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Effects of processing on the chemical composition of rice

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RESEARCH ARTICLE

Abstract

Changes in the chemical composition of rice from harvest to packaging were searched. The phytic acid content in rice bran and final white rice was 64.25 and 9.66 mg/g (dry basis; db), respectively. Sugars (fructose, glucose, and sucrose), organic acids (citric and malic), and free amino acids (alanine, aspartic, and glutamic acid) decreased according to the progressing stages. The most abundant phenolic compound present in rice kernels was ferulic acid. The antioxidant capacity of rice bran was 428.97 µM Trolox equivalent (TE)/g (db), and it dropped from 126.23 to 60.76 µM TE/g (db) during processing. The L* colour value of rice samples showed a linear increase with decreasing antioxidant capacity. About half the phytic acid content and antioxidant activity was removed as a consequence of the dehulling, whitening, polishing, and grading of rice kernels.

Keywords: ferulic acid, paddy rice, process, TEAC

1. Introduction

Rice (*Oryza sativa* L.) is the one of the most important cereals that is planted and consumed around the world. Moreover, it is a staple food for most Asian countries, providing 20% of the energy supply in the diet (Frank *et al.*, 2012). Harvested rice is known as paddy rice. It can be consumed as whole grain, flour, and fermented products after dehulling (Oli *et al.*, 2014).

Paddy rice is comprised of three parts: (1) the coat (hull and bran); (2) the embryo; and (3) the endosperm. Rice bran is the nutritionally most important part of the seed. It contains bioactive substances such as vitamins, tocopherols, tocotrienols, and oryzanol. Rice bran also contains phenolic compounds and vitamins B, E, and K (Farahmand *et al.*, 2015; Kim and Han, 2012; Madamba and Yabes, 2005).

Bran is a waste product of rice that is obtained in milling processing and formed from the pericarp and aleuronic layer. It comprises 10% of the rice seed weight (Garcia *et al.*, 2012). Although rice bran has high nutritional properties, it is totally removed from rice during polishing, whitening, and milling processes and is only used in oil extraction and animal feeding (Pradeep *et al.*, 2014). It has been reported that the milling of rice causes a loss of nutrients and their bioavailability and affects edible properties such as paste viscosity (Liang *et al.*, 2008).

Numerous studies have focused on differences in chemical composition between the parts (bran, endosperm, and embryo) of rice (Kim *et al.*, 2012) and its different varieties (Min *et al.*, 2014; Mohan *et al.*, 2010). There are also a few publications on the chemical and physical changes that occur during the rice production process (Garcia-Estepa *et al.*, 1999; Liang *et al.*, 2008; Ti *et al.*, 2014). The main aim of the current work was to define changes in the chemical composition of rice from harvested paddy rice to packaged white rice and to determine the effects of commercial processing on rice quality.

2. Materials and methods

Materials

Rice kernels from *Oryza sativa* L. cv. Baldo was used because this variety is commonly consumed in Turkey. Standards for sugars (fructose, glucose, and sucrose),

organic acids (lactic, malic, succinic, and citric acid), amino acids (alanine, glycine, serine, threonine, aspartic acid, and glutamic acid), and phenolic acids (vanillic, coumaric, gallic, and ferulic) as well as internal standards (p-hydroxybenzoic acid and phenyl-β-D-glucopyranoside), oximant reagent (hydroxylamine hydrochloride), silylation reagent (N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS); 99:1), and pyridine were purchased from Sigma (Taufkirchen, Germany).

2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and ethylene diamine tetraacetic acid (EDTA) were obtained from Sigma and sulphosalicylic acid, iron (III) chloride, and sodium sulphate were provided by Merck (Darmstadt, Germany).

Methods

Sampling

Samples taken from 12 different stages of the production process (Figure 1) were obtained from a local rice milling factory (Tat Company) in Mersin, Turkey. Temperature and relative humidity in the milling plant were 25 °C and 62.5%, respectively. Every 15 min, 250 g samples were taken at 12 different stages of the milling process. Samples were taken five times and, after thorough mixing, 100 g were used for analysis. The samples were grounded in a laboratory blender (MKM-6000; Bosch, Stuttgart, Germany) and stored in plastic bags at +4 °C until use.

Determination of moisture content, water activity, and total ash

Using 2 g samples, moisture and total ash content were determined according to AOAC (1990) and AACC (2000), respectively. The water activity of the samples was measured using a water activity meter (Aqualab 4TE; Decagon Devices, Pullman, WA, USA) at 25 °C. The results were expressed as dry basis (db).

Determination of phytic acid content

The phytic acid content of rice samples was determined by a complexometric method according to Febles *et al.* (2001). For this purpose, a 1 g rice sample was extracted with 20 ml 0.4 M HCl solution (prepared in 5% Na_2SO_4) with an orbital shaker for 90 min at ambient temperature. The extract was centrifuged at 10,000 rpm for 5 min, and then 10 ml extract was taken in a tube and incubated in a boiling water bath for 15 min after adding 10 ml 0.4 M HCl, 10 ml Fe(III) solution, and 10 ml 20% sulphosalicylic acid. It was cooled under tap water until the ferric-phytate precipitate was accumulated. Next, 10 ml of the clean upper phase was placed in a beaker, 100 ml hot water was added, and the pH was adjusted to 2.5 with glycocoll (approx. 1.5 g). It was titrated with 0.01 M EDTA on a magnetic stirrer until a bright yellow colour developed (Febles *et al.*, 2001). The results were expressed as db.

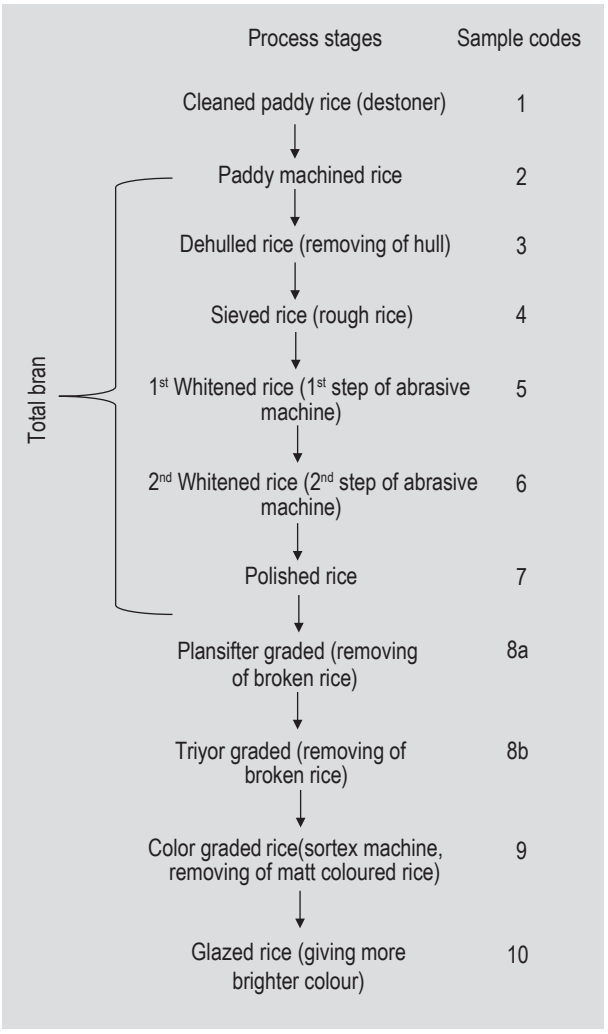

Figure 1. Stages of white rice processing with sample codes.

Simultaneous determination of sugars, organic acids, and free amino acids

The sugar, organic acid, and free amino acid content of samples were determined using a gas chromatography-mass spectrometry (GC-MS) system according to Cocchi *et al.* (2006), with some modifications. For the extraction procedure, 1 g of rice sample was homogenised with 10 ml water at 11,000 rpm (Ultraturrax T-25; IKA Labortechnik, Staufen, Germany) for 1 min. After homogenisation, the sample was centrifuged at 12,000 rpm for 10 min and the supernatant was filtered through a 0.45 mm filter (Sigma, Steinheim, Germany). From this, a 50 ml sample was taken in a vial and evaporated under N_2 flow. Then, 500 ml hydroxylamine hydrochloride (30 mg/l in pyridine) containing phenyl-β-D-glucopyranoside (2 mg/l) as an internal standard for sugars was added and incubated at

70 °C for 30 min. During incubation, sugars in the sample were converted to their oxime derivatives by this oximant reagent. After incubation, 500 ml silylation reagent (BSTFA:TMCS) containing p-hydroxybenzoic acid (2 mg/l) as an internal standard for organic acids was added and incubated at 70 °C for 30 min. During the incubation, organic acids and free amino acids were converted to their trimetilsilil (TMS) derivate.

After incubation, a 2 ml derivatised sample was injected into the gas chromatography system (Agilent 7890A GC, Agilent G4513A automatic sampler, Agilent 5975C MSD; Agilent, Wilmington, DE, USA), equipped with a column (Agilent HP-1ms, 100% dimethylpolysiloxane, 25 m × 0.32 mm × 1.05 µ). The injection was done using the 1:15 split mode. The oven temperature was started at 80 °C, increased to 210 °C at a rate of 4 °C/min, then further increased to 250 °C at a rate of 2 °C/min, and finally increased to 280 °C at a rate of 4 °C/min, where it was held for 10 min. The temperatures of the inlet and mass detector were set to 280 and 230 °C, respectively. Helium was used as the carrier gas, and its flow rate was programmed at 0.6 ml/min (Cocchi *et al.*, 2006).

The peak amounts of sugars, organic acids, and free amino acids were detected using the main ion fragments (fructose: 73, 103, 147, 217, 307 (m/z); glucose: 73, 103, 147, 205, 319; sucrose: 73, 103, 147, 217, 361, 437; lactic acid: 73, 117, 147, 191; succinic acid: 73, 147, 247, 262; malic acid: 73, 147, 233, 335; citric acid: 73, 147, 273, 465; alanine: 73, 116, 147, 281; glycine: 73, 147, 174, 284, 291; serine: 73, 100, 147, 204, 218, 306; threonine: 73, 117, 218, 291, 334; aspartic acid: 73, 100, 232, 349; glutamic acid: 73, 128, 246, 363). The quantification of sugar and organic acid content was performed using areas belonging to the internal standard and analytes. The quantification of amino acid content was determined by using the calibration of external standard solutions, prepared at concentrations of 45.45, 9.09, 4.55, 3.03, 1.52, 0.76, and 0.38 mg/kg in the final solution. Standards were dissolved in water and derivatised in the same conditions as samples after evaporation under N_2 flow. The results were expressed as db.

In the above experiments, the recovery of proline, isoleucine, alanine, glycine, serine, threonine, aspartic acid, and glutamic acid was 100.11, 80.65, 100.61, 95.40, 79.31, 80.26, 65.66, and 65.68%, respectively. Additionally, the recovery of organic acids was measured as 71.76, 83.44, 103.77 and 82.56% for lactic, succinic, malic, and citric acid, respectively.

Determination of phenolic acids

The phenolic acid content of samples was determined using the GC-MS system according to Wang *et al.* (2012) with some modifications. For the extraction of phenolic acids, a 0.5 g rice sample was extracted with 2 ml 6 M HCl for 90 min at 35 °C in an ultrasonic water bath (Sonorex; Bandelin, Berlin, Germany). The sample was centrifuged at 10,000 rpm for 5 min, and the supernatant was placed in a tube. This supernatant was extracted three times with 0.5 ml ethyl acetate and centrifuged again at 10,000 rpm for 15 min. After that, 0.5 ml extract was filtered through a 0.45 mm filter (Sigma) and 100 µl of this extract was evaporated under N_2 flow. The extract was placed in a vial and incubated at 70 °C for 4 h after adding 125 µl pyridine and 150 µl silylation reagent (BSTFA:TMCS, 99:1). After incubation, 125 µl pyridine was added to the vial, and a 1 µl sample was injected into the Agilent GC-MS system equipped with the Agilent HP-1ms column.

The oven temperature was held at 80 °C for 1 min., increased to 220 °C at a rate of 10 °C/min, and then increased to 310 °C at a rate of 20 °C/min, where it was held for 6 min at this temperature. The temperatures of the inlet and mass detector were set to 280 and 305 °C, respectively. Helium was used as a carrier gas, and the flow rate was programmed at 1.7 ml/min (Wang *et al.*, 2012). Analyses were performed in electron impact mode, and the ionisation energy was 70 eV.

For the identification of phenolic acids, analyses were conducted in the selected ion monitoring mode by selected ion monitoring of the major ion at m/*z* 458, 338, 312, and 308 for TMS-derivates of gallic, ferulic, vanillic, and coumaric acid, respectively. The quantification of phenolic acid content was determined using the calibration of standard solutions, prepared at a concentration of 125, 31.25, 15.62, 7.81, 3.9, and 1.95 mg/kg in the final solution. Standards were dissolved in pyridine and derivatised using the same conditions as samples after evaporation under N_2 flow. The results were expressed as db. The recovery rates of vanillic, coumaric, gallic, and ferulic acid were 104.32, 65.18, 90.77, and 67.26%, respectively.

Determination of antioxidant activity

The antioxidant activity of rice samples was determined as the Trolox equivalent antioxidant capacity (TEAC). For the determination of antioxidant activity, a 0.175 g sample was extracted with 6.25 ml 75 mM phosphate buffer solution (PBS, pH adjusted to 7.4) with an orbital shaker for 60 min at ambient temperature. After incubation, samples were centrifuged at 10,000 rpm for 10 min and supernatants were placed in tubes. The pellets were extracted again under the same conditions after adding 3.75 ml phosphate buffer solution. The samples were centrifuged, and the upper phase of extracts was added to the first extracts.

ABTS stock solution was dissolved in water at a concentration of 7 mM. To prepare the $ABTS^+$ radical, 25 ml of this stock solution was mixed with 12.5 ml potassium persulphate (2.45 mM) and incubated at

ambient temperature for 12-16 h. Just before analysis, the ABTS+ radical solution was diluted with PBS to ensure an absorbance of 0.70 at 734 nm in the UV-Spectrometer (UV-1800; Shimadzu, Kyoto, Japan). After that, 10 µl sample extract was added to 1 ml of ABTS+ radical solution, and the inhibition rate was determined after 6 min (Kamiloglu *et al.*, 2014; Erbas *et al.*, 2009; Michalska *et al.*, 2008; Re *et al.*, 1999). The quantification of antioxidant activity was determined using the calibration of standard Trolox solutions, prepared at concentrations of 0, 5, 10, 15, and 20 µM/ml in the final solution, as well as the inhibition rates. The results were expressed as db.

Determination of colour properties

The colour properties (L*, a*, and b*) of rice samples were determined by the CIELAB system using a CR-400 Chromameter (Konica Minolta, Tokyo, Japan). Parameter L* represents the light-dark spectrum, parameter a* represents the red-green colour spectrum, and parameter b* represents the yellow-blue colour properties of samples. Ground rice samples were analysed after calibration of the chromameter. In addition, the whiteness index (WI) degree of samples was calculated according to Hsu *et al.* (2003) using Equation 1:

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

Statistical analysis

The research was carried out using two replicates obtained from 12 different stages of rice processing, and all analyses were performed in duplicate. The results were reported on db. Data were subjected to analysis of variance using Duncan's multiple-range test. All statistical calculations were performed using the SAS Statistical Software (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

Moisture content and water activity

The moisture content and water activity of the rice samples were significantly (P<0.01) affected by the processing stages (Table 1). The moisture content of rice samples increased from 13.60 to 14.57%. The water activity of rice samples was in the range of 0.66-0.70.

Rice is not conditioned before processing, and water is not used in the dehulling, whitening, or polishing stages. The slight increase in moisture content and water activity in the rice samples was probably caused by the lower moisture content in rice bran (11.87%). After that, during the cleaning and classification process, the water activity decreased slightly because of air drying.

Table 1. Moisture content (%) and water activity of rice samples.[1]

Sample code	Moisture content (%)	Water activity
Bran	11.87^{c}	0.64^{e}
1	13.60^{b}	0.68^{bc}
2	14.55^{ab}	0.69^{b}
3	15.03^{a}	0.70^{a}
4	14.82^{a}	0.70^{a}
5	14.29^{a}	0.70^{a}
6	14.69^{a}	0.68^{bc}
7	14.87^{a}	0.68^{bc}
8a	14.88^{a}	0.68^{bc}
8b	14.56^{a}	0.67^{c}
9	14.62^{a}	0.66^{d}
10	14.57^{a}	0.67^{c}
Significance	**	**

[1] Mean values followed by different superscript letters in the same column are significantly different according to Duncan's multiple range test (P<0.05). **P<0.01.

Moisture content and water activity are important factors that directly affect the shelf life of the final product. White rice is stored and sold at room temperature in markets. Small increases in moisture content may cause major loss of grains.

According to Abdullah *et al.* (2000), critical water activity and moisture content should be 0.65 and 13.0%, respectively. However, a cereal moisture content of 14% can be accepted as a safe moisture limit (Weinberg *et al.*, 2008). Additionally, it has been noted that cereals can be stored for one month at 16-17% moisture content, but for longer-term storage, this value should be 14% (Finch *et al.*, 2014).

Ash and phytic acid contents

Phytic acid (myoinositol hexa-phosphoric acid) is a well-known chelating agent that decreases the bioavailability of foods by combining metal ions. It exists mostly in the outer layers of cereals, and it has been reported that some cereal brans can contain more than 5% ash (Garcia-Estepa *et al.*, 1999). Therefore, the phytic acid level in cereal bran limits its use as a source of dietary fibre in food, although it is known that processes such as soaking, germina–tion, fermentation, and the addition of phytase decrease the phytic acid level of cereals (Garcia-Estepa *et al.*, 1999; Moongngarm and Saetung, 2010).

The total ash and phytic acid content in rice samples were significantly (P<0.01) affected by the processing stages (Figure 2), progressively dropping from 5.03 to 0.46% and 18.65 to 9.66 mg/g (db) in the final product, respectively.

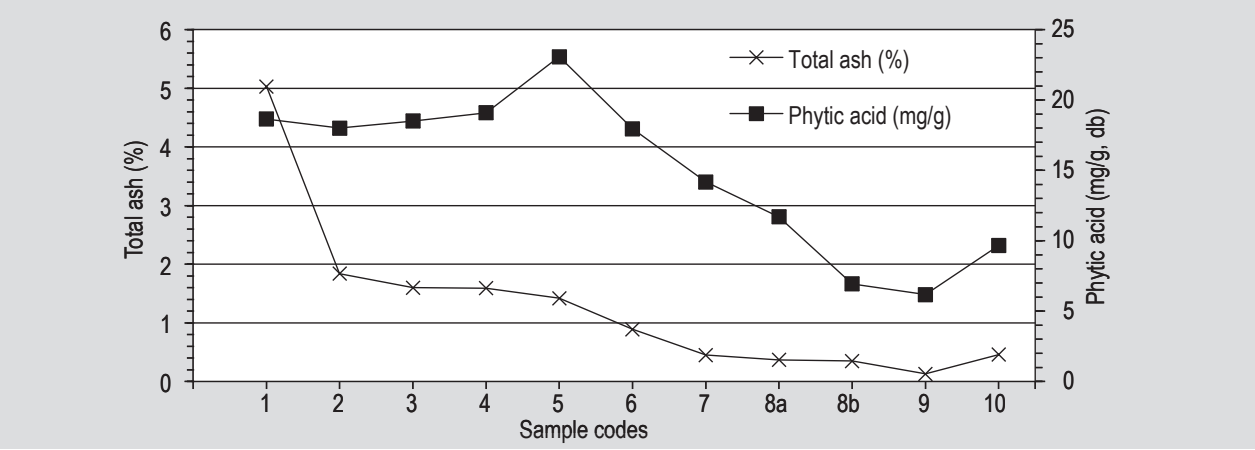

Figure 2. Phytic acid and total ash content of rice samples.

The highest ash and phytic acid content were found in rice bran (9.22% and 64.25 mg/g (db), data not shown).

The results for the phytic acid and ash content were in agreement with previous studies. The total ash content was 0.50 and 0.26% for waxy and non-waxy rice flours, respectively (Wu *et al.*, 2011). Garcia *et al.* (2012) determined that the total ash content and phytic acid content of the bran of three different rice cultivars were 7.89-11.90% and 50.5-84.8 mg/g, respectively. Elsewhere, it was determined that the phytic acid content of rice flour and bran were 5.52 and 57.71 mg/g, respectively (Garcia-Estepa *et al.*, 1999). In another study, phytic acid levels ranged from 19.65-45.13 mg/g and 3.99-7.34 mg/g for bran and brown rice samples, respectively.

It can be concluded that nearly half the phytic acid content was removed from sample 1 to 10 as a consequence of dehulling, whitening, polishing, and grading of the rice kernels. Previously, it has been reported that phytic acid content decreased from the outer to the inner layer of brown rice during milling (Wang *et al.*, 2011). In addition, extended milling caused a decrease in phytic acid content during rice flour production. When the rice samples were milled for 300 s, the phytic acid content of samples decreased at a rate of 97% (Liang *et al.*, 2008). Removal of phytic acid is nutritionally beneficial because it is a chelating agent, and its consumption may cause malnutrition (Wang *et al.*, 2011).

As expected, the total ash content of rice samples decreased from the beginning of the rice production process to the end. There was a sharp decrease in the mineral content of rice samples (90.85%) in the final product (sample 10). Wang *et al.* (2011) reported that, during the milling of three *indica* rice cultivars, Fe, Zn, Se, Mg, Ca, and Mn decreased from the outer layer of rice to the core endosperm.

Sugar content

There were significant differences in the sugar composition of rice samples (Table 2). Fructose, glucose, and sucrose levels were greatly reduced throughout the rice production process. However, there was an increase in the sucrose content of the final product (sample 10) because of the use of glazing materials.

Sucrose is the most abundant sugar in rice kernels, accounting for approximately 90% of total sugars (Frank *et al.*, 2012). In the literature, it has been determined that fructose, glucose, and sucrose content in stored rice flours was 0.246, 0.249, and 0.176 mg/g, respectively (Cao *et al.*, 2004). According to another study, the glucose, fructose,

Table 2. Sugar content of rice samples (mg/g, db).[1]

Sample code	Fructose	Glucose	Sucrose
Bran	0.352^{a}	0.608^{a}	9.766^{a}
1	0.374^{a}	0.407^{ab}	2.231^{bcd}
2	0.104^{bc}	0.208^{bc}	2.170^{bcd}
3	0.108^{bc}	0.208^{bc}	2.367^{bcd}
4	0.147^{b}	0.330^{bc}	3.302^{b}
5	0.115^{bc}	0.243^{bc}	2.809^{bc}
6	0.040^{c}	0.143^{c}	1.286^{cde}
7	0.036^{c}	0.130^{c}	0.776^{cde}
8a	0.023^{c}	0.117^{c}	0.445^{e}
8b	0.036^{c}	0.140^{c}	0.470^{e}
9	0.030^{c}	0.131^{c}	0.459^{e}
10	0.037^{c}	0.203^{bc}	1.932^{cbed}
Significance	**	*	**

[1] Mean values followed by different superscript letters in the same column are significantly different according to Duncan's multiple range test. ($P<0.05$). *$P<0.05$; **$P<0.01$.

and sucrose content was 0.4, 0.6, and 2.6 mg/g, respectively (Mohan *et al.*, 2010).

Organic acid content

The predominant organic acids found in the rice samples were citric, malic, lactic, and succinic acid. Statistical analyses indicated that, according to the milling stage, there were significant differences ($P<0.01$) in citric and malic acid, but not ($P>0.05$) in lactic and succinic acid (Table 3).

Compared to the endosperm, bran had very high levels of citric and malic acid content, with 151.3 and 17.20 mg/kg (db), respectively.

Lactic and succinic acid showed a homogenous distribution of the rice endosperm, although rice bran was rich in organic acid content. Frank *et al.* (2012) also detected succinic, fumaric, malic, and citric acid in rice.

Free amino acid content

Alanine, glycine, serine, proline, aspartic acid, glutamic acids, and asparagine were identified as free non-essential amino acids found in rice samples (Table 4). Moreover, lysine, isoleucine, and threonine were detected as free essential amino acids (Table 5). Statistical analyses indicated that there were significant differences ($P<0.01$ or $P<0.05$) in lysine, isoleucine, proline, asparagine, glycine, alanine, aspartic acid, and glutamic acid content depending on the processing stage, but not ($P>0.05$) in the serine and threonine content.

The results showed that serine is located homogenously in all layers of rice grains, and it is not affected by rice milling. The main discrepancies in glycine, proline, and asparagine content were observed between rice bran and rough rice samples. Proline and asparagine were not detected after rice dehulling and whitening. These results suggest that rice bran is a rich source of non-essential amino acids and that the elimination of bran causes the loss of some amino acids during rice processing. Higher levels of proteins, fats, vitamins, and minerals have been found in the outer layer of seeds compared to the inner layers (Saikusa *et al.*, 1994). For example, Lamberts *et al.* (2007) reported that the protein concentration decreased from the outer to the core endosperm of rice.

Moongngarm and Saetung (2010) indicated that the alanine, glycine, aspartic acid, and glutamic acid content of ungerminated rice samples was 7.48, 5.61, 7.31, and 9.61 mg/g, respectively. Similar results were determined when rice bran was extracted in hot water (Kim and Han, 2012). However, compared to the data shown here, lower amino acid concentrations have been reported elsewhere (Parrado *et al.*, 2006; Saikusa *et al.*, 1994). Such differences might be a result of the genetic characteristics of the plants and environmental factors such as soil properties.

The most abundant essential amino acid in rice was isoleucine, at 2.999 mg/g (db) in rice bran and 2.258 mg/g (db) in rough rice. Processing did not affect the isoleucine content after dehulling, so it may be concluded that this amino acid accumulates equally in the inner and outer layers of rice kernels.

Table 3. Organic acid content of rice samples (mg/kg, db).[1]

Sample code	Citric acid	Lactic acid	Succinic acid	Malic acid
Bran	151.3^{a}	99.13^{a}	2.48^{a}	17.20^{a}
1	68.2^{b}	73.77^{b}	1.11^{a}	4.07^{b}
2	64.3^{b}	70.93^{b}	2.31^{a}	2.10^{c}
3	43.9^{c}	66.29^{b}	2.80^{a}	1.80^{c}
4	42.6^{c}	68.01^{b}	2.12^{a}	2.02^{c}
5	25.6^{d}	64.56^{b}	2.02^{a}	1.84^{c}
6	21.4^{de}	57.61^{b}	2.31^{a}	1.57^{c}
7	12.5^{edf}	59.73^{b}	2.31^{a}	1.65^{c}
8a	7.52^{ef}	61.82^{b}	1.73^{a}	1.65^{c}
8b	4.39^{ef}	57.10^{b}	0.55^{a}	2.14^{c}
9	3.76^{f}	65.88^{b}	0.54^{a}	1.47^{c}
10	1.89^{f}	67.60^{b}	0.99^{a}	2.18^{c}
Significance	**	ns	ns	**

[1] Mean values followed by different superscript letters in the same column are significantly different according to Duncan's multiple range test ($P<0.05$). *$P<0.05$; **$P<0.01$; ns = not significant ($P>0.05$).

Table 4. Free non-essential amino acid content of rice samples (mg/g, db).[1]

Sample codes	Serine	Glutamic acid	Aspartic acid	Glycine	Alanine	Proline	Asparagine
Bran	2.631	1.008^{a}	0.641^{a}	0.261^{a}	0.267^{a}	0.913^{a}	2.276^{a}
1	2.548	0.792^{bac}	0.328^{bc}	0.175^{b}	0.196^{b}	0.033^{b}	0.286^{b}
2	2.273	0.557^{bdec}	0.346^{bc}	0.157^{b}	0.131b^{cd}	0.016^{b}	0.436^{b}
3	2.230	0.580^{bdec}	0.330^{bc}	0.154^{b}	0.104^{cd}	ND	0.471^{b}
4	2.287	0.827^{ba}	0.349^{bc}	0.162^{b}	0.155^{bc}	ND	0.697^{b}
5	2.671	0.709^{bdac}	0.478^{b}	0.163^{b}	0.162^{bc}	ND	0.579^{b}
6	2.080	0.371^{dec}	0.302^{bc}	0.138^{b}	0.071^{d}	ND	ND
7	2.264	0.280^{de}	0.260^{c}	0.136^{b}	0.090^{cd}	ND	ND
8a	2.091	0.247^{e}	0.196^{c}	0.124^{b}	0.062^{d}	ND	ND
8b	2.315	0.284^{de}	0.285^{bc}	0.124^{b}	0.061^{d}	ND	ND
9	2.116	0.244^{e}	0.254^{c}	0.122^{b}	0.059^{d}	ND	ND
10	2.107	0.317^{de}	0.218^{c}	0.123^{b}	0.058^{d}	ND	ND
Significance	ns	**	**	*	**	*	**

[1] Mean values followed by different superscript letters in the same column are significantly different according to Duncan's multiple range test (P<0.05). *P<0.05; **P<0.01; ns = not significant (P>0.05); ND = not detected.

Table 5. Free essential amino acid content of rice samples (mg/g, db).[1]

Sample codes	Isoleucine	Threonine	Lysine
Bran	2.999^{a}	0.542^{ab}	1.830^{a}
1	2.258^{ab}	0.699^{a}	0.644^{b}
2	1.478^{b}	0.508^{ab}	0.280^{b}
3	1.508^{b}	0.537^{ab}	0.413^{b}
4	1.811^{b}	0.566^{ab}	0.565^{b}
5	2.066^{b}	0.663^{ab}	0.510^{b}
6	1.910^{b}	0.548^{ab}	ND
7	1.937^{b}	0.492^{ab}	ND
8a	1.880^{b}	0.311^{b}	ND
8b	2.110^{b}	0.382^{ab}	ND
9	2.031^{b}	0.404^{ab}	ND
10	2.035^{b}	0.551^{ab}	ND
Significance	*	ns	**

[1] Mean values followed by different superscript letters in the same column are significantly different according to Duncan's multiple range test (P<0.05). *P<0.05; **P<0.01; ns = not significant (P>0.05); ND = not detected.

The lysine content was 1.830 and 0.644 mg/g (db) in rice bran and rough rice, respectively. Obviously, rice bran had very high lysine content, but it was not detected after processing of the rice kernels. Similar results have been obtained with other cereals (Rosenberg and Culik, 1957; Wu *et al.*, 2002).

Phenolic acid content

Vanillic, coumaric, gallic, and ferulic acids were the most abundant phenolic compounds present in rice samples. The ferulic acid content was significantly (P<0.01) reduced after debranning, which suggests that it is concentrated in the outer layers of the rice kernels (Table 6).

According to the literature, rice bran contains a great deal of ferulic acid (4-hydroxy-3-methoxycinnamic acid), which is commonly present as a cell wall component and exhibits strong antioxidant activity (Kim and Han, 2012), an anti-inflammatory effect, and a hypotensive effect (Srinivasan *et al.*, 2007). Numerous studies have reported that ferulic acid accumulates mostly in cereal bran (Shao *et al.*, 2014; Ti *et al.*, 2014; Zhou *et al.*, 2004). In addition, it has been reported that rice hull contains high levels of ferulic and coumaric acid varying from 1.51-2.04 mg/g and 4.88-6.37 mg/g, respectively (Nenadis *et al.*, 2013). It has also been determined that the ferulic acid content of rice bran and polished rice was 1.24 and 0.070 mg/g, respectively (Ti *et al.*, 2014). Zhou *et al.* (2004) reported that 70-90% of total phenolic acids were located in the rice bran of brown rice cultivars. Moreover, Liu *et al.* (2015) indicated that the concentration of phenolic acids decreased from the aleuronic layer to the endosperm in brown rice.

As expected, the white rice production process decreased total bioactive compounds such as phenolic acids. Frequent consumption of white rice may lead to an increase of some diseases. Some studies have reported that the consumption of white rice in developed countries may cause deficiencies

Table 6. Phenolic acid content (mg/kg, db) of rice samples.[1]

Sample code	Ferulic acid	Vanilic acid	Gallic acid	Coumaric acid
Bran	824.28^{a}	246.55^{c}	107.11^{e}	88.93^{a}
1	556.42^{cd}	252.27^{a}	108.43^{de}	90.87^{a}
2	359.60^{cd}	251.14^{ab}	110.19^{abc}	83.18^{b}
3	389.70^{bc}	253.00^{a}	111.41^{a}	82.39^{bc}
4	448.95^{cd}	251.26^{ab}	110.87^{ab}	80.68^{cd}
5	313.17^{cd}	246.53^{c}	108.77^{cd}	80.06^{d}
6	341.92^{cd}	247.73^{bc}	109.66^{bcd}	79.53^{de}
7	317.54^{d}	248.13^{bc}	109.87bd^{ac}	77.65^{ef}
8a	280.36^{d}	247.03^{c}	109.07^{cd}	77.32^{ef}
8b	226.31^{d}	246.52^{c}	109.18^{bcd}	77.45^{ef}
9	276.88^{d}	246.92^{c}	109.21^{bcd}	77.50^{ef}
10	226.23^{d}	246.36^{c}	109.13^{bcd}	77.12^{f}
Significance	**	**	**	**

[1] Mean values followed by different superscript letters in the same column are significantly different according to Duncan's multiple range test (P<0.05). **P<0.01.

in essential minerals, vitamins, protein, dietary fibre, and nutrition (Liu *et al.*, 2015).

Antioxidant activity

ABTS assays provide useful information about antioxidant properties in grain. The antioxidant activity of rice samples was significantly (P<0.01) affected by processing (Figure 3). The highest antioxidant activity was found in rice kernels before debranning (Figure 3) because of the high ferulic acid content, as expected. It is known from the literature that the antioxidant activities of cereals are mostly sourced from the phenolic compounds of bran and that ferulic acid is a very important phenolic compound. In addition, the presence of tocols, oryzanol, and phytosterols can contribute to these results. Antioxidant activity was reduced from 126.23 to 60.76 μM TEAC/g (db) because of the removal of the bran, which meant that 52% of antioxidant activity was lost. In addition, the antioxidant activity of rice bran was detected at 428.97 μM TEAC/g (db).

Various antioxidant activity values have been reported in different studies. It was determined that the ABTS antioxidant activity of Brazilian rice cultivars varied from 1,464-1,897 μM TEAC/g in paddy rice samples (Palombini *et al.*, 2013). Sharma *et al.* (2014) found that the antioxidant activity of brown and milled rice was 36.2 and 8.6 mmol/g, respectively. In contrast, the antioxidant activity of rice endosperm and rice bran was found to be more similar to 24.4 and 52.4 μM TEAC/g, respectively (Goufo and Trindade, 2014).

Unpolished brown rice has high phenolic acid content and antioxidant activity. As expected, the progression of processing stages decreased the concentration of several bioactive compounds. In general, whiter milled rice is desirable in markets (Lamberts *et al.*, 2007). However, whole brown rice is rarely preferred because of its poor sensory quality, hard texture, and long cooking time (Liu *et al.*, 2015). These results indicate that the consumption of brown rice is important in terms of nutritional value in spite of its low textural quality and desirability. Therefore, new rice products with a short cooking time and high sensorial quality should be developed from whole rice grain.

Colour properties

The L*, a*, and b* colour values of samples were significantly (P<0.01) affected the rice debranning process. The lightness (L*) value of paddy rice was 46.03 and 46.47 (Figure 4) and reached 80.55 for glazed rice. Lightness increased while redness and yellowness decreased during rice processing as bran was removed. Similar results were found by Lamberts *et al.* (2007), and colour pigments were uniformly distributed throughout the rice endosperm. According to Falade and Christopher (2015), the L* value of refined rice flour varied between 85.21 and 90.03.

The WI of the samples increased from 46.03 to 80.54 during the rice production process (Figure 5).

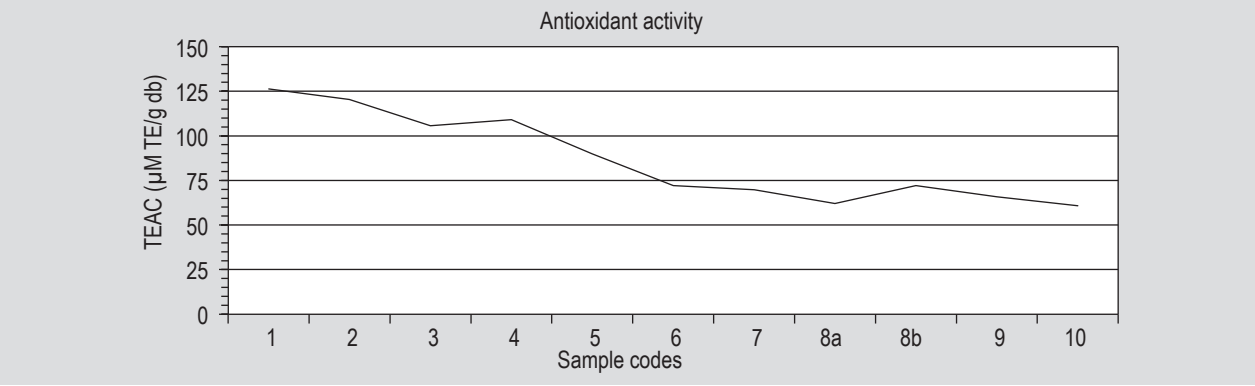

Figure 3. Trolox equivalent antioxidant capacity (TEAC) antioxidant activity of rice samples

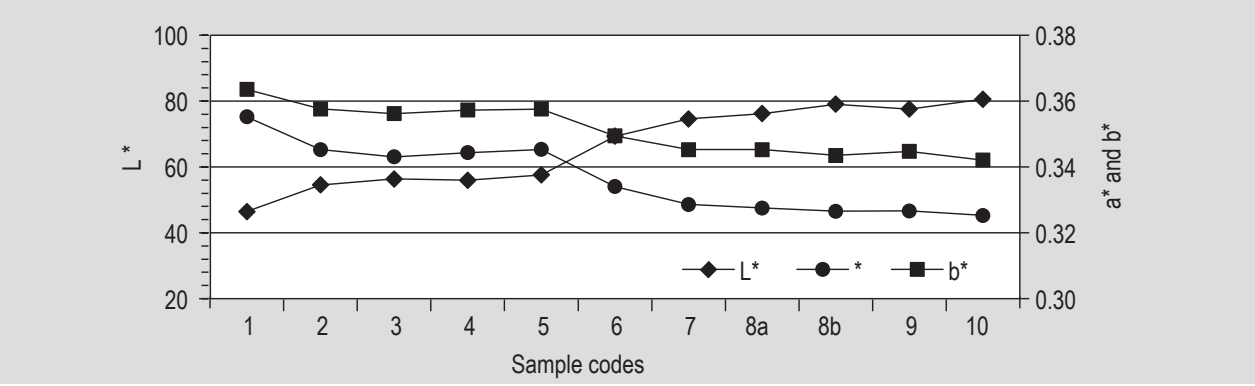

Figure 4. L*, a*, and b* colour values of rice samples. L* represents the light-dark, a* the red-green, and b* the yellow-blue properties of the samples.

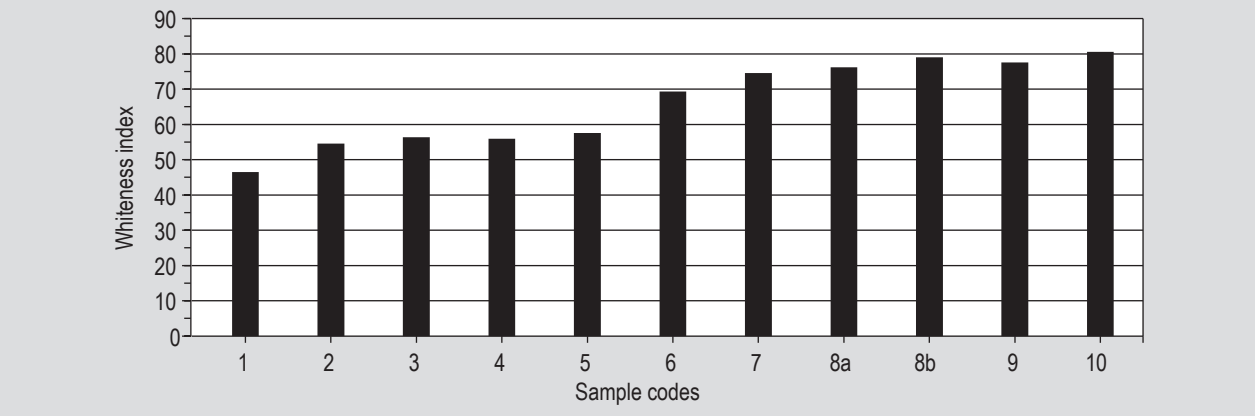

Figure 5. Whiteness index (WI) of rice samples.

4. Conclusions

According to the data shown, rice debranning mainly caused the loss of several compounds, especially sugars, some organic acids, phenolic compounds (mainly ferulic acid), and both essential and non-essential amino acids. Free proline, asparagine, and lysine were not detected after rice dehulling and whitening. In particular, about half of the antioxidant activity and phytic acid content in rice were lost because of bran removal.

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