC, EPC, GPC, and IBPC) of the prepared samples were analyzed.

Types and content of phenolic acids in four forms of phenolic compounds. The four extract fractions (free, esterified, glycosylated, and insoluble-bound phenolic compounds) were analyzed according to the method of Arruda et al. (2018) with slight modification. A high-performance liquid chromatographic (HPLC) system (Shimadzu, Kyoto, Japan) equipped with a C-18 column (ZORBAX® HPLC column, 5-μm particle size, 4.6 mm × 250 mm, Agilent Technologies, Inc., Santa Clara, CA, USA) was used to separate the phenolic acids. The samples were eluted with a gradient model using 100 % methanol as Solvent A and ultra-pure water (0.1 % formic acid) as Solvent B. A 10-μL aliquot of each sample was injected into the column at a flow rate of 1 mL/min. A 35-min solvent gradient was used with the following volume ratios: 0–13.5 min, 85–66 % B; 13.5–21.5 min, 66–60 % B; 21.5–23 min, 60–54 % B; 23–25.5 min, 54 % B; 25.5–27.5 min, 54–85 % B and 27.5–35 min, 85 % B. Each phenolic acid was identified by the retention time of the standard solutions and quantified using the peak area. The four extracted fractions of the free phenolic compounds, esterified phenolic compounds, glycosylated phenolic compounds and insoluble-bound phenolic compounds were used to analyze the type and content of phenolic acids: free phenolic acid (FPA), esterified phenolic acid (EPA), glycosylated phenolic acid (GPA) and insoluble-bound phenolic acid (IBPA), respectively. The total phenolic acid (TPA) was the sum of the FPA, EPA, GPA and IBPA.

Maillard reaction

Protein content. The protein content of the wheat flours was analyzed according to the AOAC Method 979.09, with a nitrogen to protein conversion factor of 6.25 (AOAC, 2002).

Analysis of the MRPs. An MRP analysis was carried out using a method reported by Michalska et al. (2008) with minor modifications. The indicators included the intermediate Maillard products (IMPs), soluble tryptophan (Tryp), fluorescence of advanced MRPs (FI) and melanoidin (MI).

1) Intermediate Maillard products

The fluorescence of IMPs (F_{IMPs}), expressed as arbitrary fluorescence units (AFU), was determined using a fluorescence detector (Model SpectraMax® i3x, Molecular Devices LLC, San Jose, CA, USA) at an λ_{em} and λ_{ex} of 438 and 353 nm, respectively.

2) Index of the fluorescence of advanced MRPs

The fluorescence of soluble tryptophan (F_{Tryp}), expressed in arbitrary fluorescence units (AFU), was measured by a fluorescence detector (Model SpectraMax® i3x, Molecular Devices LLC, San Jose, CA, USA) at an λ_{em} and λ_{ex} of 340 and 290 nm, respectively. The FI (%) was calculated as follows:

$$FI(\%) = \frac{F_{IMPs}}{F_{Tryp}}$$

where F_{IMPs} is the AFU of the IMPs and F_{Tryp} is the AFU of soluble tryptophan.

3) Melanoidin index

Melanoidins are the final products of the Maillard reaction and classified as a major group of compounds. These products consist of nitrogen and furan rings, resulting in a brown color, the intensity of which was evaluated by measuring the absorbance of the sample at 420 nm using a UV–vis spectrophotometer (Cary 60, Agilent Technology, Inc., Santa Clara, CA, USA). The MI was expressed as absorbance units (AU).

Determination of the DPPH scavenging activity

Evaluation of the scavenging activity of the four forms of phenolic compounds in roasted wheat flours. The DPPH scavenging activity of the samples was measured using a procedure reported by Tian et al. (2021) with minor modifications. Each of the four forms of extracted phenolic compounds (100 μL) was reacted with 100 μL of a DPPH solution (0.15 mM in 80 % ethanol) in a 96-well microplate at 25 °C for 30 min in the dark. The absorbance of the solution was read at 517 nm on a microplate absorbance reader (iMark™680, Bio-Rad Laboratories, Inc., Hercules, CA, USA). Trolox was used as an antioxidant standard. An equal volume of 80 % ethanol to that of the sample solution was used as a blank control. The results were expressed as μmol Trolox equivalent (TE)/100 g DW. The free phenolic DPPH scavenging activity (FDSA), esterified phenolic DPPH scavenging activity (EDSA), glycosylated phenolic DPPH scavenging activity (GDSA), and insoluble-bound phenolic DPPH scavenging activity (IBDSA) were determined. The total phenolic DPPH scavenging activity (TDSA) was the sum of the FDSA, EDSA, GDSA, and IBDSA.

Evaluation of scavenging activity of the ethanol extracts of roasted wheat flours. The 80 % ethanol extraction method described in Section 2.4.1 was used to obtain extracts for determining the antioxidant activity of the roasted wheat flours. The DPPH scavenging activity of the ethanol extract (EEDSA) was determined using the method described in Section 2.4.6.1.

Statistical analysis

The data were analyzed using IBM® SPSS® Modeler 18.0 for Windows. The differences in the mean values obtained for wheat flours subjected to different treatments were determined using the one-way analysis of variance (ANOVA), followed by Duncan's test at $p \leq 0.05$ or $p \leq 0.01$ significance levels. The results are presented as the mean ± standard deviation (SD).

Results

Contents and phenolic acid compositions of the four forms of phenolic compounds

Contents of the four forms of phenolic compounds in unroasted wheat flours

An in-depth analysis was performed on the phenolic compounds in unroasted wheat flours with three maturities. Fig. 1(a) presents the contents of the four forms of phenolic compounds in these wheat flours. The contents of all four forms of phenolic compounds in the unroasted wheat flours decreased with the wheat-grain development. The free phenolic content (FPC) of the DAF-15 unroasted flours of 54.25 mg GAE/100 g DW was higher than the unroasted DAF-30 (46.63 mg GAE/100 g DW) and DAF-45 (42.65 mg GAE/100 g DW) wheat flours. The esterified phenolic content (EPC) ranged from 62.82 to 30.23 mg GAE/100 g DW. The glycosylated phenolic content (GPC) of the DAF-15 unroasted flours was 3 times that of the other two groups of wheat flours. The insoluble-bound phenolic content (IBPC) was 206.57 mg GAE/100 g DW, 157.55 mg GAE/100 g DW, and 97.03 mg GAE/100 g DW for the unroasted DAF-15, DAF-30, and DAF-45 wheat flours, respectively.

TPC of the unroasted wheat flours was 504.80 mg GAE/100 g DW for the DAF-15 flours, 317.91 mg GAE/100 g DW for the DAF-30 flours and 233.83 mg GAE/100 g DW for the DAF-45 flours, as shown in Fig. 1(a). The insoluble-bound phenolic content (IBPC) was the main content of the wheat flours with three maturities, especially the DAF-15 and DAF-30 flours. Our results showed IBPCs of up to 52.19 % (DAF-15), 64.85 % (DAF-30), and 54.07 % (DAF-45).

Effect of roasting treatment on the contents of the four forms of phenolic compounds

The TPC reached 593.55 mg GAE/100 g DW for the DAF-15 wheat flours roasted at 120 °C, which was 89.75 mg GAE/100 g DW higher than that of the unroasted DAF-15 wheat flours. A slightly higher TPC

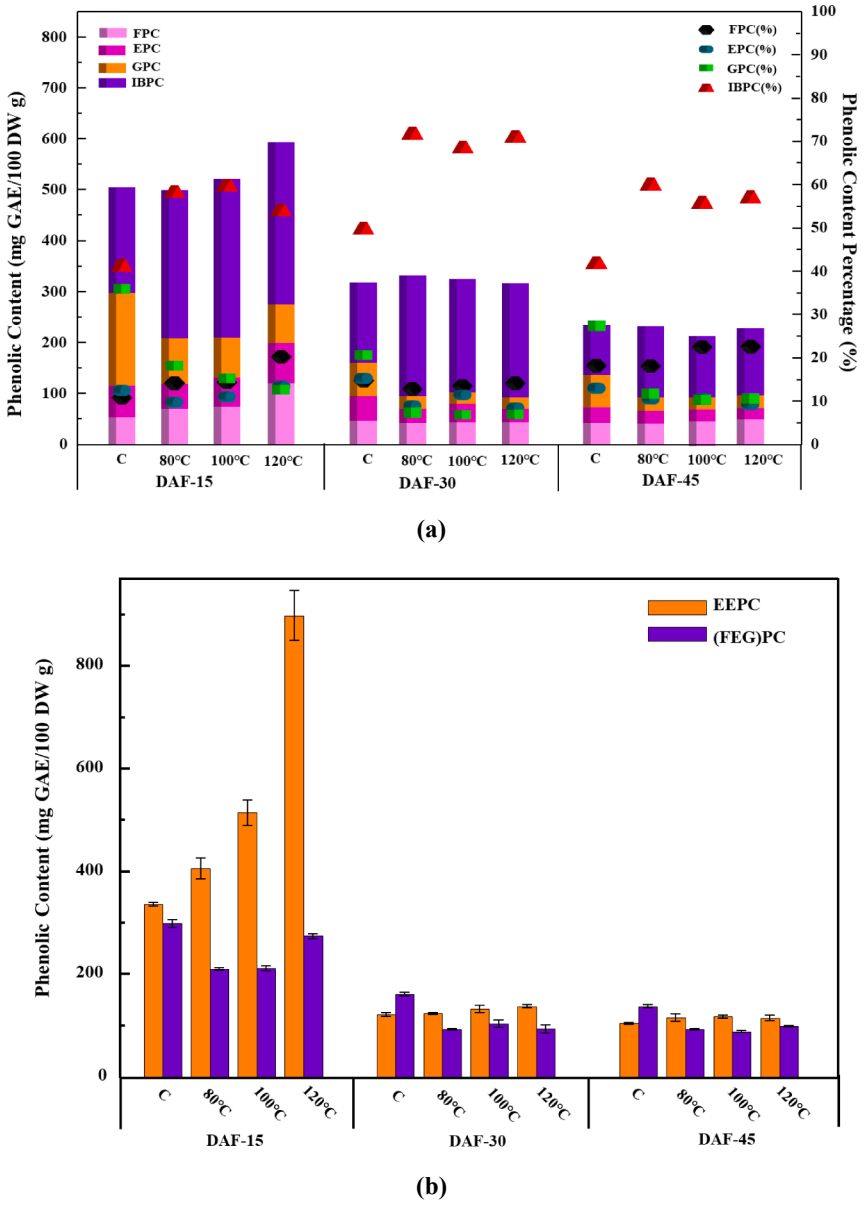

Fig. 1. Four forms phenolic content of non-thermal and roasted wheat flours (a), EEPC and (FEG)PC in unroasted and roasted wheat flours (b).

was obtained for DAF-15 wheat flours roasted at 80 °C/100 °C. However, no significant reduction in the TPC was found for DAF-30 or DAF-45 wheat flours with increasing roasting temperature, as shown in Fig. 1 (a). The TPC was thus determined: however, how did roasting change the contents of the four forms of phenolic compounds in wheat flours with three maturities? The contents of the four forms of phenolic compounds were determined, and the results are shown in Fig. 1(a). The FPC and IBTC of the DAF-15 wheat flours increased with the roasting temperature. The FPC of the DAF-15 wheat flours ranged from 54.25 to 120 mg GAE/100 g DW, and the corresponding IBTC ranged from 504.80 to 593.55 mg GAE/100 g DW. The EPC of the DAF-15 wheat flours decreased to 48.35 mg GAE/100 g DW after roasting at 80 °C and then increased to 79.65 mg GAE/100 g DW after roasting at 100 °C. However, the GPC of the DAF-15 wheat flours decreased from 181.16 mg GAE/100 g DW to 75.01 GAE mg/100 g DW with increasing roasting temperature. The FPC of the DAF-30 wheat flours remained constant with increasing roasting temperature. The EPC and GPC of the DAF-30 wheat flours were generally lower than those for the DAF-15 wheat flours, in contrast to the IBPC results. The highest phenolic content of the DAF-30 wheat flour was IBPC after roasting at 120 °C (222.91 mg GAE/100 g DW), and the corresponding lowest phenolic content was GPC (21.85 mg GAE/100 g DW) for wheat flours roasted at 120 °C. The FPC and EPC of DAF-45 wheat flours changed slightly with increasing roasting temperature. As the roasting temperature increased, the GPC of DAF-45 wheat flours decreased from 63.91 to 24.70 mg GAE/100 g DW and the IBPC increased from 97.03 to 130.31 mg GAE/100 g DW. The GPC dropped by 19.53 %, 13.34 %, and 15.59 % for the DAF-15, DAF-30, and DAF-45 flours, respectively, roasted at 80 °C and then remained constant with increasing roasting temperature. However, the change in the IBPC was opposite to that of the GPC and increased by 18.61 %, 21.41 %, and 18.19 %, respectively, for the DAF-15, DAF-30, and DAF-45 flours roasted at 80 °C and then changed similarly to the GPC. A correlation analysis was carried out to determine the relationship among the maturity degrees (MD), roasting temperature (RT), and contents of the four forms of phenolic compounds. The F values for the FPC, EPC, GPC, IBPC, and TPC showed these contents varied highly and significantly with the MD ($p \leq 0.01$). The F values for FPC, GPC, and IBPC showed these contents varied highly and significantly with the RT ($p \leq 0.01$)

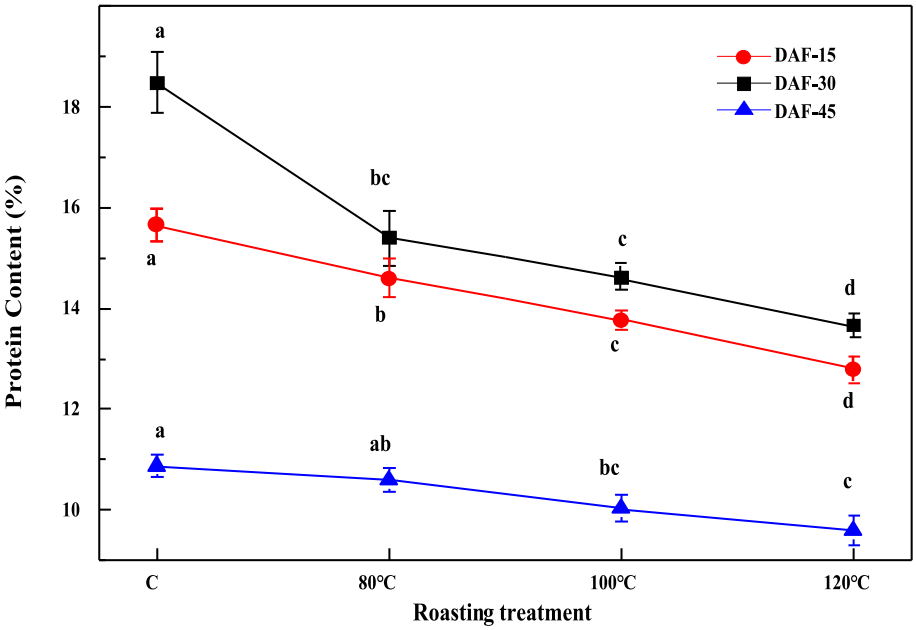

Fig. 2. Protein content of unroasted and roasted wheat flours.

Table 1
Maillard reaction products of non-thermal treatment and thermal treatment wheat flour.

	F_{IMPs} (Arbitrary Fluorescence units × 10^7)			F_{Tryp} (Arbitrary Fluorescence units × 10^7)			FI			MI (Arbitrary absorbance units)		
	DAF-15	DAF-30	DAF-45	DAF-15	DAF-30	DAF-45	DAF-15	DAF-30	DAF-45	DAF-15	DAF-30	DAF-45
C	2.33 ± 0.16^{d}	0.29 ± 0.02^{c}	0.27 ± 0.00^{c}	0.78 ± 0.02^{a}	1.58 ± 0.04^{c}	2.63 ± 0.02^{c}	2.99 ± 0.16^{d}	0.18 ± 0.02^{c}	0.10 ± 0.00^{c}	0.18 ± 0.01^{d}	0.06 ± 0.01^{b}	0.05 ± 0.00^{b}
80℃	2.80 ± 0.13^{c}	0.42 ± 0.01^{b}	0.35 ± 0.01^{b}	0.67 ± 0.02^{b}	1.95 ± 0.03^{ab}	3.35 ± 0.03^{a}	4.15 ± 0.08^{c}	0.22 ± 0.01^{c}	0.11 ± 0.00^{b}	0.37 ± 0.01^{c}	0.08 ± 0.00^{a}	0.05 ± 0.00^{b}
100℃	3.15 ± 0.10^{a}	0.46 ± 0.02^{b}	0.36 ± 0.02^{b}	0.47 ± 0.02^{c}	1.86 ± 0.10^{b}	3.04 ± 0.07^{b}	6.65 ± 0.08^{b}	0.25 ± 0.01^{b}	0.11 ± 0.00^{b}	0.50 ± 0.02^{b}	0.08 ± 0.00^{a}	0.05 ± 0.00^{ab}
120℃	2.84 ± 0.11^{bc}	0.67 ± 0.04^{a}	0.40 ± 0.01^{a}	0.28 ± 0.01^{d}	1.61 ± 0.04^{c}	2.86 ± 0.11^{b}	10.05 ± 0.12^{a}	0.42 ± 0.02^{a}	0.14 ± 0.00^{a}	1.40 ± 0.04^{a}	0.08 ± 0.01^{a}	0.06 ± 0.00^{a}

Three measurements ± SD. C = Control (non-thermal treatment), DAF-15 = 15 Days after flowering, DAF-30 = 30 Days after flowering, DAF-45 = 45 Days after flowering, F_{IMPs} = Fluorescence of intermediate Maillard reaction products, F_{Tryp} = Fluorescence of free tryptophan, FI = the ration of F_{IMPs} and F_{Tryp}, MI = Melanoidin index, The same letters are not significantly different at $p \leq 0.05$ as determined by Duncan's multiple tests.

(Table S3). The MD exhibited a highly negative significant correlation with the FPC, EPC, GPC, BPC, and TPC ($r = -0.658$, $r = -0.819$, $r = -0.653$, $r = -0.880$, $r = -0.948$, $p \leq 0.01$). The FPC, GPC and IBPC were significantly related with the RT ($r = 0.374$, $p \leq 0.05$; $r = -0.479$, $p \leq 0.01$; $r = 0.339$, $p \leq 0.05$, respectively). However, the roasting treatment had a complex effect on the contents of the four forms of phenolic compounds because of the structures of these compounds, as shown in Table 2. The EPC and TPC were not significantly correlated with the RT ($p \geq 0.05$).

Type and content of phenolic acids in the four forms of phenolic compounds

Table S2 shows the type and content of different phenolic acids in the four forms of phenolic compounds determined in this study. Eight phenolic acids were detected in the free phenolic compounds in the DAF-15 wheat flours. Six phenolic acids (gallic acid, protocatechuic acid, vanillic acid, syringic acid, *p*-coumaric acid, and ferulic acid) were detected in the free phenolic compounds in the DAF-30 wheat flours. Four phenolic acids (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) were detected in the free phenolic compounds in the DAF-45 wheat flours. Six phenolic acids (gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and ferulic acid) were detected in the esterified phenolic compounds in the DAF-15 wheat flours. Five phenolic acids (protocatechuic acid, syringic acid, syringic acid, *p*-coumaric acid, and ferulic acid) were detected in the esterified phenolic compound in the DAF-30 wheat flours. Four phenolic acids (protocatechuic acid, syringic acid, syringic acid, and ferulic acid) were detected in the esterified phenolic compounds in the DAF-45 wheat flours. Six phenolic acids (protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, and ferulic acid) were detected in the glycosylated phenolic compound in the DAF-15 wheat flours. Four phenolic acids (protocatechuic acid, syringic acid, *p*-coumaric acid, and ferulic acid) were detected in the glycosylated phenolic compound in the DAF-30 wheat flours. Five phenolic acids (protocatechuic acid, 4-hydroxybenzoic acid, syringic acid, *p*-coumaric acid, and ferulic acid) were detected in the glycosylated phenolic compound in the DAF-45 wheat flours. The results showed that there were abundant phenolic acids in the free, esterified, and glycosylated forms in the DAF-15 wheat flours. Eight phenolic acids were detected in the insoluble-bound phenolic compounds in the DAF-15, DAF-30, and DAF-45 wheat flours. The F values for the eight phenolic acids in four forms showed that these contents highly and significantly varied with the MD and RT ($p \leq 0.05$), expect for ferulic acid in insoluble-bound form with RT ($p \geq 0.05$), as shown in Table S4. Table S5 shows that the major phenolic acids, such as syringic acid and ferulic acid, in FPA in the wheat flours were susceptible to heat. 4-Hydroxybenzoic acid in EPA in the wheat flours was destroyed upon roasting in the present study. Roasting

Table 2
Pearson correlation coefficients between the various properties of roasted and unroasted wheat flour.

	Pro	FPC	EPC	GPC	IBPC	TPC	(FEG)PC	EEPC	FPA	EPA	GPA	IBPA
MD	-0.623**	-0.658**	-0.819**	-0.653**	-0.880**	-0.948**	-0.810**	-0.742**	-0.793**	-0.689**	-0.721**	-0.946**
RT	-0.421*	0.374*	-0.046	-0.479**	0.339*	0.079	-0.195	0.337*	-0.077	-0.438**	0.233	-0.049
Pro		0.051	0.422*	0.392*	0.371*	0.413*	0.364*	0.102	0.248	0.514**	0.115	0.642**
FPC			0.784**	0.334*	0.744**	0.815**	0.708**	0.963**	0.604**	0.342*	0.904**	0.597**
EPC				0.695**	0.676**	0.897**	0.919**	0.863**	0.629**	0.624**	0.805**	0.744**
GPC					0.307	0.677**	0.895**	0.449**	0.629**	0.748**	0.509**	0.626**
IBPC						0.889**	0.586**	0.797**	0.733**	0.481**	0.729**	0.808**
TPC							0.892**	0.889**	0.822**	0.677**	0.855**	0.878**
(FEG)PC								0.787**	0.731**	0.723**	0.793**	0.756**
EEPC									0.676**	0.437**	0.915**	0.675**
FPA										0.787**	0.724**	0.698**
EPA											0.495**	0.600**
GPA												0.610**
IBPA												
TPA												
F*IMPs*												
F*Tryp*												
FI												
MI												
FDSA												
EDSA												
GDSA												
IBDSA												
TDSA												
(FEG)DSA												

MD = Mature degree, RT = Roasting temperature, Pro = Protein content, PC = phenolic content, FPC = Free PC, EPC = Esterified PC, GPC = Glycosylated PC, IBPC = Insoluble-bound PC, TPC = Sum of (FPC + EPC + GPC + IBPC), (FEG)PC = Sum of (FPC + EPC + GPC), EEPC = Ethanol extraction PC, PA = phenolic acids, FPA = Free PA, EPA = Esterified PA, GPA = Glycosylated PA, IBPA = Insoluble-bound PA, TPA = Sum of (FPA + EPA + GPA + IBPA), F_{IMPs} = Fluorescence of intermediate Maillard reaction products, F_{Tryp} = Fluorescence of free tryptophan, FI = the ration of F_{IMPs} and F_{Tryp}, MI = Melanoidin index, DSA = DPPH scavenging activity, FDSA = Free phenolic DSA, EDSA = Esterified phenolic DSA, GDSA = Glycosylated phenolic DSA, IBDSA = Insoluble-bound phenolic DSA, TDSA = Sum of (FDSA + EDSA + GDSA + IBDSA), (FEG)DSA = Sum of (FDSA + EDSA + GDSA), EEDSA = Ethanol extraction DSA.
*$p \leq 0.05$.
**$p \leq 0.01$.

the wheat flours caused the reduction of gallic acid, *p*-coumaric acid, and ferulic acid in GPA, in contrast to syringic acid. Gallic acid and caffeic acid in IBPA in wheat flours were significantly negatively correlated with the RT ($p \leq 0.01$). Ferulic acid was predominant in IBPA in the wheat flours with three degrees of maturity and was thermostable to heat because of being an insoluble-bound form (Table S2, Table S5). Ferulic acid was also found to be the main phenolic acid in the wheat flours with three maturities (Table S2).

The content of insoluble-bound ferulic acid was 62.45 mg/100 g DW for the DAF-45 wheat flours, which was lower than that of the DAF-15 (251.25 mg/100 g DW) and DAF-30 (158.28 mg/100 g DW) wheat flours. The TPA ranged from 307.34 to 338.32 mg/100 g DW for the roasted DAF-15 wheat flours. The TPA ranged from 139.60 to 169.55 mg/100 g DW for the roasted DAF-30 wheat flours. The TPA ranged from 44.75 to 74.23 mg/100 g DW for the roasted DAF-45 wheat flours. The TPA content in the wheat flours was highest for the DAF-15 flours and lowest for the DAF-45 flours.

Table 2 shows that FPA, EPA, GPA, IBPA and TPA were highly negatively correlated with the MD (r = −0.793, −0.689, −0.721, −0.946, and −0.966, respectively; $p \leq 0.01$). The contents of FPA, EPA, GPA, IBPA, and TPA were significantly positively correlated with the phenolic contents (FPC, EPC, GPC, IBPC and TPC) (Table 2). These results indicate that the four forms of phenolic compounds contribute significantly to the reducing capacity of wheat flours.

The EPA content was highly negatively correlated with the RT (r = −0.438; $p \leq 0.01$). The contents of the other three phenolic acid forms (FPA, GPA and IBPA) were not significantly correlated with the RT.

Phenolic content of the ethanol extract

Fig. 1(b) shows the reducing capacity of the ethanol extracts: with increasing roasting temperature, the EEPC of all the roasted samples increased compared to that of the unroasted samples, whereas the (FEG)PC generally decreased slightly after roasting. The EEPC of the DAF-15 wheat flours dramatically increased after roasting treatment, ranging from 335.78 to 898.31 mg GAE/100 g DW, whereas the (FEG)PC ranged from 209.45 to 298.23 mg GAE/100 g DW. The EEPC of the DAF-30 and DAF-45 wheat flours increased slightly after roasting. The F values for the EEPC showed that this content highly and significantly varied with the MD and RT ($p \leq 0.01$), as shown in Table S3. The EEPC was highly negatively correlated with the MD (r = −0.780; $p \leq 0.01$) and positively correlated with the RT (r = 0.418; $p \leq 0.05$). These results showed that the (FEG)PC was not only the main contributor to the EEPC, especially for the roasted DAF-15 wheat flours. The Maillard reaction products in the roasted wheat flours were further studied, as detailed below.

Maillard reaction

Protein content

Protein is an important component of wheat and a major index for the Maillard reaction. Fig. 2 shows that the DAF-30 flours had the highest protein content (18.47 %) among the unroasted wheat flours. By comparison, the protein content of the DAF-15 and DAF-45 wheat flours were 2.84 % and 7.61 % lower, respectively. In our study, roasting treatment significantly reduced the protein content in all flours with different maturities (Fig. 2). The protein in the DAF-30 wheat flours roasted at 80 °C was more sensitive to thermal treatment than the other two groups, where the protein content decreased by 16.72 % compared to a 6.65 % decrease for the DAF-15 wheat flours. There was no significant change in the protein content of the DAF-45 wheat flours upon thermal treatment. Roasting the DAF-15 and DAF-30 wheat flours at 120 °C reduced the protein content by 2.56 % and 6.10 %, respectively. Roasting the DAF-45 wheat flours at 120 °C caused the protein content to drop by 1.44 %. The present results showed that roasting at high

TPA	F*IMPs*	F*Tryp*	FI	MI	FDSA	EDSA	GDSA	IBDSA	TDSA	(FEG)DSA	EEDSA
-0.966**	-0.873**	0.977**	-0.760**	-0.610**	-0.722**	-0.787**	-0.915**	-0.980**	-0.984**	-0.962**	-0.852**
0.006	0.113	-0.049	0.338*	0.386*	0.333*	-0.101	0.051	0.052	0.060	0.074	0.358*
0.560**	0.216	-0.598**	0.103	0.026	0.190	0.486**	0.414*	0.589**	0.544**	0.444**	0.228
0.716**	0.781**	-0.714**	0.956**	0.974**	0.903**	0.297	0.562**	0.680**	0.675**	0.645**	0.894**
0.817**	0.802**	-0.841**	0.852**	0.788**	0.798**	0.528**	0.682**	0.826**	0.813**	0.763**	0.815**
0.654**	0.656**	-0.627**	0.457**	0.273	0.302	0.568**	0.708**	0.629**	0.649**	0.666**	0.419*
0.852**	0.838**	-0.867**	0.820**	0.720**	0.803**	0.661**	0.790**	0.889**	0.892**	0.870**	0.923**
0.943**	0.945**	-0.945**	0.902**	0.776**	0.822**	0.693**	0.882**	0.950**	0.956**	0.938**	0.930**
0.828**	0.845**	-0.816**	0.787**	0.663**	0.661**	0.574**	0.780**	0.804**	0.811**	0.800**	0.736**
0.786**	0.861**	-0.778**	0.989**	0.967**	0.920**	0.433**	0.647**	0.761**	0.763**	0.744**	0.940**
0.774**	0.902**	-0.750**	0.706**	0.519**	0.542**	0.731**	0.853**	0.783**	0.820**	0.863**	0.742**
0.637**	0.672**	-0.604**	0.454**	0.269	0.258	0.761**	0.679**	0.731**	0.729**	0.703**	0.466**
0.755**	0.798**	-0.743**	0.881**	0.893**	0.839**	0.454**	0.652**	0.732**	0.740**	0.733**	0.836**
0.980**	0.793**	-0.927**	0.701**	0.550**	0.677**	0.700**	0.855**	0.914**	0.915**	0.888**	0.778**
	0.863**	-0.953**	0.799**	0.673**	0.762**	0.704**	0.878**	0.943**	0.947**	0.924**	0.853**
		-0.859**	0.897**	0.718**	0.741**	0.701**	0.901**	0.875**	0.904**	0.930**	0.916**
			-0.796**	-0.667**	-0.770**	-0.662**	-0.888**	-0.944**	-0.945**	-0.917**	-0.878**
				0.943**	0.915**	0.455**	0.685**	0.778**	0.784**	0.771**	0.959**
					0.928**	0.256	0.469**	0.633**	0.623**	0.586**	0.870**
						0.345*	0.553**	0.711**	0.708**	0.681**	0.885**
							0.766**	0.802**	0.829**	0.854**	0.558**
								0.895**	0.928**	0.960**	0.785**
									0.994**	0.954**	0.865**
										0.980**	0.870**
											0.854**

temperatures reduced the protein content of the wheat flours with three maturities. This conclusion is supported by the results presented in Table 3, which show that the protein content is significantly negatively correlated with the RT ($r = -0.364$; $p \leq 0.01$).

Maillard reaction products (MRPs)

Table 1 shows the major MRPs produced during roasting treatment, as well as the fluorescence of the intermediate MRPs (F_{IMPs}) and free tryptophan (F_{Tryp}), FI (the ratio of F_{IMPs} to F_{Tryp}) and MI. Roasting caused the fluorescence of the intermediate MRPs in the DAF-15 wheat flours to increase from 2.80×10^7 to 3.15×10^7 AFU. The fluorescence absorbance was higher for the roasted groups than the unroasted groups ($p \leq 0.05$) (Table 1). Comparing the results for the MRPs for flours with different degrees of maturity and roasting treatment conditions shows that the fluorescence absorbance of the intermediate MRPs in the DAF-15 wheat flours was 10 times higher than for the wheat flours at the other two maturities, indicating that the intermediate MRPs formed most easily in the most immature wheat flours. The F_{IMPs} and MD exhibited a significantly negative correlation ($r = -0.877$; $p \leq 0.01$). The F_{IMPs} and RT exhibited a positive correlation ($r = 0.114$; $p \geq 0.05$) (Table 2), which suggests that IMP production increased with the RT. The F_{Tryp} decreased with increasing RT ($r = -0.049$; $p \geq 0.05$), especially for the DAF-15 wheat flours, which implied that the protein was degraded due to the high RT ($r = -0.598$; $p \leq 0.01$) (Table 2). The F_{IMPs} was significantly positively correlated with the FPC, EPC, GPC, IBPC and TPC ($r = 0.781$, 0.802, 0.656, 0.837 and 0.945, respectively; $p \leq 0.01$). These results show that the intermediate MRPs contributed to the TPC.

The FI was positively correlated with the RT ($r = 0.268$; $p \geq 0.05$). The FI of the intermediate MRPs increased after the wheat flours were subjected to thermal treatment. The FI was negatively correlated with the MD ($r = -0.777$; $p \leq 0.01$). In this study, the FI was positively correlated with the FPC, EPC, GPC, IBPC and TPC ($r = 0.956$, 0.852, 0.457, 0.820 and 902, respectively; $p \leq 0.01$). This result showed that the intermediate MRPs contributed to the reducing capacity of the roasted wheat flours.

Table 1 shows that the MI increased with the RT. The MI of the DAF-45 wheat flours ranged from 0.05 to 0.06 AU. By comparison, the MI of the DAF-15 wheat flours ranged from 0.18 to 1.40 AU. The MI was negatively correlated with the MD ($r = -0.610$; $p \leq 0.01$). The MI varied ($p \leq 0.05$) among the wheat flours with three maturities roasted at 120 °C (Table 1). The MI was significantly positively correlated with the FPC, EPC, IBPC and TPC ($r = 0.974$, 0.788, 0.720 and 0.776, respectively; $p \leq 0.01$).

Antioxidant activity

Although the aforementioned results showed the effect of roasting treatment on the phenolic content and Maillard reaction products, the effect of roasting treatment on the free radical scavenging activity of the wheat flours of three maturities remained to be determined. Thus, an in-depth evaluation of the scavenging activity of the four forms of phenolic compounds and ethanol extracts of roasted wheat flours was carried out.

DPPH scavenging activity of the four forms of phenolic compounds

Fig. 3(a) is a heatmap showing the activities of the four forms of phenolic compounds obtained from different roasting treatments on the DPPH free radical scavenging. The insoluble-bound phenolic compounds showed the highest radical scavenging activity, which was strongest for the DAF-15 wheat flours. These results suggested that the insoluble-bound phenolic extracts were the predominant antioxidant in the wheat flours with three maturities, in agreement with the IBPC results. The four forms of phenolic compounds in the extracts of the DAF-15 wheat flours showed higher free radical scavenging activity than the wheat flours with the other two maturities. Table 2 shows that the radical scavenging activity of the free phenolic compounds was positively correlated with the RT ($r = 0.333$; $p \leq 0.05$), whereas the radical scavenging activity of the other three forms of phenolic compounds was not correlated with the RT. An analysis of the data also revealed that the TDSA was significantly correlated with the FPC, EPC, GPC, IBPC and TPC ($r = 0.675$, 0.813, 0.649, 0.892 and 0.956, respectively; $p \leq 0.01$).

DPPH scavenging activity of the ethanol extracts

Fig. 3(b) shows that the EEDSA was higher than the (FEG)DSA in all the roasted samples. The EEDSA was highest (278.73 μmol TE/100 g DW) in the DAF-15 wheat flours at roasted at 120 °C and lowest (80.80 μmol TE/100 g DW) in the unroasted DAF-45 wheat flours. The EEDSA was significantly positively correlated with the RT ($r = 0.358$; $p \leq 0.05$), whereas the (FEG)DSA was not correlated with the RT ($r = 0.074$; $p \geq$

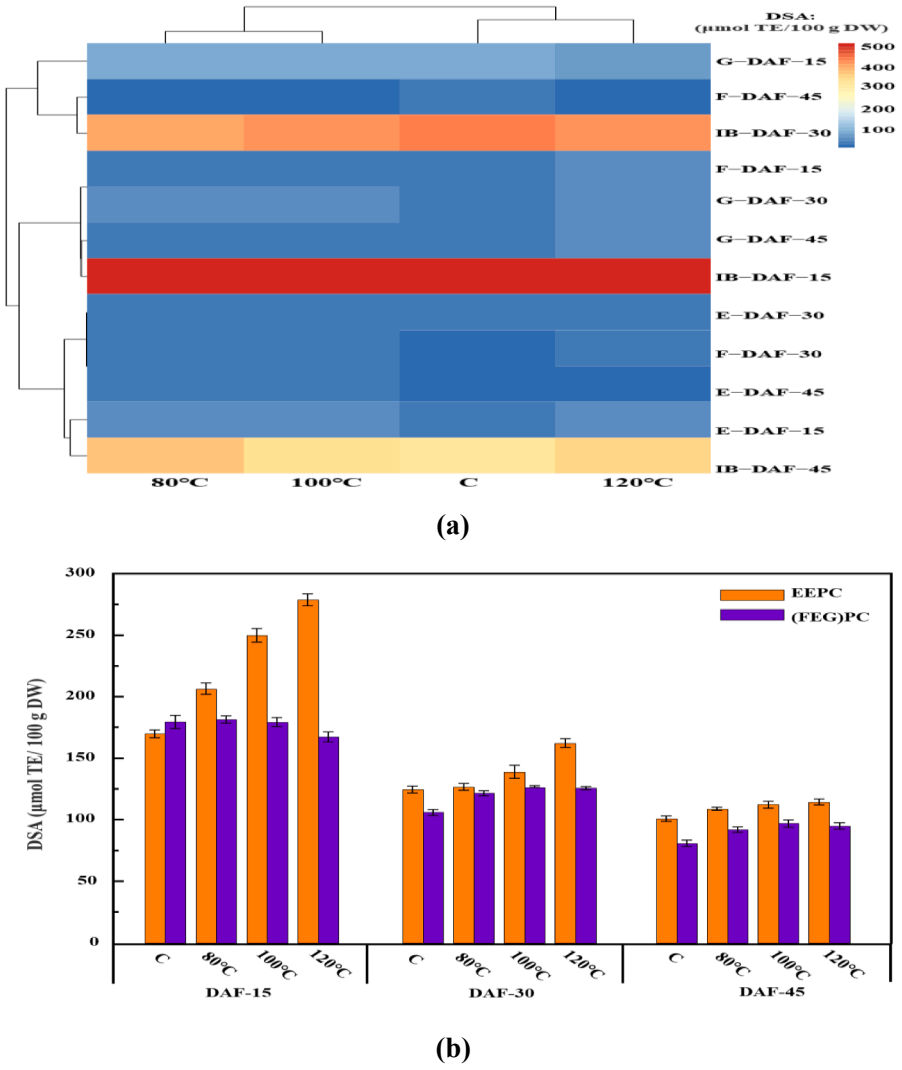

Fig. 3. Heatmap displaying DPPH scavenging activity of unroasted and roasted wheat flours (a), EEDSA and (FEG)DSA in unroasted and roasted wheat flours (b).

0.05) and remained relatively constant in wheat flours with different RT. The EEDSA was positively correlated with the FI and MI ($r = 0.959$, $r = 0.870$; $p \leq 0.01$, respectively).

Principal component analysis of various parameters

Principal component analysis (PCA) was used to identify correlations among all the measured elements in the roasted and unroasted wheat flours of different maturities, as shown in Table S6. A total variability of 74.14 % and 10.91 % was determined from the contributions of Principal Component 1 (PC1) and Principal Component 2 (PC2), respectively. Fig. 4(a) is a plot between the first two principal component scores showing the effects of the roasting treatment on the flours with three maturities: the three distinct groups are Group 1 (DAF-15), Group 2 (DAF-30) and Group 3 (DAF-45). Fig. 4(b) is a plot between the first two principal component scores, where the following three independent groups represent the effect of the RT on the wheat flours with different maturities: Group 1 (control), Group 2 (80 °C), Group 3 (100 °C) and Group 4 (120 °C). A correlation analysis showed that the F_{Tryp} was typically negatively correlated with the F_{IMPS} and all other indices, as shown in Fig. 4(c). These results suggested that both the MD and RT significantly affected the production of antioxidant compounds and MRPs during the roasting treatment. A correlation analysis showed that the F_{Tryp} was typically negatively correlated with the F_{IMPS} and all other indices, as shown in Fig. 4(c). Protein was a major resource for MRPs. The results showed that the protein content was negatively correlated with the MI, FDSA, FPC, EEPC and FI. However, the protein content was more closely related to the GPC and EPA than to the other forms of phenolic compounds. The TPC was more closely related to the IBPC and EPC than the GPC and FPC, suggesting that the IBPC and EPC were the predominant reasons for the change in the TPC of the wheat flours upon roasting treatment. The FI was positively correlated with the MI, indicating that the Maillard reaction resulted in a reaction between the decomposed protein and reducing sugars, increasing melanoidin formation.

Discussion

Previous studies on immature wheat flours have been focused on the free or bound forms, and there is a lack of data for the esterified and glycosylated forms. Kim (2016) reported a total phenolic content (TPC) of immature wheat flours of 5.32 mg GAE/g. A similar TPC was measured in our study (504.80 mg GAE/100 g DW for unroasted DAF-15 wheat flours). Santos et al. (2019) reported that the TPC ranged from 179.8 to 408.0 mg GAE/100 g DW in different genotypes of immature wheat and decreased in all genotypes during wheat grain development. This trend was also observed in our results. Most previous studies have shown that IBPC (60–78.4 %) was the dominant content of mature

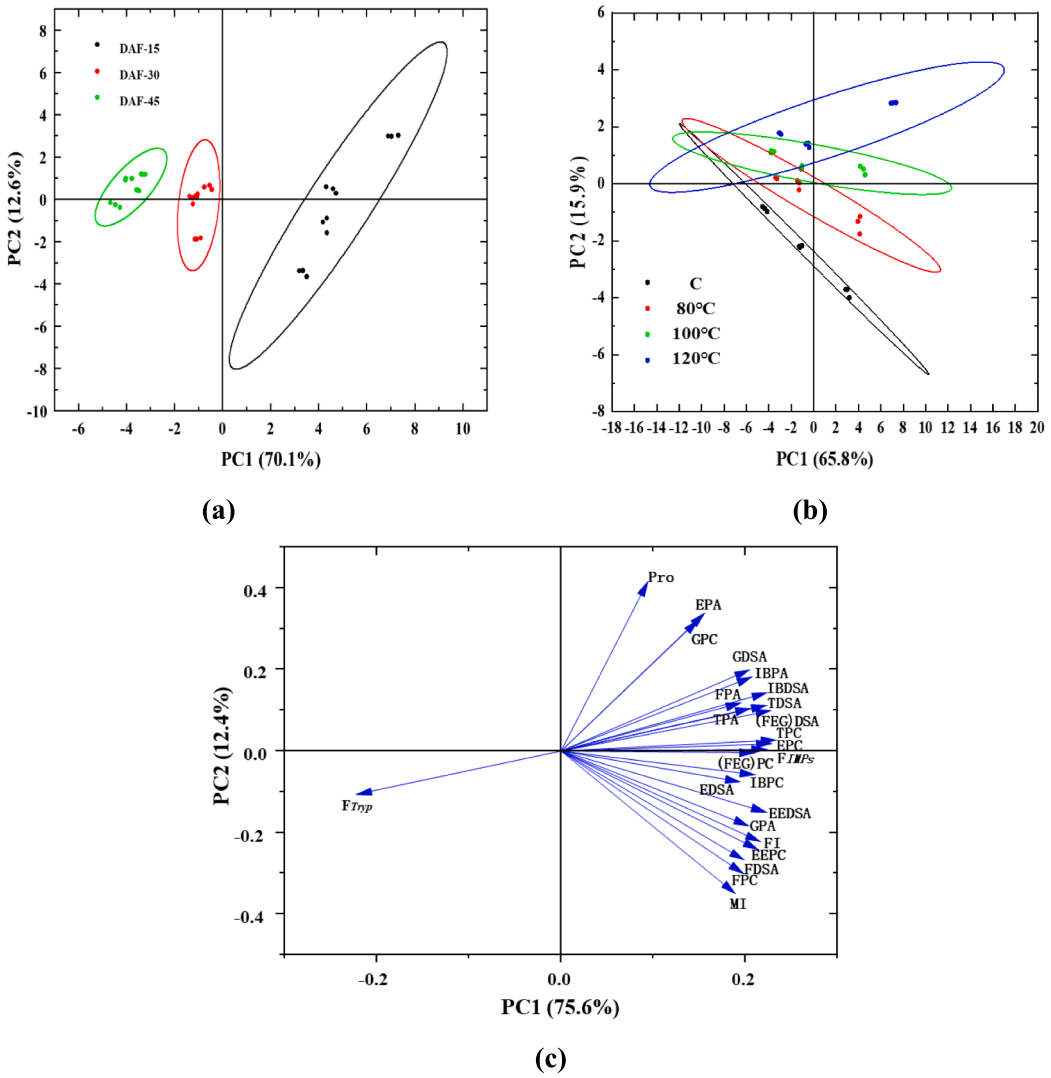

Fig. 4. Principal component analysis (PCA), score plot: mature degree (a), roasting temperature (b), and loading plots (c) describing relationship among different properties of wheat flours with three maturities.

wheat flours (Chen et al., 2017; Santos et al., 2022). These results corroborated our data. Zhang et al. (2021) found that GPC was the dominant content (128.71 μg GAE/g) in C. oleifera oil. However, IBPC was the most abundant content rather than GPC in the wheat flours with three maturities.

Zielinski et al. (2009) reported a decrease in the content of extractable phenolic compounds of buckwheat roasted at 160 °C compared to unroasted buckwheat. In the present study, it showed that roasting treatment dramatically reduced the GPC of the wheat flours with three maturities, which demonstrated the decrease in the content of the extractable phenolic compounds resulted from the decrease in the GPC content. A 59 % phenolic content has been reported for the bound form of whole wheat grain (Liyana-Pathirana and Shahidi, 2006). Major in bound form was also showed in present study. It showed that roasting treatment dramatically increased the IBPC of the wheat flours with three maturities, which suggested that thermal processing promoted the release of insoluble phenolic compounds. A similar effect was reported in a previous study (Tian et al., 2021) and was explained in terms of the transformation of phenolic compounds bound to the matrix into extractable phenolic compounds by alkaline hydrolysis after thermal processing. The increases in the contents of free phenolic, esterified phenolic and glycosylated phenolic compounds all resulted from the dissociation of these compounds from the food matrix by food processing, where these compounds play an essential role in promoting human health, such as by metabolism in alimentary canals and notable biological activity (Shahidi and Yeo, 2016). Thermal treatment increases the phenolic content of the grain matrix (Okarter, 2012). In the present study, roasting increased the total phenolic content or changed the contents of the four forms of phenolic compounds in wheat flours (free, esterified, glycosylated, and insoluble-bound), especially the DAF-15 flours. The aforementioned effects positively or negatively alter the contents of phenolic compounds, probably affecting their bioactivity and therefore, impact on human health (Abdel-Aal and Rabalski, 2013).

For phenolic acids in wheat flours, ferulic acid was abound in flour with three degrees. A similar previous study also showed that ferulic acid was the most abundant phenolic acid in 22 genotypes of durum wheat, with contents ranging from 13.28 μg/g to 324.69 μg/g (Di Loreto et al., 2018). The phenolic acid content in flours was reduced during wheat grains development in the present results. This correlation resulted from the rapid formation of amino acids, such as phenylalanine and tyrosine, during the initial stages of wheat seed development that were precursors for the formation of phenolic compounds (Zhen et al., 2016). The FPA, EPA, GPA, and IBPA contents of the DAF-15 wheat flours were considerably higher than of the DAF-30 and DAF-45 flours, which was in line with the trends for the FPC, EPC, GPC, and IBPC of the wheat flours. IBPA was the most abundant phenolic form in the wheat

flours with three maturities, and similar results were obtained for the IBPC. The TPA content increased with the roasting temperature. The release of ferulic acid from the insoluble-bound form in wheat flours made up for the loss of TPA due to the roasting treatment. The results showed that EPA was the most sensitive to roasting heat among the four phenolic acid forms. In previous studies, the thermal deterioration of phenolic acids was found to depend on the type and number of functional groups, particularly methoxyl and hydroxyl groups, in the aromatic ring (Carciochi et al., 2016). In the present study, the EPA content was found to be significantly reduced after thermal treatment, whereas the contents of the other three forms of phenolic compounds were sensitive to, but not negatively correlated, with heating ($p \geq 0.05$). Therefore, we proposed that thermal treatment broke down cell walls to release insoluble-bound phenolic acids with intact molecular structures. By contrast, phenolic acids in the esterified form were more sensitive to heat and decomposed during the roasting treatment.

The protein content initially increases and then decreases at the last stage of wheat grains development. Similar results were found that the protein content of Immature Wheat Grain-2 (17.84 %, DAF-36) was found to be higher than that of Immature Wheat Grain-1 (13.53 %, DAF-26) in a study by Aktaş et al. (2015) on the physical and chemical properties of immature wheat flours. The reduction in the protein content of the flours with three degrees after roasting attributed to the denaturation and associated with the involvement of amino acids in Maillard reactions during the thermal process (Bekedam et al., 2008). Roasting treatment produces reaction between free amino group of proteins and reducing sugar forming brown polymers called melanoidins. Meanwhile FI was increased with roaring temperature improve in present study, especially for wheat flour at DAF-15. It is in agreement with those of previous studies, in which roasting buckwheat groat at 160 °C for 50 min increased the FI (Wronkowska et al., 2016; Zielinski et al., 2009). The presence of melanoidins in wheat flour after roasting can increase the MI: Michalska et al. (2008) found that the MI was higher in the bread crust than the crumb of whole rye. These results are supported by those from a previous study, in which roasting buckwheat groat at 160 °C for 50 min increased the MI index from 0.5 to 0.7 AU (Wronkowska et al., 2016). The results of this study suggested that roasting treatment increased the formation of MRPs in wheat flours, which contributed to the reducing capacity of the flours. The MRPs provided a reaction substrate, such that roasting produced the highest melanoidin levels in the most immature flour. MRPs are produced by the rapid formation of proteins and monosaccharides during the initial stages of the Maillard reaction (Iametti et al., 2006; Verspreet et al., 2013; Zhen et al., 2016).

Similar results were obtained by Verardo et al. (2018) and Bai et al. (2021) for barley, where the antioxidant capacity was found to be positively correlated with the content of insoluble-bound phenolic compounds. Tian et al. (2021) also reported increased DPPH radical scavenging activity of both soluble and bound fractions after baking wheat bread. Free phenolics in buckwheat were found to possess the highest antioxidant activity among all forms of phenolic compounds, whereas the bound phenolics in Kainth fruit had higher antioxidant activities than other phenolic classes (Zhu et al., 2019; Lou et al., 2020). Insoluble-bound phenolic extracts were demonstrated to have the highest antioxidant properties because of being the major insoluble-bound form in the wheat flours with three maturities. In the present study, antioxidant activity of ethanol extraction had higher than that of extractable phenolic compounds. A similar result was also shown in reducing power of ethanol extraction and extractable phenolic compounds. These results demonstrated that the formation of MRPs also promoted the antioxidant activity of the wheat flours. MRPs possess electron donors and hydroxyl groups that can act as reducing agents by donating hydrogen atoms to quench free radicals (Vhangani and Van Wyk, 2016; Wang et al., 2013), thereby increasing the antioxidant activity of wheat flours. The results of the present study suggested that MRPs contribute to the antioxidant activity of wheat flours.

Conclusion

Roasting treatment increased the phenolic content and antioxidant activity of wheat flours, which promoted the formation of Maillard reaction products. The highest number of antioxidant and reducing compounds were produced in DAF-15 wheat flours after roasting treatment, reaching a maximum at a roasting temperature of 120 °C in the present study. In wheat flours with three maturities, phenolic compounds were predominantly present in an insoluble-bound form, followed by glycosylated, esterified and free forms. The results of this study demonstrate the impact of the maturity degree and roasting temperature on bioactive compounds in wheat flours.

CRediT authorship contribution statement

Chao Zhang: Conceptualization, Data curation, Writing – original draft, Software, Writing – review & editing. **Xiaoxue Guo:** Visualization, Formal analysis. **Ruijia Guo:** Formal analysis, Investigation. **Lin Zhu:** Conceptulization, Methodology. **Xinrong Qiu:** Visualization, Validation. **Xiaoxhan Yu:** Writing – review & editing. **Jun Chai:** Writing – review & editing. **Chunhe Gu:** Visualization, Funding aquisition, Supervision, Writing – review & editing. **Zhen Feng:** Conceptualization, Funding aquisition, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100548.

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