# The impact of cooking method on the phenolic composition, total antioxidant activity and starch digestibility of rice (*Oryza sativa* L.)

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## Abstract

This study investigated changes in the phenolic composition, total antioxidant activity (TAA) and starch digestibility in white and brown rice due to three different cooking procedures, and subsequent reheating of cooked rice after storage. Among the analyzed samples, brown rice showed the highest TAA and phenolic content (622.5 mg/kg DW). All cooking methods resulted in significant decrease of phenolic content and TAA of rice ($p < 0.05$). The greatest loss was observed after processing in rice cooker, which reduced phenolic content of both brown and polished rice by ~30% and ABTS radical-scavenging activity by 20 and 28%, respectively. In general, the levels of polyphenols and TAA of cooked rice tended to further decline after storage and reheating, but to a much lesser extent when rice was prepared using microwaves. The application of in vitro digestion system disclosed that the microwave cooking resulted in the highest starch digestibility among cooking methods used.

## Practical applications

Rice is one of the most commonly consumed staple foods worldwide. Scientific and epidemiological studies have showed that their phytochemicals exhibit antioxidant, anti-inflammatory, antihypertensive and chemopreventive effects. Therefore, their high consumption, easy availability throughout the year and use as an additive to meat and high-fat foods may make rice, especially in the form of whole grains, potentially important chemopreventive component of the diet. The appropriate cooking procedure of rice is crucial for preservation of bioactive compounds as well as digestion of starch and thus duration of the glycemic response. Preferably, this study is focused on the evaluation of the effect of cooking methods on the health-related quality of rice. The results provide practical advice that the consumption of freshly cooked rice ensures its highest nutritional quality, while rice microwaving is recommended both when cooked rice will be reheated after storage (e.g., in restaurants) and accelerated starch digestion is in favor.

## 1 | INTRODUCTION

Cereals are estimated to provide about 42% of daily energy supply required by human organism. Globally, 19% of this caloric demand is covered by rice (*Oryza sativa* L.), which is a major foodstuff for more than half of the world's population (Setyaningsih, Saputro, Palma, & Barroso, 2015). With the expanding human population, especially in regions where rice is or has become recently a main staple food, also the demand for its increased production is expected. Such a continent is Africa, where rice consumption has been on increase for past three decades, because its preparation takes much less time than that of traditional African starch sources, for example, cassava roots or sorghum that additionally may contain residues of toxic cyanogenic components when not processed properly.

Currently, there are more than 120,000 rice varieties recognized worldwide, but their properties not always ensure economically sound quality and productivity (Wang et al., 2014). To ensure world food security, many companies work on creation of new hybrid strains that are bred to withstand pest, to resist unfavorable environmental conditions and to produce more grains (Zeigler, 2014). With the exception of rice genetically modified to synthesize β-carotene, known as "golden rice" that was developed to address vitamin A deficiencies, relatively

little attention is paid to the health promoting value of this crop. However, besides the contribution of rice to the total human calorie intake, as well as providing nutrients such as proteins (20–31 g/kg cooked rice; 65–75 g/kg raw rice) and mineral elements (P, K, Na, Ca, Mg, Fe, Zn, Cu, and Mn) (USDA National Nutrient Database; http://ndb.nal.usda.gov), rice contains also non-nutrients with proven benefits for human health (Deng et al., 2013). These are tryptophan derivatives (Setyaningsih et al., 2015), but in particular phenolic compounds whose most emphasized bioactivity is high antioxidant capacity and capability of scavenging free radicals (Qiu, Liu, & Beta, 2010). As a consequence, a number of studies on the determination of phenolic composition and total antioxidant activity (TAA) of rice were carried out (Walter et al., 2013; Zaupa, Calani, Del Rio, Brighenti, & Pellegrini, 2015; Zhang, Shao, Bao, & Beta, 2015; Zhou, Robards, Helliwell, & Blanchard, 2004).

Phenolic compounds that occur in rice grains include anthocyanins, flavan-3-ols, flavanols, phenolic acids and their aldehydes (Qiu et al., 2010; Setyaningsih et al., 2015; Zaupa et al., 2015). The most abundant are phenolic acids derived from hydroxybenzoic acid and hydroxycinnamic acid (Goufo & Trindade, 2014). Phenolic compounds display many beneficial effects on human health. Scientific and epidemiological studies of bioactive properties of whole grain rice and dietary rice bran showed that their phytochemicals exhibit antioxidant, anti-infladigesmmatory (Shao & Bao, 2015), antihypertensive (Massaretto, Madureira Alves, Mussi de Mira, Karaoglanovic Carmona, & Lanfer Marquez, 2011) and chemopreventive effects against several types of cancer, such as colon, breast, lung and liver cancer (Dipti et al., 2012; Henderson et al., 2012; Mannan, Sarker, Kabir, Rahman, & Alam, 2014). Moreover, the polyphenols distributed in bran layer of rice may reduce the risk of some chronic diseases including cardiovascular disorders, type II diabetes and obesity, as well as play an important role in the inhibition of cholesterol oxidation (Dipti et al., 2012; Shao & Bao, 2015).

Previous studies have shown that thermal treatment of cereals generally cause a significant reduction in the phenolic content and antioxidant capacity mainly due to their release from bound forms, degradation, polymerization, oxidation and conversion to Maillard reaction products (Massaretto et al., 2011). Considering the effect of rice cooking, a decrease of phenolic compounds in pigmented and non-pigmented rice was also observed, with a higher reduction in pigmented varieties (Finocchiaro et al., 2007). For example, Massaretto et al. (2011) reported a 50% loss of total phenolic content in red rice, whereas no significant effect was recorded in white one. A detrimental effect of thermal treatment was also noted on anthocyanins of black rice, especially in roasted or pressure cooked samples in which respectively 94 and 80% of total anthocyanins were degraded, for example, cyanidin-3-glucoside into protocatechuic acid (Hiemori, Koh, & Mitchell, 2009; Surh & Koh, 2014). Similarly, domestic cooking methods of different rice varieties caused a significant decrease in the level of phenolic compounds, with the highest lost (27–38%) in samples cooked by boiling (Zaupa et al., 2015). Apparently, much less is known about the influence of microwave cooking on rice polyphenols, although it has been shown that type of processing is less destructive for this type of phytochemicals (Piasek et al., 2011).

In recent years, the digestibility of starchy food products, such as rice, is widely studied due to its direct correlation with the rate and the duration of the glycemic response in humans. Previous reports indicated that to manage the diet for patients, raw or partially cooked starch could be considered as a low glycemic index (GI) food resource, as GI is easily changed by starch gelatinization (Singh, Dartois, & Kaur, 2010). Generally, rice is consumed after gelatinization by adding water during cooking process, which results in increased enzymatic hydrolysis and thus more efficient starch digestion. In contrast, it was observed that storage of cooked rice at the refrigerated temperatures may lead to a reduction in starch digestibility and estimated GI. However, there are only few papers dealing with the influence of thermal processing of the rice grains on their starch digestibility (Tamura, Singh, Kaur, & Ogawa, 2016). For example, Khatoon and Prakash (2006) who studied the nutritional quality of microwave and pressure cooked rice found that the amount of hydrolyzed starch did not differ significantly among cooking methods used, while Li, Han, Xu, Xiong, and Zhao (2014) observed higher starch digestibility of microwaved rice compared to that heated in conductive way.

As there are only few reports on the fate of individual phytochemicals in rice after the traditional thermal processing and hardly any for rice submitted to microwave cooking, the main purpose of this study was to determine the composition of phenolic compounds and TAA in unpolished and polished rice and to evaluate the effect of different domestic cooking methods (i.e., electro-domestic rice cooker [RC], microwave oven [MC], and boiling in water [BW]) as well as reheating of cooked rice after storage on the stability of these bioactive phytochemicals. To compare the starch digestibility of rice prepared by various cooking procedures, an in vitro gastro-intestinal digestion was also performed. To the best of our knowledge, this study is the first that systematically examines the relationship between culinary procedure and rice starch digestibility.

## 2 | MATERIALS AND METHODS

### 2.1 | Chemicals and biochemicals

The determination of TAA were carried out with the use of the following chemicals obtained from Sigma-Aldrich (Poznań, Poland): trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a standard antioxidant, radicals ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt), and DPPH (2,2-diphenyl-1-picrylhydrazyl), as well as Folin–Ciocalteu reagent (FCR) supplied by Merck (Darmstadt, Germany).

For chromatographic analyses, HPLC-grade methanol and formic acid from Merck (Darmstadt, Germany) were used. Water was purified using QPLUS185 system from EMD Millipore (Billerica, MA, USA). The quantification of polyphenols was based on standard curves generated for phenolic compounds (concentration range 0.05–10 mg/L) expected to occur in rice: caffeic acid (CAF), chlorogenic acid (CHL), isovanillic acid (IVA), *p*-coumaric acid (*p*-COU), *p*-hydroxybenzaldehyde (*p*-HB), and syringic acid (SYR) obtained from Sigma-Aldrich, ferulic acid (FER), *p*-hydroxybenzoic acid (*p*-HBA), protocatechuic acid (PRO),

protocatechuic aldehyde (PRA), sinapic acid (SIN), vanillic acid (VAA), vanillin (VAN) obtained from Fluka (Buchs, Switzerland), and isoferulic acid (IFA) purchased from Extrasynthese (Genay, France). Stock solutions of standards were prepared in aqueous methanol 50:50 (vol/vol) and stored in a freezer at −20 °C until use.

The digestibility of rice samples was examined with the aid of enzymes recommended by Minekus et al. (2014). Pepsin from porcine gastric mucosa (powder, ≥250 U/mg solid), pancreatin from porcine pancreas (8× USP), amyloglucosidase from *Aspergillus niger*, (powder, 70 U/mg), glucose oxidase/peroxidase reagent (assay kit GAGO20) were derived from Sigma-Aldrich. Hemoglobin from bovine blood, porcine bile extract, 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), *p*-toluenesulfonyl-L-arginine methyl ester (TAME), and inorganic salts of appropriate grade were also purchased from Sigma-Aldrich.

## 2.2 | Sample preparation

Two types of rice samples were used in this study: polished and unpolished rice. The samples of milled rice included basmati rice of Sun Clad brand (aromatic, white, long grain rice grown in India) and Thai jasmine rice of Golden Phoenix brand (aromatic, long grain rice from Thailand). The whole grain brown rice produced by Beta Food Company from Thailand was used as a sample of unpolished rice throughout this study. All rice samples were purchased in local grocery shops, Gdańsk, Poland. The uncooked rice samples (50 g) were powdered by laboratory mill for 1 min prior to extraction. The milling process was stopped after 30 s to avoid overheating. The fine rice powder was stored at −20 °C until analysis.

Samples of jasmine, basmati and brown rice grains for the study of the effect of thermal treatment were weighed 150, 150, and 100 g, respectively. They were washed on a sieve with tap water three times. Then purified water was added to rice with a ratio of rice and water 1:2 (wt/vol) and soaked for 10 min. Rice was cooked using three different cooking methods, that is, in RC for 21 min (jasmine and basmati) and 25 min (brown); in MC at power 450 W for 20 min (jasmine and basmati) and 25 min (brown); and BW for 24 min (jasmine and basmati) and 30 min (brown). The cooking time was set in accordance with the manufacturer's recommendations and to provide the same cooking degree of rice (fully cooked) using all these methods. Finally, cooked rice samples were left to cool down and then transferred to stripped plastic bags. All cooked rice samples were divided into three portions. First portion was kept at −20 °C until analysis. Second and third portion were stored in a refrigerator (4 °C) for 6 and 24 hr, respectively, prior to reheating process. The remaining samples were reheated in the microwave oven at 450 W for 3 min. All samples were frozen and freeze dried.

## 2.3 | Extraction of phenolic compounds

Each rice sample (10 g) was weighed and placed in an extraction tube. Then, 50 mL of solvent was added into the extraction vessel. The ultrasound-assisted extraction (UAE) was carried out under conditions optimized in the previous study (Saputro i.e. pers. comm., 1 September 2015, The GDCh Scientific Forum Chemistry [Chemie 2015], Dresden, Germany). The UAE conditions were as follows: solvent (80% methanol); amplitude (47%); cycle (0.4 s); pH of solvent (4.25); temperature (45 °C); solvent-to-sample ratio (5:1) (vol/wt); and extraction time (25 min). After extraction, the samples were centrifuged at 4,500 rpm, 4 °C for 6 min. The clear supernatant was collected and dried using vacuum rotary evaporator at 65 °C. The extract was then adjusted with methanol to 1 mL and centrifuged at 10,000 rpm, 4 °C for 5 min.

## 2.4 | Determination of phenolic compounds

The analyses of rice phenolic compounds were carried out using an Agilent 1200 series HPLC system coupled with diode array detector (DAD). The Kinetex XB-C18 100A column (150 mm × 4.6 mm, 5 μm particle size) was used for the separation of rice phenolic compounds. The mobile phase was a binary solvent system consisting of phase A (water with 4.8% formic acid) and phase B (methanol). The flow rate was 0.8 mL/min and the injection volume was 10 μL. Elution was conducted with a linear gradient program as follows: 5–50% B in 20 min, 50–100% B in 5 min, and held at 100% B for 5 min. Absorbance spectra were recorded between 190 and 700 nm every 2 s with a bandwidth of 4 nm, while the chromatograms were monitored at 254, 270, and 325 nm to monitor hydroxybenzoic acids, phenolic aldehydes, and hydroxycinnamic acids, respectively.

## 2.5 | Determination of total antioxidant activity by spectrophotometry

TAA of rice samples was determined by the spectrophotometric batch assays employing ABTS, DPPH, and FCR reagents as reported by Kusznierewicz et al. (2012). All determinations were carried out in 48-well plates, and absorbance was measured using a TECAN Infinite M200 spectrophotometer (Tecan Group Ltd., Männedorf, Switzerland). The series of dilutions of trolox standard solution was carried out to give a final concentration within the range of 0.1–1.0, 0.05–0.3, and 0.1–1.4 mg/mL for ABTS, DPPH and FCR assays, respectively. Subsequently, the appropriate volume (10, 30, and 100 μL) of rice extracts or trolox solutions were reacted with 1 mL of ABTS, DPPH or FCR solutions, and then the absorbance was measured after 10 min at 734, 515, and 750 nm, respectively. TAA of rice samples was expressed as trolox equivalents (mg TE/g DW) on the basis of the calibration curves.

## 2.6 | In vitro starch digestion protocol

A harmonized in vitro starch digestion of rice samples was carried out using a method reported by Minekus et al. (2014). The electrolyte stock solutions of simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were prepared as given in Table 1. The enzyme activity of pepsin is based on certificate of analysis from supplier (503 U/mg). The trypsin activity in pancreatin is based on TAME assay (5.06 U/mg) as described in Supporting Information. The amount of pancreatin added was based on the trypsin assay and amounted to 100 TAME units per mL of intestinal phase content.

**TABLE 1** Preparation of electrolyte stock solutions of digestion fluids: SSF, SGF, and SIF[a]

| Component | Stock concentration (mol/L) | SSF, pH 7 | | SGF, pH 3 | | SIF, pH 7 | |
|---|---|---|---|---|---|---|---|
| | | Volume of stock added to 0.4 L (mL)[b] | Final concentration (mmol/L) | Volume of stock added to 0.4 L (mL)[b] | Final concentration (mmol/L) | Volume of stock added to 0.4 L (mL)[b] | Final concentration (mmol/L) |
| KCl | 0.5 | 15.1 | 15.1 | 6.9 | 6.9 | 6.8 | 6.8 |
| $KH_2PO_4$ | 0.5 | 3.7 | 3.7 | 0.9 | 0.9 | 0.8 | 0.8 |
| $NaHCO_3$ | 1.0 | 6.8 | 13.6 | 12.5 | 25.0 | 42.5 | 85.0 |
| NaCl | 2.0 | – | – | 11.8 | 47.2 | 9.6 | 38.4 |
| $MgCl_2(H_2O)_6$ | 0.15 | 0.5 | 0.15 | 0.4 | 0.12 | 1.1 | 0.33 |
| $(NH_4)_2CO_3$ | 0.5 | 0.06 | 0.06 | 0.5 | 0.5 | – | – |
| HCl[c] | 6.0 | 0.09 | 1.1 | 1.3 | 15.6 | 0.7 | 8.4 |

Abbreviations: SGF = simulated gastric fluid; SIF = simulated intestinal fluid; SSG = simulated salivary fluid.
[a]The electrolyte stock solutions are 1.25× concentrated. The addition of enzymes, bile salts, $CaCl_2$, and water will result in the correct electrolyte concentration in the final digestion mixture.
[b]The amounts are calculated for a final volume of 500 mL for each digestion fluid.
[c]HCl was added for pH adjustment.

#### 2.6.1 | Oral phase

The cooked rice (20 g) was minced using an electric mincer. The sample was weighed (5 g) and transferred into falcon tube of 50 mL. Each preparation was carried out in duplicate, where one of the tubes was used for gastric phase analysis and another tube was used for intestinal phase analysis. The rice samples were diluted with 4 mL of SSF electrolyte stock solution, followed by 25 μL of 0.3 mol/L $CaCl_2$ and 0.975 mL of purified water. Then, the reaction mixture was incubated at 37 °C for 2 min.

#### 2.6.2 | Gastric phase

Preparation of the porcine pepsin solution was carried out by dissolving 600 mg of porcine pepsin in 7.55 mL of purified water to obtain activity of 2,000 U/mL in the final mixture of gastric digesta. After oral phase, duplicate digests were pooled and mixed with 8 mL of SGF electrolyte stock solution, 1 mL of porcine pepsin solution, 5 μL of 0.3 mol/L $CaCl_2$, 0.23 mL of 5 mol/L HCl, and 0.765 mL of purified water to reach pH 3 in the final solution. Then, the reaction mixtures were incubated at 37 °C for 2 hr. The gastric phase sampling was carried out after 30 and 120 min (G-30 and G-120) incubation in duplicate (2.5 mL each). Subsequently, sodium bicarbonate (1 mol/L) was added into samples in order to stop the reactions of gastric phase. The samples were frozen in liquid nitrogen and stored at −80 °C until further analyses.

#### 2.6.3 | Intestinal phase

Preparation of the pancreatin solution was carried out by dissolving 11.12 g of pancreatin in 70.31 mL of SIF electrolyte stock solution to reach activity of 100 U/mL TAME in the final mixture of intestinal digesta. Preparation of the bile solution was carried out by dissolving bile extract in purified water to reach the concentration of 10 mmol/L in the final mixture. Bile solution was stirred at 37 °C for 30 min. The liquid "food" digested during gastric step (20 mL) was mixed with 11 mL of SIF electrolyte stock solution followed by 5 mL of pancreatin solution, 2.44 mL of bile solution, 40 μL of 0.3 mol/L $CaCl_2$, 35 μL of 5 mol/L NaOH, and 1.486 mL of purified water to reach finally pH 7. Then, reaction mixtures were incubated at 37 °C for 2 hr. The sampling (2 mL each) was carried out after the incubation for 30, 60, 90, and 120 min (I-30, I-60, I-90, and I-120). Subsequently, 8 mL of ethanol was added into the samples and they were immediately frozen in liquid nitrogen in order to stop the enzymatic reactions of intestinal phase.

### 2.7 | Glucose assay

Immediately before the assay, the samples collected during the in vitro digestion were thawed and centrifuged at 5,000 rpm, 4 °C for 10 min. The gastric phase samples (G-30 and G-120), intestinal phase sample (I-30) and intestinal phase samples (I-60, I-90, and I-120) were diluted 5, 50, and 100 times, respectively with purified water. Then, the glucose released during digestion was determined based on the protocol from Sigma-Aldrich applied by other researchers (Tamura et al., 2016) and expressed as hydrolyzed starch during the amount of time elapsed. Detailed procedure of the glucose assay is outlined in Supporting Information.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Determination of phenolic compounds in raw and cooked rice

The validated UAE/HPLC-DAD method (Supporting Information Table S1) was applied to extract and analyze polished (jasmine and basmati) and unpolished (brown) rice grains. The phenolic profiles of raw and cooked rice samples were evaluated to establish the effect of cooking process on the stability of polyphenols.

Phenolic compounds in rice extract were tentatively identified by matching retention time of detected peaks with that of phenolic standards. Based on these parameters, rice extracts were found to contain

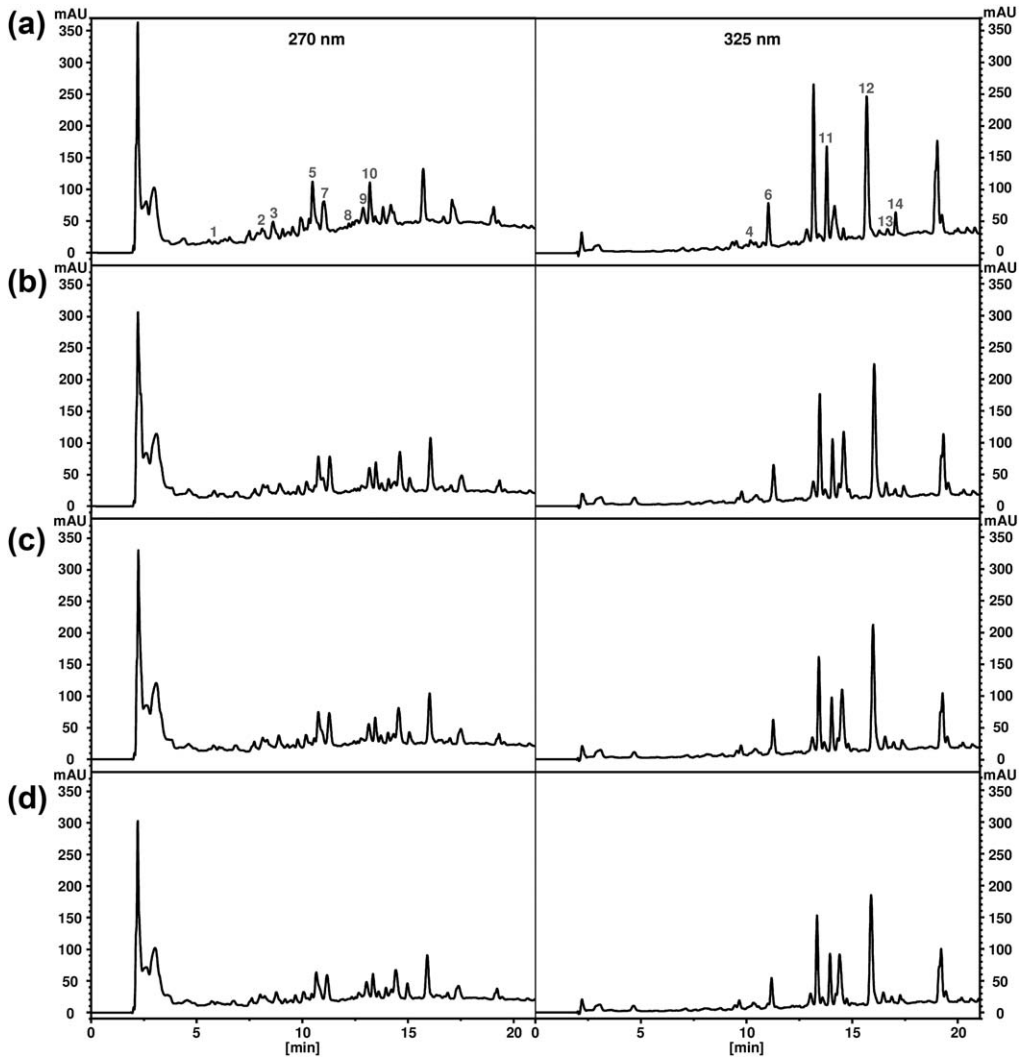


**FIGURE 1** The HPLC-DAD chromatograms obtained at the detection wavelength of 270 nm (left) and 325 nm (right) for brown rice in the form of (a) raw grains, (b) processed in electro-domestic rice cooker, and samples reheated after (c) 6 hr, and (d) 24 hr of storage. The numbers refer to the peak appearance in the chromatogram: (1) protocatechuic acid (PRO), (2) protocatechuic aldehyde (PRA), (3) *p*-hydroxybenzoic acid (*p*-HBA), (4) chlorogenic acid (CHL), (5) *p*-hydroxybenzaldehyde (*p*-HB), (6) caffeic acid (CAF), (7) vanillic acid (VAA), (8) isovanillic acid (IVA), (9) syringic acid (SYR), (10) vanillin (VAN), (11) *p*-coumaric acid (*p*-COU), (12) ferulic acid (FER), (13) sinapic acid (SIN), and (14) isoferulic acid (IFA)

14 phenolic compounds (Figure 1) that were identified and quantified. The achieved differences between retention times of phenolic standards and polyphenols detected in rice extracts were below 0.8%. Afterwards, the identity of the peaks was confirmed by comparing their spectra with spectral profile of individual standards, as well as by spiking method.

The levels of polyphenols in raw rice samples are shown in Table 2. The results disclosed that brown rice exhibited the highest total content of phenolic compounds (630.2 mg/kg DW), followed by jasmine (175.6 mg/kg DW), and basmati rice (171.3 mg/kg DW). These levels mainly corresponded to the amount of ferulic acid that contributed up to 33% of the total phenolic compounds in the grains. For that reason, ferulic acid was the most predominant compound in the rice grain samples evaluated. In several previous studies, ferulic acid was also found to be the most abundant phenolic acid determined in all varieties of rice with the concentration ranging from 3.4 to 84.0 and from 12.4 to 362.0 mg/kg DW in non-pigmented and pigmented rice grains, respectively (Shao & Bao, 2015; Tian, Nakamura, & Kayahara, 2004; Zaupa et al., 2015; Zhou et al., 2004).

As can be seen in Table 2, the second most abundant compounds were *p*-coumaric acid, *p*-hydroxybenzoic acid and syringic acid, constituting about 15–17% of the phenolic fraction depending on the variety of rice. The concentration of *p*-coumaric and ferulic acids found in the three rice varieties studied is in accordance with that reported for some varieties of brown (155.1–362.0 mg FER/kg DW; 22.0–152.0 mg *p*-COU/kg DW) and white rice (53.3–84.0 mg FER/kg DW; 3.6–11.0 mg *p*-COU/kg DW) (Tian et al., 2004; Zhou et al., 2004) However, Vichapong, Sookserm, Srijesdaruk, Swatsitang, and Srijaranai (2010), analyzing unpolished and polished Thai brown rice, observed a lower level of these compounds equal to 6.8–108.1 and 2.2–83.3 mg/

**TABLE 2** The content of individual phenolic compounds (mg/kg DW)[a] in rice cooked by different methods and reheated in microwave

| Rice variety | Cooking method | | Aldehydes[b] | | Hydroxybenzoic acids[b] | | | | | | Hydroxycinnamic acids[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | PRA | *p*-HB | VAN | PRO | *p*-HBA | VAA | IVA | SYR | CHL | CAF | *p*-COU | FER | SIN | IFA |
| Brown | Raw | | 12.66 ± 0.51 | 36.82 ± 3.26 | 57.84 ± 3.73 | 8.68 ± 0.52 | 29.49 ± 3.15 | 78.81 ± 1.42 | 4.60 ± 0.16 | 24.99 ± 1.79 | 17.75 ± 2.48 | 46.77 ± 0.08 | 91.32 ± 7.17 | 198.88 ± 7.51 | 7.85 ± 0.34 | 13.79 ± 1.76 |
| | BW | (1) | 11.01 ± 1.35 | 29.71 ± 2.97 | 37.37 ± 2.04 | 6.09 ± 0.51 | 24.25 ± 1.80 | 65.97 ± 1.92 | 3.82 ± 0.17 | 8.01 ± 0.72 | 15.46 ± 1.66 | 38.14 ± 4.88 | 73.02 ± 5.06 | 159.24 ± 0.41 | 6.89 ± 0.05 | 7.96 ± 0.31 |
| | | (2) | 7.77 ± 0.87 | 26.49 ± 1.32 | 31.50 ± 1.34 | 5.50 ± 0.02 | 23.89 ± 0.36 | 61.89 ± 1.74 | 3.70 ± 0.31 | 7.45 ± 0.23 | 10.46 ± 0.22 | 25.03 ± 0.47 | 65.40 ± 1.93 | 147.42 ± 4.79 | 6.33 ± 0.23 | 6.58 ± 0.46 |
| | | (3) | 4.41 ± 0.26 | 25.25 ± 1.51 | 30.48 ± 1.00 | 5.37 ± 0.30 | 22.76 ± 1.47 | 59.41 ± 1.63 | 3.09 ± 0.13 | 7.31 ± 0.47 | 9.82 ± 0.80 | 24.76 ± 1.12 | 61.87 ± 2.66 | 146.58 ± 1.67 | 6.18 ± 0.21 | 5.03 ± 0.32 |
| | RC | (1) | 8.61 ± 1.05 | 26.10 ± 1.55 | 36.45 ± 2.89 | 5.30 ± 0.09 | 21.49 ± 1.39 | 62.87 ± 3.58 | 2.99 ± 0.18 | 7.25 ± 0.76 | 13.42 ± 1.48 | 33.65 ± 2.44 | 64.90 ± 0.92 | 152.78 ± 7.24 | 6.59 ± 0.26 | 6.22 ± 1.11 |
| | | (2) | 8.07 ± 0.64 | 22.52 ± 1.46 | 28.95 ± 1.88 | 4.72 ± 0.11 | 20.10 ± 1.25 | 58.49 ± 1.58 | 2.77 ± 0.48 | 6.42 ± 0.97 | 11.41 ± 0.39 | 24.05 ± 1.92 | 64.47 ± 0.53 | 141.70 ± 4.06 | 5.87 ± 0.76 | 4.94 ± 0.25 |
| | | (3) | 7.32 ± 0.10 | 18.90 ± 0.21 | 25.22 ± 2.25 | 4.65 ± 0.01 | 18.87 ± 1.33 | 44.91 ± 2.55 | 2.78 ± 0.05 | 5.87 ± 0.49 | 8.00 ± 0.06 | 20.01 ± 0.91 | 48.46 ± 3.49 | 112.10 ± 5.77 | 5.23 ± 0.01 | 4.44 ± 0.42 |
| | MC | (1) | 10.43 ± 1.12 | 26.88 ± 1.83 | 35.14 ± 0.95 | 6.91 ± 0.47 | 22.48 ± 2.23 | 67.12 ± 6.25 | 3.48 ± 0.38 | 8.02 ± 1.28 | 12.95 ± 0.49 | 34.60 ± 3.12 | 66.25 ± 0.84 | 158.15 ± 0.62 | 6.86 ± 0.42 | 6.94 ± 0.51 |
| | | (2) | 8.85 ± 0.67 | 21.29 ± 0.74 | 32.80 ± 0.44 | 5.45 ± 0.59 | 22.19 ± 1.21 | 60.76 ± 2.52 | 3.25 ± 0.06 | 6.70 ± 0.45 | 9.78 ± 0.25 | 23.98 ± 0.50 | 65.00 ± 2.16 | 146.68 ± 5.80 | 6.13 ± 0.04 | 4.42 ± 0.18 |
| | | (3) | 6.33 ± 0.52 | 16.96 ± 0.25 | 23.89 ± 1.40 | 4.60 ± 0.22 | 15.03 ± 0.35 | 38.40 ± 3.57 | 2.69 ± 0.17 | 5.35 ± 0.29 | 6.52 ± 0.28 | 18.05 ± 0.89 | 40.11 ± 2.54 | 127.17 ± 5.63 | 4.35 ± 0.19 | 4.37 ± 0.26 |
| Jasmine | Raw | | 6.26 ± 0.96 | 13.00 ± 1.09 | 17.17 ± 0.25 | ND | 28.14 ± 0.35 | 22.18 ± 0.09 | 2.46 ± 0.18 | 5.50 ± 0.29 | 5.45 ± 0.07 | 5.64 ± 0.11 | 15.33 ± 1.44 | 48.46 ± 2.76 | 6.04 ± 0.68 | TR |
| | BW | (1) | 4.86 ± 0.08 | 7.15 ± 0.07 | 14.40 ± 1.10 | ND | 12.49 ± 0.35 | 20.01 ± 0.17 | 1.96 ± 0.04 | 4.65 ± 0.20 | 4.06 ± 0.04 | 3.94 ± 0.05 | 13.88 ± 0.39 | 35.79 ± 1.09 | 2.64 ± 0.03 | TR |
| | | (2) | 3.39 ± 0.15 | 6.24 ± 0.56 | 12.34 ± 0.51 | ND | 10.85 ± 0.93 | 16.62 ± 1.33 | 1.95 ± 0.01 | 4.21 ± 0.01 | 3.77 ± 0.27 | 3.85 ± 0.39 | 12.51 ± 1.19 | 29.10 ± 3.04 | 2.41 ± 0.17 | TR |
| | | (3) | 3.14 ± 0.18 | 5.78 ± 0.13 | 12.61 ± 0.46 | ND | 9.40 ± 0.40 | 15.19 ± 0.17 | 1.92 ± 0.14 | 4.01 ± 0.08 | 3.74 ± 0.03 | 3.27 ± 0.04 | 10.49 ± 0.69 | 24.40 ± 0.91 | 2.36 ± 0.16 | TR |
| | RC | (1) | 3.95 ± 0.20 | 5.13 ± 0.87 | 12.93 ± 0.48 | ND | 13.50 ± 1.02 | 20.13 ± 1.17 | 2.07 ± 0.20 | 4.74 ± 0.51 | 3.65 ± 0.23 | 2.70 ± 0.09 | 13.88 ± 0.57 | 34.38 ± 1.40 | 4.86 ± 0.12 | TR |
| | | (2) | 3.02 ± 0.22 | 4.42 ± 0.09 | 11.96 ± 1.04 | ND | 12.38 ± 0.09 | 17.73 ± 0.39 | 1.96 ± 0.34 | 4.50 ± 0.05 | 3.56 ± 0.05 | 2.66 ± 0.20 | 13.46 ± 0.23 | 30.78 ± 0.35 | 4.49 ± 0.24 | TR |
| | | (3) | 2.85 ± 0.15 | 4.25 ± 0.55 | 11.72 ± 0.09 | ND | 10.34 ± 0.61 | 15.71 ± 0.57 | 2.00 ± 0.04 | 4.17 ± 0.07 | 3.46 ± 0.01 | 2.48 ± 0.15 | 10.91 ± 0.26 | 25.98 ± 2.13 | 4.23 ± 0.37 | TR |
| | MC | (1) | 5.01 ± 0.87 | 6.79 ± 0.43 | 14.80 ± 0.23 | ND | 11.29 ± 0.32 | 17.65 ± 0.81 | 2.03 ± 0.09 | 4.24 ± 0.24 | 3.94 ± 0.11 | 4.20 ± 0.06 | 12.96 ± 0.91 | 30.46 ± 1.35 | 4.72 ± 0.45 | TR |
| | | (2) | 4.43 ± 0.57 | 5.94 ± 0.19 | 12.07 ± 0.52 | ND | 11.23 ± 0.46 | 17.00 ± 0.34 | 1.82 ± 0.01 | 4.13 ± 0.09 | 3.72 ± 0.11 | 3.61 ± 0.02 | 12.60 ± 1.19 | 29.59 ± 0.90 | 3.44 ± 0.19 | TR |
| | | (3) | 3.16 ± 0.02 | 5.73 ± 0.72 | 11.97 ± 0.57 | ND | 10.76 ± 1.02 | 17.09 ± 1.14 | 1.81 ± 0.21 | 3.97 ± 0.12 | 3.46 ± 0.07 | 2.94 ± 0.17 | 12.21 ± 0.57 | 27.63 ± 1.54 | 2.93 ± 0.14 | TR |
| Basmati | Raw | | 16.30 ± 0.70 | 4.99 ± 0.33 | 8.12 ± 0.41 | ND | 7.09 ± 0.34 | 8.09 ± 0.35 | 2.38 ± 0.17 | 28.41 ± 2.11 | 15.26 ± 1.06 | 4.90 ± 0.16 | 12.23 ± 1.34 | 56.16 ± 3.16 | 7.43 ± 0.05 | TR |
| | BW | (1) | 13.00 ± 1.77 | 4.37 ± 0.17 | 6.13 ± 0.84 | ND | 6.39 ± 0.38 | 6.03 ± 0.57 | 1.84 ± 0.05 | 25.16 ± 0.52 | 11.71 ± 0.25 | 4.03 ± 0.27 | 9.53 ± 0.68 | 45.63 ± 2.74 | 6.61 ± 0.44 | TR |
| | | (2) | 8.74 ± 0.36 | 4.00 ± 0.16 | 5.71 ± 0.16 | ND | 4.66 ± 0.76 | 5.27 ± 0.40 | 1.75 ± 0.07 | 21.27 ± 1.54 | 9.81 ± 1.38 | 2.94 ± 0.01 | 9.30 ± 0.71 | 40.92 ± 2.00 | 4.49 ± 0.36 | TR |
| | | (3) | 8.91 ± 0.50 | 3.73 ± 0.50 | 5.12 ± 0.20 | ND | 4.33 ± 0.50 | 4.84 ± 0.30 | 1.74 ± 0.13 | 19.83 ± 1.67 | 8.87 ± 0.95 | 2.76 ± 0.01 | 7.93 ± 0.69 | 34.42 ± 2.65 | 3.00 ± 0.21 | TR |
| | RC | (1) | 8.00 ± 2.39 | 3.63 ± 0.38 | 6.00 ± 0.21 | ND | 4.84 ± 0.81 | 5.34 ± 0.83 | 1.90 ± 0.14 | 22.48 ± 1.38 | 9.38 ± 0.42 | 3.26 ± 0.15 | 9.20 ± 0.38 | 41.42 ± 3.70 | 4.06 ± 0.39 | TR |
| | | (2) | 7.63 ± 0.75 | 3.48 ± 0.09 | 5.86 ± 0.43 | ND | 4.60 ± 0.15 | 4.99 ± 0.32 | 1.81 ± 0.01 | 18.74 ± 1.27 | 8.83 ± 0.12 | 2.91 ± 0.12 | 8.28 ± 1.01 | 36.00 ± 2.50 | 2.93 ± 0.23 | TR |
| | | (3) | 5.62 ± 0.33 | 3.42 ± 0.06 | 5.11 ± 0.74 | ND | 4.05 ± 0.25 | 4.55 ± 0.09 | 1.57 ± 0.06 | 16.27 ± 0.73 | 7.79 ± 0.31 | 2.60 ± 0.02 | 6.71 ± 0.18 | 31.64 ± 2.23 | 2.96 ± 0.27 | TR |
| | MC | (1) | 11.00 ± 2.53 | 4.52 ± 0.42 | 7.17 ± 0.57 | ND | 5.51 ± 0.16 | 7.10 ± 0.41 | 2.05 ± 0.05 | 23.64 ± 0.60 | 11.28 ± 0.66 | 3.20 ± 0.36 | 11.00 ± 0.76 | 48.53 ± 3.76 | 4.66 ± 0.17 | TR |
| | | (2) | 10.50 ± 0.46 | 3.98 ± 0.27 | 6.20 ± 0.27 | ND | 5.27 ± 0.46 | 5.81 ± 0.05 | 1.92 ± 0.15 | 21.70 ± 1.40 | 10.20 ± 0.82 | 2.84 ± 0.15 | 10.37 ± 0.72 | 43.82 ± 2.45 | 2.97 ± 0.19 | TR |
| | | (3) | 9.42 ± 1.23 | 3.47 ± 0.09 | 6.28 ± 0.15 | ND | 5.36 ± 0.13 | 5.21 ± 0.15 | 1.89 ± 0.06 | 21.30 ± 0.30 | 8.98 ± 0.24 | 2.78 ± 0.01 | 9.10 ± 0.04 | 41.39 ± 0.97 | 2.58 ± 0.06 | TR |

Abbreviations: BW = boiling in water; MC = microwave oven; RC = rice cooker; ND = not detected (<LOD); TR = trace amount; (1) = freshly cooked rice; (2) = cooked rice reheated after storage in a fridge for 6 hr; (3) = cooked rice reheated after storage in a fridge for 24 hr.

[a]Values are expressed as mean ± standard deviation of triplicate measurements.

[b]The name of individual phenolic compounds abbreviated as in Figure 1.

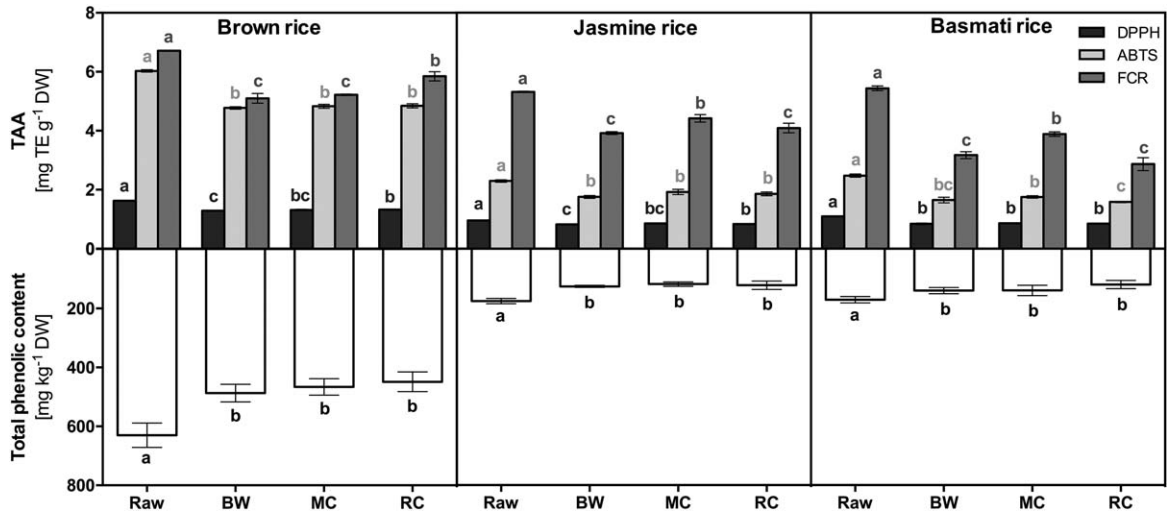


**FIGURE 2** The levels of TAA and polyphenols content determined by HPLC in different rice varieties, raw (Raw) and cooked in a rice cooker (RC), microwave oven (MC), or boiling water (BW). For a given rice variety submitted to indicated cooking methods, the results obtained from the same spectrophotometric assay or HPLC analysis marked with different letters are significantly different (Fisher's LSD test; $p < 0.05$)

kg DW of ferulic and *p*-coumaric acids, respectively. Meanwhile, the study of Italian white rice variety showed a significantly higher total content of both phenolic acids (~5–10-fold), including free and bound forms (Zaupa et al., 2015). These comparative data on ferulic and *p*-coumaric acids, which serve as main substrates to form the basic skeleton of all flavonoid derivatives in rice grain (Shao & Bao, 2015), indicated that their contents in white and brown rice, not surprisingly, depend strongly on the variety, geographical origin, type of processing as well as extraction method used. The levels of *p*-hydroxybenzoic and syringic acids, dominant components of phenolic profile of the jasmine and basmati rice grains, are higher than that reported in other studies of nonpigmented (white) varieties (Shao, Xu, Sun, Bao, & Beta, 2014; Tian et al., 2004; Vichapong et al., 2010; Zaupa et al., 2015; Zhou et al., 2004). The obtained results showed that vanillic acid contributing up to 12.6% of total phenolics was also found at higher concentrations compared to previous literature reports on white (0.3–8.5 mg/kg DW) and colored rice grains (0.4–15.0 mg/kg DW) (Shao & Bao, 2015; Tian et al., 2004; Vichapong et al., 2010; Zhou et al., 2004).

In contrary, the studied rice varieties were characterized by trace levels of protocatechuic and isovanillic acids (<2% of the total phenolics in grain). To the best of our knowledge, the presence of isovanillic acid in rice grains have not been reported before. Moreover, protocatechuic acid, the most abundant free phenolic acid found in pigmented rice (red and black varieties) (Shao & Bao, 2015), was not detected in the present study of polished rice samples using the HPLC-DAD, but it can be quantified in whole grain brown rice (8.7 mg/kg DW). With the exception of both these acids, the other phenolic compounds constituted a medium portion (2.0–9.8%) of the phenolic profile of analyzed rice samples. Among them, vanillin has been indicated as one of the major aroma constituents of basmati, glutinous and fine-grained rice, as well as cooked rice (Nadaf, Mathure, & Jawali, 2016). It was observed that the content of this phenolic aldehyde in all studied rice varieties (8.1–57.8 mg/kg DW) was above its odor threshold (OT = 0.058 mg/kg) and thus could contribute significantly to the aroma of these samples. Only few researchers focused on the evaluation of vanillin content in rice grains and reported very low amounts, equal to 0.03–0.40 mg/kg (Mathure, Wakte, Jawali, & Nadaf, 2011), in contrast to our findings. Our results emphasize the role of vanillin as an important ingredient of rice aroma and flavor besides 2-acetyl-1-pyrroline with characteristic sweet, cooked rice and popcorn like aroma (OT = 0.001 mg/kg).

In general, the concentration of phenolic compounds evidently decreases when the grains are subjected to polishing process. This finding is reasonable taking into consideration that the rice bran is removed during polishing process (Zhang et al., 2015). Another study on the determination of phenolic compounds in different parts of rice grain confirmed that phenolic acids in bran ensure the highest contribution to the total phenolic content in the grain compared to endosperm and embryo (Shao & Bao, 2015; Shao et al., 2014). Hence, bran removal process during polishing of dehulled rice to obtain milled rice, the form that is generally consumed, reduces the concentration of phenolic compounds in the grain. In present study, the level of total polyphenols in unpolished grains was 3.5-fold higher than in polished ones.

## 3.2 | The impact of cooking on phenolic composition

The results concerning the content of total and individual polyphenols in the studied rice varieties subjected to different cooking process are shown in Figure 2 and Table 2. In general, the levels of polyphenols in both polished and unpolished rice tended to decline after cooking. In order to determine the significance of differences between the level of polyphenols in raw and cooked rice samples, the statistical analysis based on one-way analysis of variance (ANOVA) was performed. Significant differences in the content of phenolic compounds in rice

samples were found and thus Fisher's least significant difference (LSD) test was applied. LSD revealed that the initial levels of polyphenols in rice grains were significantly higher than the ones in cooked rice (brown rice, $p = 0.0001$; jasmine rice, $p = 0.001$; and basmati rice, $p = 0.025$), regardless of cooking method (Figure 1). Among three processing methods, cooking using RC caused the highest reduction of phenolic content (29–31%), followed by microwaving (18–33%), and boiling (18–28%). However, the differences in the loss of polyphenols depending on the cooking method used were not statistically significant. Previous study on the evaluation of the effect of thermal treatment on rice phenolics also confirmed that domestic cooking causes a significant loss of phenolic compounds in rice grains prepared by boiling (27–38%) and risotto (8–33%) procedures (Zaupa et al., 2015), which is in accordance with our findings. In addition, the lowest reduction of total phenolic content during cooking process, especially by microwaves and boiling was observed in basmati rice, while the highest was found in jasmine rice.

Considering the impact of rice cooking on the level of phenolic acids, the reduction of hydroxycinnamates (20–30%) in brown and jasmine varieties was higher than hydroxybenzoates (26–40%), while the opposite effect was observed for basmati rice. Only in unpolished brown rice, syringic and isoferulic acids were significantly affected by all thermal processing methods, with the highest lost found in grains cooked using RC. As shown in Figure 1 and Table 2, their concentration dropped two- and threefold, respectively. In the case of jasmine rice, the cooking process resulted in the highest loss of *p*-hydroxybenzoic acid (52–60%), as well as its corresponding aldehyde (*p*-HB), regardless of the type of thermal treatment used. On the other hand, vanillic acid was found to be the most thermally stable hydroxybenzoic acid in both jasmine and brown rice grains cooked by different methods; its content decline did not exceed 20%. Regarding the hydroxycinnamic acids, sinapic acid and *p*-coumaric acid were the least sensitive to thermal treatment in brown and jasmine varieties, respectively. Their degradation was hardly observed, especially during boiling, probably due to the binding of these phenolic acids to the cell wall polysaccharides and lignin (Zaupa et al., 2015; Zhou et al., 2004). Taking into account the effect of cooking on individual phenolic compounds in basmati rice, it was observed that their contents were affected differently between the three processing methods. For example, the concentration of sinapic acid and protocatechuic aldehyde decreased during cooking in RC and MC by half and one-third, respectively, whereas BW affected their level to a much lesser extent (11 and 20%) as shown in Table 2.

In the case of three phenolic aldehydes, the thermal treatment of studied rice varieties using RC caused the highest loss of these compounds (34–40%). In general, these compounds behaved differently among varieties and between the three processing methods used. Only the content of vanillin in brown rice and *p*-hydroxybenzoic aldehyde in jasmine rice were affected by all cooking methods to a comparable extent.

Rice is also often consumed after reheating the cooked rice. Therefore, the content of phenolic compounds in the rice samples stored at 4 °C and then reheated in a MC after 6 and 24 hr, were also evaluated (Table 2). As shown in Figure 3 the levels of polyphenols in the reheated samples were lower than in freshly cooked rice. The reduction of these phytochemicals due to reheating was 7–15 and 12–33% for cooked rice reheated after 6 and 24 hr, respectively. The results of one-way ANOVA indicted that reheating of jasmine and basmati rice prepared by MC did not affect the level of phenolic compounds in statistically significant way. Similarly, the detrimental effect of reheating process on phenolic content in basmati rice previously cooked in RC was not observed. Nevertheless, statistically significant loss of polyphenols was noted in all samples of brown rice reheated after 6 and 24 hr ($F \geq 2.937$, $p \leq 0.019$), regardless of the cooking method as shown in Figure 3. In the case of jasmine and basmati rice boiled in water as well as jasmine grains prepared using RC, only the reheating after longer storage (24 hr) resulted in a significant decrease of phenolic content ($F \geq 2.789$, $p \leq 0.024$), with the highest lost (23–25%) determined in samples cooked by boiling. Therefore, it is advisable to consume the rice right after it was cooked. In addition, the obtained results suggest that preparation of polished rice varieties using microwaves leads to a higher stability of phenolic phytochemicals during storage and reheating process in comparison to other cooking methods (9 and 14% loss after 6 and 24 hr of storage). Thus, consumption of reheated rice that was previously prepared in a MC is also recommended.

Summarizing the effect of rice cooking on the phenolic composition, the thermal processing, especially cooking using RC, generally resulted in a decrease in the content of such compounds. As described before, the reduction of phenolic content in rice grain is probably associated with the instability of these compounds at high temperature during thermal process (Liazid, Palma, Brigui, & Barroso, 2007), and thus may provide their release from bound forms, degradation, polymerization and the formation of Maillard reaction products (Massaretto et al., 2011; Zaupa et al., 2015). Furthermore, the presence of oxygen and moisture, in higher temperature could accelerate the oxidative degradation of phenolic compounds (Min, McClung, & Chen, 2014). Considering the complete absorption of the cooking water by the rice grains during all three cooking procedures used, it can be stated that the losses of phenolic compounds resulted mainly from the thermal and oxidative degradation.

### 3.3 | The impact of cooking method on antioxidant capacity

TAA of three rice samples in a form of raw and cooked grains was measured employing ABTS, DPPH radicals and FCR assay (Figure 2). The results disclosed that, for raw rice samples, brown rice exhibited the highest TAA (1.62, 6.03, and 6.71 mg TE/g DW for DPPH, ABTS and FCR assay, respectively), followed by basmati (1.10, 2.48, and 5.44 mg TE/g DW for DPPH, ABTS and FCR assay, respectively) and jasmine varieties (0.96, 2.29, and 5.32 mg TE/g DW for DPPH, ABTS and FCR assay, respectively). As shown in Figure 2, polished rice grains were characterized by comparable level of antioxidant activity, while unpolished brown rice exhibited significantly higher value of TAA, especially when determined as ABTS radical scavenging activity. A total of 2.5-fold lower TAA of white (undermilled and milled) rice grains

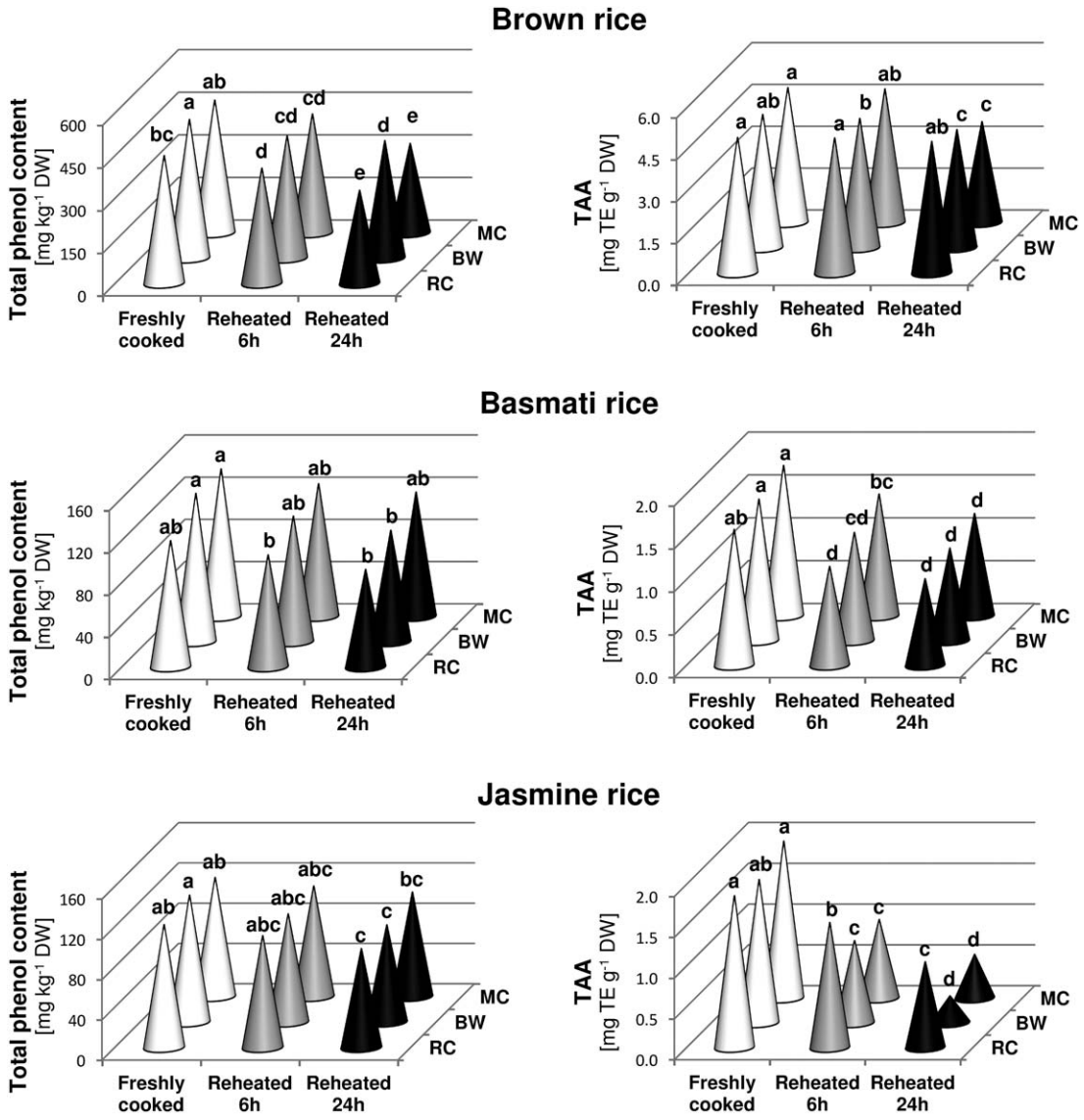


**FIGURE 3** The reduction of polyphenol contents (left panels) and TAA (right panels) by reheating rice samples prepared in: rice cooker (RC), boiling water (BW), or microwave oven (MC) and stored in a refrigerator for 6 and 24 hr. TAA was determined by ABTS assay. Different letters for the results obtained for the same rice variety correspond to significant differences (Fisher's LSD test; $p < 0.05$) among cooking methods and storage time

compared to the brown ones were also reported by Finocchiaro et al. (2007) using ABTS assay. Furthermore, our results obtained for basmati and jasmine rice samples are consistent with TAA of white rice varieties (0.60–0.87 mg TE/g DW) evaluated by DPPH spectrophotometric test during previous studies (Zhang et al., 2015).

The different cooking methods of the rice grains also affected TAA (Figure 2), which was similarly altered as the total phenolic content. The one-way ANOVA ($p < 0.05$) revealed that the three cooking processes produced a significant reduction of TAA of rice. Among the three processing methods, rice cooking in RC caused the highest reduction of TAA, ranging from 13 to 47% according to DPPH, ABTS, and FCR assays, followed by preparation of rice in boiling water (14–42%), and MC (11–29%). In brown and white boiled rice, a loss of 20–30% of the initial antioxidant activity, measured by ABTS assay, was also observed by Finocchiaro et al. (2007). These reductions of TAA of cooked rice were probably due to the decline of phenolic level in grains during cooking. The strong positive correlation observed between the TAA and total content of phenolic compounds in cooked rice samples, as confirmed by the Pearson coefficient (ABTS $r = 0.993$; DPPH $r = 0.993$; FCR $r = 0.836$), also indicate that these compounds are the main components responsible for the TAA of rice. A similar relationship for rice grains (Surh & Koh, 2014; Walter et al., 2013), as well as other cereals such as wheat, oats, sorghum, and flaxseed was reported by other researches (Choi, Jeong, & Lee, 2007; Velioglu, Mazza, Gao, & Oomah, 1998).

The level of TAA of cooked rice reheated after 6 and 24 hr of prior refrigeration were also evaluated. In general, the reheating of rice resulted in a decrease in TAA in all three varieties (Figure 3). The

reduction of ABTS radical scavenging activity ranged from 0.3 to 50 and 3 to 81% for rice samples reheated after storage in a fridge for 6 and 24 hr, respectively. As shown in Figure 3, statistically insignificant effect of reheating on the level of TAA was only observed in brown rice cooked in RC. Similarly, the one-way ANOVA revealed that reheating of brown rice prepared by two other cooking methods did not significantly affect the TAA only after shorter storage period of 6 hr. These findings are not correlated with the results showing the impact of reheating on the phenolic content in brown rice, which tended to decline during this process. The observed opposite effect was probably due to diversified contribution of phenolic compounds to total antioxidant potential of plant foods, such as rice grains, as well as the possibility of synergistic, additive, and antagonistic interactions that may occur between polyphenols and other components of sample matrix during processing as also indicated by Finocchiaro et al. (2007). In the case of polished rice varieties, the reheating process caused a statistically significant decline of TAA values for both basmati ($F \geq 2.475$, $p \leq 0.038$) and jasmine ($F \geq 4.390$, $p \leq 0.002$) rice grains, regardless of the storage time and thermal processing method applied. The highest reduction in the level of TAA due to reheating of rice was observed in samples prepared by boiling which agrees well with the loss of polyphenols (Figure 3). A previous study revealing the decrease of TAA of pigmented and non-pigmented rice grains submitted to the thermal treatment suggested that such reduction was due to the degradation or leaching of soluble compounds, mainly free phenolics (Zaupa et al., 2015). However, the absorption of all the cooking water by the rice during thermal treatment in our study indicated that decrease of TAA is most likely related to the thermal and oxidative degradation of phenolic compounds.

## 3.4 | In vitro starch digestibility

In this part of study, one sample of each type of rice was analyzed. The brown rice was used as unpolished rice sample. Due to the growing volume of jasmine rice trade in the last decade, from 1.7 million tons in 2005 to 2.5 million tons in 2013 (Mohanty, 2013), this variety was selected as polished rice sample for starch digestion analysis.

Figure 4 shows the changes in the digestion of starch in homogenized cooked rice samples submitted to various cooking procedures during the simulated in vitro digestion. Throughout the course of simulated gastric digestion (up to 120 min; G-120), no starch hydrolysis was observed, which was attributed to the absence of amylases in the gastric juice and is consistent with the results of previous studies (Tamura et al., 2016).

Afterwards, the starch hydrolysis occurred due to the action of pancreatic enzymes fed into the simulated intestinal digestion. Starch digestion in homogenized samples for RC, MC and BW during the process sharply increased from nearly-zero level (~0.7 g/kg fresh weight [FW]) to 50.1, 56.7, and 42.4 g/kg FW of jasmine rice and 42.8, 49.0, and 38.0 g/kg FW of brown rice after 30 min of simulated intestinal digestion (I-30), respectively. The results also indicated that the amount of hydrolyzed starch in cooked jasmine rice samples occurred at a comparable level regardless of the cooking method used. Khatoon and

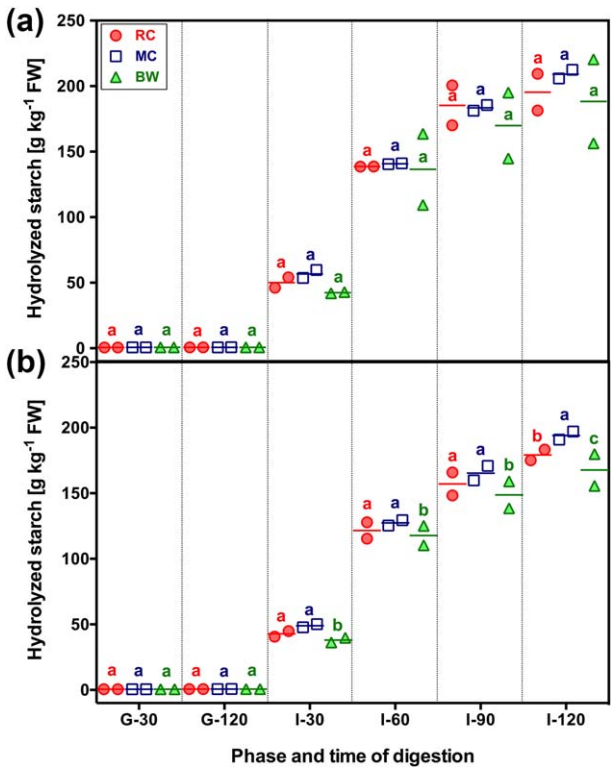


**FIGURE 4** Comparison of starch digestion for (a) polished (jasmine) and (b) unpolished (brown) rice submitted to various cooking processes: RC—in a rice cooker, MC—in a microwave oven, and BW—boiled in water. The digesta samples were collected after a given time (in minutes) of the gastric (G) and intestinal (I) phase. The results of two independent in vitro digestion trials are shown. For a given rice type and time of gastrointestinal digestion, the results of starch digestibility marked with different letters are significantly different ($p < 0.05$) among cooking methods used

Prakash (2006) also reported similar findings for microwave and pressure cooked rice. Nevertheless, among the three different cooking methods, the highest amount of digested starch in polished jasmine rice (209.1 g/kg FW) was reproducibly determined for sample cooked in MC. Similarly, the microwave cooking of unpolished rice resulted in a significantly higher (194.0 g/kg FW; $p < 0.05$) starch digestibility after 2 hr of simulated intestinal digestion (I-120) in comparison to other culinary procedures, as shown in Figure 4b. This finding is probably due to differences in heat transfer during rice cooking. Thermal radiation occurs in the MC, while the conduction takes place during BW or RC procedure. This results in a greater swelling and smaller gaps between granulates of rice starch heated by microwave and thus higher starch digestibility as suggested by Li et al. (2014). Hence, it is advisable to cook rice with the aid of a MC when accelerated starch digestion is in favor. A corresponding study on starch digestion of cooked rice prepared by different cooking time also confirmed that the cooking process increased the rate of hydrolysis by gelatinizing the starch which makes it available for enzymatic reactions (Tamura et al., 2016). Additionally, it has been suggested that the mechanical processes, including chewing, may enhance the interaction of starch with amylases that leads to higher rates of starch hydrolysis.

## 4 | CONCLUSIONS

In the present study, the validated UAE/HPLC-DAD procedure was successfully applied to determine and monitor the phenolic composition of unpolished and polished rice before and after cooking by three different methods, as well as after reheating refrigerated cooked rice. As expected, brown rice grains showed the highest TAA and phenolic content among the analyzed samples. However, in all rice samples studied cooking process had detrimental effect on the content of polyphenols with their greatest loss and the highest drop in TAA caused by thermal processing in the rice cooker. In the case of reheating, generally the levels of phenolic compounds and TAA tended to decline even more. Therefore, it is advisable to consume the rice right after it was cooked. The processing of polished rice using microwaves turned out to ensure higher stability of phenolic phytochemicals during storage and reheating process in comparison to other cooking methods. Our results also suggest that the consumption of whole-grain rice should be recommended in habitual diet to increase the daily intake of phenolic antioxidants. In addition, reproducibly the highest level of digested starch was observed for microwaved rice. Hence, it is recommended to cook rice with the aid of MC to preserve phenolic phytochemicals and when accelerated starch digestion is in favor.


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