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Nutrient Components and Antioxidant Activity in Hassawi Rice and Basmati Rice Varieties

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محتوى الأرز الحساوي والأرز البسمتي من المغذيات ومضادات الأكسدة

منيرة قاسم المسلّم ورندة مقبل القرشي

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Antioxidant activity, Basmati rice, flavonoids, Hassawi rice, nutrients, phenolic compounds

أرز بسمتي، أرز حساوي، فلافونويدات، مركبات فينولية، مغذيات، نشاط مضاد الأكسدة

ABSTRACT

Hassawi rice (*Oryza Sativa L.*) is an indigenous reddish-brown rice of the Indica variety grown and traditionally consumed in the Al-Ahsa oasis in the Eastern Province of Saudi Arabia. The main objectives of this study were to assess the nutrients and antioxidant activity in Hassawi rice in comparison to those in Basmati rice. The results revealed that Hassawi rice had significantly higher phenolic compound and flavonoid contents ($p<0.01$) and antioxidant activity ($p<0.05$) than those of Basmati rice. The macro- and micro-mineral and water-soluble vitamin contents were also significantly higher ($p<0.05$) in Hassawi rice than in Basmati rice (with the exception of niacin, selenium, and boron). This study concluded that Hassawi rice plays an important role in the human diet as a nutritional and functional food. Further study is required to evaluate the benefits of Hassawi rice on human health.

الملخص

الأرز الحساوي (*Oryza Sativa L.*) هو أرز بني محمر أصلي من صنف إنديكا المزروع والمستهلك تقليديًا في واحة الأحساء في المنطقة الشرقية بالمملكة العربية السعودية. والهدف الرئيس لهذه الدراسة هو تقييم المغذيات ونشاط مضادات الأكسدة في الأرز الحساوي مقارنة بالأرز البسمتي. وقد أظهرت النتائج أن الأرز الحساوي يحتوي على نسبة أعلى من الفينولات والفلافونويدات ($p<0.01$) ونشاط مضادات الأكسدة ($p<0.05$) مقارنة بتلك الموجودة في الأرز البسمتي. كما أن محتوى الفيتامينات الذائبة في الماء والأملاح المعدنية الكبرى والصغرى كانت أعلى بشكل ملحوظ ($p<0.05$) في الأرز الحساوي بالمقارنة بالأرز البسمتي (باستثناء النياسين والسيلينيوم والبورون). وقد خلصت الدراسة إلى أن الأرز الحساوي يلعب دورًا هامًا في النظام الغذائي البشري كغذاء وظيفي. وتوصي الدراسة بإجراء المزيد من البحث لتقييم فوائد الأرز الحساوي على صحة الإنسان.

1. Introduction

Rice (*Oryza sativa*) is one of the most important cereal food crops worldwide (Wang and Li, 2005) and is considered a main source of both carbohydrates (CHOs) and proteins for approximately two-thirds of the world's population (Ciulu *et al*, 2018). In addition to the CHO and protein contents, pigmented rice can be a source of bioactive compounds, including phenolic compounds (Ito and Lacerda, 2019; Samyor *et al.*, 2017). Bioactive compounds from natural sources have recently attracted increasing attention from the scientific community due to their medicinal and pharmaceutical applications (Porrini and Riso, 2008; Guaadaoui *et al.*, 2014; Biesalski *et al.*, 2009). Bioactive compounds exert an effective role in protecting the human body from chronic degenerative diseases due to their ability to scavenge any intermediates of free radicals resulting from oxidative species (Van Horn *et al.*, 2008; Temple, 2000). Studies have found that whole grain rice (e.g., brown, black, and purple) contained higher contents of phenolic compounds than white rice (Tian *et al.*, 2004; Shao and Bao, 2015).

In Saudi Arabia, a wide range of rice varieties are available, including Hassawi rice. Hassawi rice (*Oryza sativa* L.) is an indigenous, reddish-brown rice of the *Indica* variety. It has been cultivated in the Al-Ahsa area of the Eastern Province of Saudi Arabia for hundreds of years, and it is characterized by its wide adaptability to soil salinity and drought (Bimpong *et al.*, 2014). Several studies have been carried out on Hassawi rice in terms of its cultivation (Al-Khayri *et al.*, 2000; Al-Noaim *et al.*, 2004; Al-Saeedi *et al.*, 1999; Bimpong *et al.*, 2014), nutritional composition (Al-Bahrany, 2002; Al-Mssallem *et al.*, 2010; 2011; Hadid and Elsheikh, 2012), genomics (Zhang *et al.*, 2012) and clinical effects on blood glucose and insulin responses (Al-Mssallem *et al.*, 2011). Nutritionally, Hassawi rice has a lower total CHO content (Al-Mssallem *et al.*, 2011) and higher total protein content (Al-Mssallem and Al-Mssallem 1997; Hadid and Elsheikh, 2012) than white long-grain rice. Furthermore, the levels of ash, proximate fat and non-starch polysaccharides are higher in Hassawi rice than in white long grain rice (Al-Bahrany, 2002; Al-Mssallem *et al.*, 2011; Hadid and Elsheikh, 2012). In addition, Hassawi rice had a moderate impact on blood glucose and insulin responses (Al-Mssallem *et al.*, 2011).

To our knowledge, data on the contents of phenolic compounds in Hassawi rice and their antioxidant activity are not available. As such, the objective of this study was to investigate and compare the nutrients and total phenolic compounds in Hassawi rice and Basmati rice. Our hypothesis was that Hassawi rice has higher nutritional and functional properties than widely consumed white Basmati rice.

2. Materials and Methods

2.1. Preparation of the Rice:

Hassawi rice and white Basmati rice were collected from a local market in Al-Ahsa, Eastern Province, Saudi Arabia. The samples were ground to a fine powder in a mechanical blender, sieved (100 mesh) to a uniform size and then frozen at -20 °C until analysis.

2.2. Non-starch Polysaccharide, Mineral and Vitamin Analyses:

Non-starch polysaccharides (NSPs) were determined using an ANKOM dietary fibre analyser (AOAC-991.43). The mineral content was analysed using inductively coupled plasma-mass spectrometry (ICP-MS). Vitamin B analysis was carried out using ELISA (Biopharm

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P1006, P1007, and P1004 for B1, B2, and B3, respectively), while vitamin C was analysed using HPLC-PDA (M-H079). All analyses were carried out in accordance with the AOAC procedures at the Inspection Diagnostics Analysis Consultation (IDAC) Laboratory, Riyadh, Saudi Arabia.

2.3. Determination of Antioxidant Activity and Phenolic Compounds:

2.3.1. Preparation of the rice extract

The powdered samples (10 g) were mixed with 100 ml of 80% methanol, vortexed for 15 min and kept at room temperature for 1 h. The mixture was then centrifuged at 3000 rpm for 5 min at room temperature. This process was repeated three times, and then supernatants were collected and filtered through 0.45 µm filter paper. The solvents were evaporated by a rotary evaporator (IKA RV 10 Control, Werke GmbH and Co. KG, Germany) at 40 °C, and then the extracts were adjusted to pH 2 with 1% HCl. The extracts were protected from light by foil and immediately subjected to analysis of total phenolic acid and flavonoid contents and antioxidant activity.

2.3.2. Determination of total phenolic content

The total phenolic acid content was assessed by applying the Folin-Ciocalteu method and using gallic acid as a standard (Sun *et al.*, 2002). A 0.5 ml aliquot of a sample extract or standards was mixed with 0.5 ml of Folin-Ciocalteu reagent (SOMATCO, Saudi Arabia). After incubation for 3 min at room temperature, 4 ml of saturated sodium carbonate solution was added, and the tube was placed on a shaker for 25 min at room temperature. The absorbance of the sample was measured at 650 nm using a UV-1800 spectrophotometer (Shimadzu, China). The total phenolic content was calculated using a calibration curve constructed with the standard (gallic acid), and the results are expressed as gallic acid equivalents (GAE) mg/g DW (mean ± SD; n = 3, triplicate analysis).

2.3.3. Total flavonoid content

The total flavonoid content was measured using a spectrophotometric assay based on aluminium complex formation (Silva *et al.*, 2015; Ostertag *et al.*, 2010; Pękal *et al.*, 2014;). Briefly, 1 ml of sample extract was added into a 15 ml tube containing 4 ml of distilled water. The mixture was mixed with 0.3 ml of 5% sodium nitrite and incubated for 5 min at room temperature. Then, 0.3 ml of 10% $AlCl_3$ was added, and after an additional 6 min, 2 ml of 1 M sodium hydroxide was added, and the total volume was brought to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 512 nm using a UV-VIS spectrophotometer. Total flavonoids are expressed as catechin equivalents (CE) mg/g DW (mean ± SD; n = 3, triplicate analysis).

2.3.4. Determination of antioxidant activity

The antioxidant activity of the rice extracts was determined using the 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay (Amarowicz *et al.*, 2004). In this assay, 1 ml of sample extract was mixed with 1 ml of 0.1 mM DPPH in methanol. The absorbance was measured after 30 min of incubation at room temperature in a dark room at 515 nm using a UV-VIS spectrophotometer. The result was calculated as a percentage using the following equation:

$$\text{DPPH scavenged (\%)} = ((A_{con} - A_{test})/A_{con}) \times 100$$

where A_{con} is the absorbance of the control reaction (DPPH) and A_{test} is the absorbance of the rice extract.

2.4. Statistical Analysis:

Statistical analysis was performed using Statistical Package for Social Sciences software (SPSS software, Version 23.0). Calibration curves of total phenolic compounds and flavonoids were generated with Microsoft Office Excel 2019. The results are expressed as percentages and means ± SD, unless stated otherwise. Differences in antioxidant activity and total phenolic acid and flavonoid contents between Hassawi rice and Basmati rice were evaluated by paired t-tests. All data were examined using a two-tailed approach, with a level of $p<0.05$ considered significant.

3. Results

3.1. Non-Starch Polysaccharide, Mineral and Vitamin Analyses:

Table 1 illustrates the non-starch polysaccharide, macro- and micro-mineral, and water-soluble vitamin contents of Hassawi rice and Basmati rice. Hassawi rice had a considerable amount of non-starch polysaccharides, with a content that was 6 times that found in Basmati rice (6.22 vs 0.96% DW). It was obvious that the most abundant macro-minerals were phosphorus and potassium in both types of rice. The calcium, potassium, and phosphorus contents in Hassawi rice were almost double those observed in Basmati rice. Zinc, on the other hand, was the most abundant micro-mineral in both types of rice, followed by iron. Zinc and copper were found in Hassawi rice at a content that was double that in Basmati rice. The lead, mercury, arsenic, and cadmium contents were not detectable, with detection limits of 0.004, 0.005, 0.08, and 0.004 ppm, respectively.

For thiamine and riboflavin, Hassawi rice had significantly higher contents of these water-soluble vitamins ($p<0.01$), the levels of which were almost double those found in Basmati rice (Table 1). However, the niacin content was higher in Basmati rice than in Hassawi rice (1.26 vs 0.73 mg/100 g). Vitamin C was not detected, and its limit of quantitation was < 0.01 mg/100 g.

Table 1 Non-starch polysaccharide, macro- and micro-mineral, and water-soluble vitamin contents in Hassawi rice and Basmati rice (raw matter).

Nutrient content	Hassawi rice	Basmati rice	p-value
Non-starch polysaccharides (g/100 g)			
Non-starch polysaccharides (NSPs)	6.22±0.14	0.96±0.03	0.000
Macro-minerals (ppm)			
Calcium, Ca	110.82±17.57	42.15±9.52	0.005
Phosphorus, P	3326.44±28.93	1146.75±6.48	0.000
Potassium, K	2732.65±27.84	1327.54±14.79	0.000
Sodium, Na	25.37±6.32	19.39±6.48	0.021
Micro-minerals (ppm)			
Zinc, Zn	30.67±7.24	12.83±2.38	0.024
Iron, Fe	13.69±0.38	6.68±4.99	0.153
Copper, Cu	3.38±0.06	1.57±0.04	0.000
Selenium, Se	0.16±0.01	0.19±0.01	0.053
Lead, Pb	ND	ND	
Mercury, Hg	ND	ND	
Arsenic, As	ND	ND	
Boron, B	2.60±0.82	3.76±0.22	0.188
Cadmium, Cd	ND	ND	
Water-soluble vitamins (mg/100 g)			
Thiamine, B1	0.53±0.02	0.18±0.01	0.004
Riboflavin, B2	0.17±0.01	0.08±0.01	0.004
Niacin, B3	0.73±0.01	1.26±0.05	0.005
Vitamin, C	ND	ND	

3.2. Antioxidant Activity and Phenolic Compounds in Rice Extracts:

Table 2 shows the total phenolic acid and flavonoid contents of the two rice samples. The results show significant differences between Hassawi rice and Basmati rice in both total phenolic acid and flavonoid contents. The Hassawi rice had a higher total phenolic acid content (118±2.6 GAE mg/g) than Basmati rice (31±1.0 GAE mg/g) ($p<0.001$). In contrast, Hassawi rice exhibited a higher total flavonoid content (61.66±1.52 CE mg/g) than Basmati rice (8.66±1.52 CE mg/g) ($p<0.001$), as shown in Table 2.

3.3. Antioxidant Activity of Dry Rice Extracts:

The rice extracts in this study were measured by the DPPH-free radical scavenging activity assay (Table 2). The Hassawi rice showed the highest free-radical scavenging activity of 76.4%, while the Basmati rice exhibited a significantly lower antioxidant activity (29.3%) ($p<0.05$).

Table 2: Total phenolic acid and flavonoid contents and antioxidant activity of Hassawi rice and white Basmati rice extracts (mean±SD).

Variable	Hassawi rice	Basmati rice	p-value
Total phenolic acids (GAE mg/g)	118±2.64	31±1.00	0.000
Total flavonoids (CE mg/g)	61.66±1.52	8.66±1.52	0.001
Antioxidant activities (%)	76.43 ± 3.76	29.35± 5.54	0.013

4. Discussion

Hassawi rice is a variety of rice commonly consumed in the eastern part of Saudi Arabia, and it is considered to have a better nutritional quality than that of other types of white rice.

In this study, the bioactive compounds in Hassawi rice included nutritive and non-nutritive bioactive components. The nutritive bioactive components in Hassawi rice included NSPs and selenium. It was obvious that NSPs in Hassawi rice were approximately 548% higher than those in Basmati rice. However, when NSPs in Hassawi rice were compared with those found in the literature for brown rice, the NSP content was approximately 53% higher in Hassawi rice than in brown rice (USDA, 2016). This considerable level of NSPs in Hassawi rice indicates the importance of this type of rice as a source of dietary NSPs. Similarly, Al-Mssallem *et al.* found that the NSP content in Hassawi rice was almost double that found in Uncle Ben's rice (Al-Mssallem *et al.*, 2011). The high content of NSPs in Hassawi rice could be explained by the fact that Hassawi rice is an unprocessed rice that retains its outer bran layer prior to consumption. Thus, Hassawi rice contains higher levels of NSPs and nutrients than Basmati rice, which is typically bleached and stripped of its bran prior to consumption.

Selenium was also detected in Hassawi rice and is considered the second most abundant nutritive bioactive compound in Hassawi rice after NSPs. In addition to its nutritional value, selenium possesses antioxidant and anti-inflammatory effects and protects the human body from oxidative stress (Rayman, 2012).

In terms of non-nutritive bioactive compounds, our results revealed that the contents of phenolic acids and flavonoids and the antioxidant activity in Hassawi rice were higher than those found in white Basmati rice. The higher content of phenolic compounds and antioxidant activity in Hassawi rice were partially attributed to its reddish-brown colour. Similar findings have been reported for pigmented rice, which has been shown to possess more phenolic compounds than white rice (Deng *et al.*, 2013). This feature of Hassawi rice confirmed its functional properties in addition to its nutritional value. Studies have reported that the high consumption of fruits, vegetables, and whole grains plays an essential role in preventing damage to the human body from oxidative stress (Miller and Engel, 2006; Miller *et al*, 2000; Panala *et al*, 2009; Stratil *et al*, 2007). Thus, regular consumption of such pigmented rice can contribute to improving human health and protecting the human body against chronic degenerative diseases (Gao *et al.*, 2018; Muntana and Prasong, 2010; Samyor *et al.*, 2017).

Although Hassawi rice is used in its natural form and is not fortified with nutrients, its contents of some minerals and vitamins were higher than those of white Basmati rice. In this study, phosphorus was the most abundant mineral in Hassawi rice, followed by potassium, and these findings were consistent with those previously reported in the literature (Al-Bahrany, 2002).

The results showed that the most abundant water-soluble vitamin in both types of rice was niacin, the content of which was higher in Basmati rice than in Hassawi rice. This could be explained by the fact that most processed rice is fortified by the addition of some vitamins and minerals. However, the contents of both thiamine and riboflavin were higher in Hassawi rice than in Basmati rice. These two water-soluble vitamins were also higher in Hassawi rice than in long-grain white rice according to the literature (USDA, 2016).

In conclusion, Hassawi rice has been shown to possess higher nutrient and total phenolic compound contents and antioxidant activity than Basmati rice, so we could fully accept our hypothesis that Hassawi rice would have higher nutritional and functional properties than Basmati rice.

To our knowledge, this study is the first to investigate the antioxidant activity and phenolic compounds in Hassawi rice. Further studies are needed to investigate the prebiotic activity of Hassawi rice NSPs and phenolic compounds in promoting the growth of beneficial gut bacteria, which may be considered important in preventing some degenerative diseases and improving general human health.

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Bios

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