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Chemical, nutritional and sensory profiles of different pigmented rice varieties impacted by cooking process

Perfis químicos, nutricionais e sensoriais de diferentes variedades de arroz pigmentado impactadas pelo processo de cozimento

Perfiles químicos, nutricionales y sensoriales de diferentes variedades de arroz pigmentado afectadas por el proceso de cocción

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Abstract

Pigmented rice has several health benefits associated with the presence of bioactive compounds and its hypoallergenic properties. The cooking process, as it involves high temperatures, can cause changes in the phytochemical and antioxidant profile. Thus, the objective was to study the nutritional and sensory properties of pigmented rice, the effect of the cooking process, comparing black and red rice genotypes developed by the Institution of Agricultural Research and Rural Extension of Santa Catarina (Epagri) to commercial varieties found in the Brazilian market. Epagri´s black rice had higher levels of protein, fat, ash, fiber and fatty acids than commercial black rice. The levels of phenolic compounds and antioxidants were higher in the Epagri rice genotypes than in the commercial varieties for both pigmented rice. When comparing both pigmented rice, black rice showed higher chemical composition, phytochemical profile and antioxidant properties than red rice. Cooking did not affect commercial black rice phenolic and caused a 28% reduction in Epagri black rice, while red rice reduced 60%. Flavonoids reduced 50% in commercial and Epagri genotypes after cooking. Proanthocyanidins were reduced by 79 and 64% in commercial and Epagri red rice, respectively, and anthocyanins were reduced by 56 and 51% in commercial and Epagri black rice, respectively. Regarding the sensory analysis, raw and cooked pigmented rice showed 70 and 80% of acceptability, respectively.
Keywords: Black rice; Red rice; Phytochemicals; Cooking; Consumer acceptability.

Resumo

O arroz pigmentado apresenta diversos benefícios à saúde associados à presença de compostos bioativos e por suas propriedades hipoalergênicas. O processo de cozimento, por envolver altas temperaturas, pode causar alteração no perfil fitoquímico e antioxidante. Assim, objetivou-se estudar as propriedades nutricionais e sensoriais do arroz

pigmentado, o efeito do processo de cozimento, comparando genótipos de arroz-preto e vermelho desenvolvidos pela Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri) com variedades comerciais encontradas no mercado brasileiro. O arroz-preto desenvolvido pela Epagri apresentou maiores teores de proteína, gordura, cinzas, fibras e ácidos graxos do que o arroz-preto comercial. Os teores de compostos fenólicos e antioxidantes foram maiores nos genótipos de arroz da Epagri do que nas variedades comerciais para ambos os arrozes pigmentados. Ao comparar ambos os arrozes pigmentados, o arroz-preto apresentou maior composição química, perfil fitoquímico e propriedades antioxidantes do que o arroz-vermelho. O cozimento não afetou os fenólicos do arroz-preto comercial e causou uma redução de 28% no arroz-preto Epagri, enquanto no arroz-vermelho reduziu 60%. Os flavonóides reduziram 50% nos genótipos comercial e Epagri após o cozimento. As proantocianidinas foram reduzidas em 79 e 64% nos genótipos de arroz-vermelho comercial e Epagri, respectivamente, e as antocianinas diminuíram em 56 e 51% no arroz-preto comercial e Epagri, respectivamente. Em relação à análise sensorial, o arroz pigmentado cru e cozido apresentaram 70 e 80% de aceitabilidade, respectivamente.
Palavras-chave: Arroz-preto; Arroz-vermelho; Fitoquimicos; Cozimento; Aceitabilidade do consumidor.

Resumen
El arroz pigmentado tiene varios beneficios para la salud asociados con la presencia de compuestos bioactivos y sus propiedades hipoalergénicas. El proceso de cocción, al involucrar altas temperaturas, puede provocar cambios en el perfil fitoquímico y antioxidante. Así, el objetivo fue estudiar las propiedades nutricionales y sensoriales del arroz pigmentado, el efecto del proceso de cocción, comparando los genotipos de arroz negro y rojo cultivados por la Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri) con variedades comerciales encontradas en el mercado brasileño. El arroz negro Epagri exhibió niveles más altos de proteínas, grasas, cenizas, fibra y ácidos grasos que el arroz negro comercial. Los niveles de compuestos fenólicos y antioxidantes fueron mayores en los genotipos de arroz Epagri que en las variedades comerciales para ambos arroces pigmentados. Al comparar ambos arroces pigmentados, el arroz negro mostró mayor composición química, perfil fitoquímico y propiedades antioxidantes que el arroz rojo. La cocción no afectó los compuestos fenólicos del arroz negro comercial y provocó una reducción del 28% en el arroz negro Epagri, mientras que el arroz rojo redujo un 60%. Los flavonoides se redujeron en un 50% en los genotipos comercial y Epagri después de la cocción. Las proantocianidinas se redujeron en un 79 y 64% en arroz rojo comercial y Epagri, respectivamente, y las antocianinas se redujeron en un 56 y 51% en arroz negro comercial y Epagri, respectivamente. En cuanto al análisis sensorial, el arroz pigmentado crudo y cocido mostró 70 y 80% de aceptabilidad, respectivamente.
Palabras clave: Arroz negro; Arroz rojo; Fitoquímicos; Cocción; Aceptabilidad del consumidor.

1. Introduction

The consumption of rice is essential due to its important nutritional properties, especially in countries such as Brazil where the population consumes it daily. Rice cultivars with pigmented pericarp became popular due to their special composition related to health benefits. In Brazil, red and black rice are important crops and are classified as the same species as the traditional white rice known as *Oryza sativa* L. (Martins et al., 2021).

Black and red rice crops present in their bran layer higher contents of anthocyanin and proanthocyanidins, respectively, which are responsible for the characteristic natural color of the grain. These phenolic compounds show great antioxidant properties that play important role in human health by decreasing oxidative stress, preventing hypocholesterolemia and related metabolic syndromes (Aalim et al., 2021; Wang et al., 2020; Shao et al., 2018).

In addition, pigmented rice has significantly higher levels of protein, dietary fiber and bioactive compounds than non-pigmented grains, which makes the study of its acceptability very interesting (Tiozon et al., 2021; Meza et al., 2021 and 2019; Melini et al., 2019; Samyor et al., 2017). Rice grain must to be cooked for consumption. However, the cooking process involves high heating temperatures, which can cause the degradation of bioactive compounds and antioxidant properties in pigmented and white rice (Thuengtung & Ogawa, 2020; Massaretto et al., 2011). The impact of cooking on phenolic profile of black rice was observed by reduction in anthocyanins contents, which were described by Hiemori (2009) and Zaupa et al. (2015). Although, the literature has extensively described the quality of pigmented rice, the effect of cooking on the phytochemical composition and sensory analysis of red and black rice has not yet been explored.

Therefore, this study focused on the better understanding about the pigmented rice genotypes cultivated in Brazil. It is important to highlight that few studies using the Brazilian pigmented rice have been described. In this context, this work aimed to evaluate the nutritional and phytochemical characteristics of pigmented grains; the effect of cooking process on bioactive compounds and the sensory acceptance test of raw and cooked black and red rice, comparing the commercial varieties to the new genotypes developed by Epagri, in Brazil.

2. Methodology

2.1 Pigmented rice

Commercial samples of whole black (BRC, n = 5) and red rice (RRC, n = 5) (*Oryza sativa* L.) were obtained from the market of São Paulo, SP – Brazil. Cultivars and genotypes of whole black (BRE, n = 10) and red rice (RRE, n = 4) (*Oryza sativa* L.) were provided by Epagri/Itajaí Experimental Station (Wickert et al., 2014). The rice cultivars were registered at the Ministry of Agriculture, Cattle and Supplying under number 30233 for red rice (*Oryza sativa* var. SCS119 Rubi) and number 30234 for black rice (*Oryza sativa* var. SCS120 Ônix). Samples were stored in plastic bottles at 4˚C ± 2˚C protected from light until use. The samples were milled with an Analytical Mill A10, Kinematica GAC (Luzern, Switzerland) until reaching a homogeneous powder (≥ 80 mesh, ≤ 177 µm).

2.2 Cooking parameters

The black and red rice were prepared in the proportion of 1:5 and 1:4 of rice:water, respectively. The cooking times were defined as 45 and 35 minutes for black and red rice, respectively. Pigmented rice were cooked with 2.5% NaCl and soy oil (15 mL).

2.3 Proximate composition of the raw samples

Protein, fat, ash and dietary fiber levels of raw samples were determined, in triplicate, according to the standard methods of the Association of Official Analytical Chemists – AOAC (2012). Digestible carbohydrates were calculated by difference, subtracting from one hundred the contents of moisture, ash, protein, fat, and dietary fiber. Results were expressed as g/100g on a dry basis (d.b.).

2.4 Fatty acids profile of the raw samples

Extraction of fatty acids were conducted by using hexane, 0.5M KOH in methanol and 1.0M H_2SO_4 in metanol at 80 ºC during 1h. The methyl esters formed were recovered with hexane. Analysis was performed using a 17A Shimadzu GC. Fatty acids were separated on a SP2340 capillary column (60 m length × 0.25 mm i.d. × 0.25 µm film thickness), and hydrogen was used as the carrier gas at a constant flow rate of 17 mL/min. The injection volume was 0.5 µL in split mode (1:100) at 240°C injector temperature. The oven temperature was initially at 140°C, held for 5 minutes, increased to 240°C at 4°C/min, and held for 10 minutes. The fatty acids composition was determined by comparing the retention time and the peak area to respective methyl esters standards (47885-U FAME mix, Supelco, EUA). Results were expressed in g per 100g of sample. All samples were analyzed in triplicate.

2.5 Anthocyanins profile of the raw and cooked samples

The extraction was conducted mixing 5.0 g of black rice with 50 mL of acidified methanol (99% methanol: 1% 1N HCl) during 8 h under refrigeration (Degenhardt et al., 2000). The extracts were filtered, evaporated at 30 º C and dried on nitrogen gas. The dried anthocyanin extract was ressuspended in 0.5% HCl. The supernatant (15 mL) was diluted with 50 mL of 10% CH_2O_2. To remove sugars, the extract was applied in Amberlite-XAD 7 column (25 cm x 3 cm) (Sigma-Aldrich,

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Germany), with 1 L of water deionized. The elution was made with 500 mL acidified methanol (19 metanol:1 CH_2COOH, v/v). The eluate was concentrated in rotative evaporator and stored at -35°C. The quantification of anthocyanins were performed by High-Performance Liquid Chromatography (HPLC) using a Shimadzu LC-20AD chromatograph equipped with a diode-array detector DAD (SPD-M20, Shimadzu), a vacuum degasser, quaternary pumps, an automated sample injector and a column C_{18} Shim-pack CLC-ODS (250mm×4.6 mm, 5 µm, Shimadzu) column.

2.6 Bioactive compounds of the raw and cooked samples

2.6.1 Extraction

Methanolic extracts were prepared by mixing 1 to 2 g of samples with 15 mL 70% methanol (Massaretto et al., 2011), in triplicate, and used to determine the total phenolics (TPC), total flavonoids (TFC), total proanthocyanidins (TPAC), and the antioxidant capacity (oxygen radical absorbance capacity – ORAC and 1,1-diphenyl-2-picrylhydrazyl – DPPH).

2.6.2 Total phenolics

TPC was determined using the Folin-Ciocalteu colorimetric method (Singleton et al.,1999). Methanolic extract (500 µL) was diluted with 500 µL Folin-Ciocalteu reagent and 4 mL 0.5M NaOH, and the absorbance was measured at 760 nm. A ferulic acid standard curve was used to calculate the TPC, and the results were expressed as mg ferulic acid equivalent per 100 g of sample.

2.6.3 Total flavonoids

The methanolic extract (250 µL) was mixed with 1250 µL distilled water, 75 µL 5% sodium nitrite, and 150 µL 10% aluminum chloride. After 5 minutes of reaction, 500 µL 1M NaOH was added and the absorbance was measured at 510 nm (Xu & Chang, 2007). A catechin standard curve was used to calculate the TFC, and the results were expressed as mg catechin equivalent per 100 g of sample.

2.6.4 Total proanthocyanidins

The methanolic extract of red rice (500 µL) was mixed with 3000 µL 4% vanillin methanol solution and 1500 µL concentrated HCl, and the absorbance was measured at 500 nm (Xu & Chang, 2007). A catechin standard curve was used to calculate the TPAC, and the results were expressed as mg catechin equivalent per 100 g sample.

2.6.5 Total anthocyanins

For the extraction of total anthocyanins (TAC), 1 g of black rice flour was mixed with 50 mL acidified methanol (methanol:1 N HCl (85:15 v/v) in triplicate, and the absorbance (*A*) of the solution was measured at 535 nm (Abdel-Aal & Hucl, 2003). TAC was calculated according to Eq. 1, and the results were expressed as mg equivalent cyanidin-3-*O*-glucoside per 100 g of sample.

$$TAC = {}^{A \times MW \times V \times 10^5}/_{\varepsilon \times WS} \qquad \textit{Eq. 1}$$

where: *MW*, molecular weight of cyanidin-3-*O*-glucoside (449 g/mol); *V*, total volume of anthocyanin extract (L); ε, molar absorptivity of cyanidin-3-*O*-glucoside (25.965 L.mol/cm); and *WS*, sample weight (g).

2.7 Antioxidant capacity of the raw and cooked samples

2.7.1 ORAC radical scavenging assay

The methanolic extracts (25 µL) were mixed with 300 µL 75 mM phosphate buffer (pH 7.4) and 150 µL 40 x 10^{-9} M fluorescein, and incubated at 37º C for 30 minutes. Then, 25 µL 0.15M AAPH was added, and the fluorescence was measured at excitation/emission wavelength of 493/515 nm (Zhang et al., 2018). A standard curve of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was constructed, and the results were expressed as mg Trolox equivalent per 100 g of sample.

2.7.2 DPPH radical scavenging assay

The methanolic extracts (50 µL) were mixed with 150 µL 7x10^{-5} M DPPH solution. Absorbance (*A*) readings were performed at 517 nm after the time (*t*) 0 and 30 minutes using methanol as a blank (Bhat et al., 2019). DPPH was calculated according to Eq. 2, and the results were expressed as mg Trolox equivalent per 100 g of sample.

$$DPPH = [1 - (A\ sample\ t30 / A\ control\ t0)] \times 100 \qquad \textit{Eq. 2}$$

2.8 Sensory evaluation

Sensory evaluation was conducted only with the Epagri´s rice genotypes. The analysis of the raw and cooked black and red rice was conducted with 87 untrained panelists. After cooking, each taster received the sample separately. The raw rice was evaluated for size, shape, color and overall acceptability while the cooked rice for texture, flavor, color and overall acceptability. Panelists were asked to score the samples according to a 9-point hedonic scale (9 = like extremely to 1 = dislike extremely). For purchase intention, a 5-point hedonic scale was used (5 = would certainly buy to 1 = would certainly not buy). The Research Ethics Committee of the Federal University of São Paulo, under decision number 683.687, approved this study.

2.9 Statistical analysis

All data were expressed as mean ± standard error of at least three replicates. Significant differences between samples were evaluated by one-way ANOVA (p<0.05), the Student’s *t* test, or Tukey’s test using Minitab 19.2 (Minitab Inc., PA, USA). For PCA-biplot analysis, data were normalized (by log transformation), Pareto scaled (mean-centered and divided by the square root of the standard deviation of each variable) using the Metaboanalyst 4.0 server (Chong et al., 2019).

3. Results and Discussion

3.1 Differences in dimension, proximal composition, fatty acids and phytochemical parameters between commercial and Epagri raw rice genotypes

The commercial and Epagri varieties of black and red rice were categorized by the genotype/cultivar, growing region and dimension of grains (Table 1). It was possible to observe that Epagri varieties have a tendency to show higher values of L/W parameter (length/width) than the commercial varieties, characterized by the higher lengths of the grains in black rice. However, no differences were observed between the samples of red rice.

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Table 1 - Identification of pigmented rice samples (*Oryza Sativa* L.)

SAMPLES	Genotype / Cultivar	State of Brazil / year	Length / Width	L / W
Commercial black rice				
Black rice	IAC 600	SP / 2009	5.73 / 2.58	2.22
Black rice	IAC 600	SP / 2010		
Black rice	IAC 600	SP / 2011		
Black rice	Unknown	RS / 2010	5.95 / 2.62	2.27
Black rice	Unknown	RS / 2011		
Epagri´s black rice				
Black rice	SC 606	SC / 2009	6.7 / 1.8	3.72
Black rice	SC 606	SC / 2010		
Black rice	SC 606	SC / 2011		
Black rice	SCS120 Ônix	SC / 2009	7.41 / 1.91	3.88
Black rice	SCS120 Ônix	SC / 2010		
Black rice	SCS120 Ônix	SC / 2011		
Black rice	SC 704	SC / 2011	7.54 / 2.20	3.43
Black rice	SC 705	SC / 2011	8.19 / 2.12	3.86
Black rice	SC 706	SC / 2011	8.09 / 2.26	3.58
Black rice	SC 707	SC / 2011	7.94 / 2.22	3.57
Commercial red rice				
Red rice	Unknown	SP / 2009	7.92 / 2.32	3.40
Red rice	Unknown	SP / 2010		
Red rice	Unknown	SP / 2011		
Red rice	Unknown	RS / 2010	7.45 / 2.33	3.19
Red rice	Unknown	RS / 2011		
Epagri´s red rice				
Red rice	SCS119 Rubi	SC / 2010	7.21 / 2.24	3.22
Red rice	SCS119 Rubi	SC / 2011		
Red rice	SC 709	SC / 2011	7.15 / 1.98	3.61
Red rice	SC 710	SC / 2011	6.78 / 1.97	3.44

BR, black rice; RR, red rice; L/W, length/width; SC, genotype; SCS, cultivar; SP, São Paulo; SC, Santa Catarina; RS, Rio Grande do Sul. Source: Authors (2022).

The raw pigmented rice was characterized by its chemical and fatty acid composition (Table 2). Epagri black rice showed higher levels of protein, crude fat, ash, insoluble and total fiber than the commercial black rice. The opposite behavior was observed by red rice samples, presenting greater amounts of ash, soluble, insoluble and total fiber in commercial rice varieties than Epagri rice varieties. The others chemical components analyzed as protein and crude fat did not show significate differences between the red rice samples. Regarding fatty acids profile of pigmented rice, palmitate, estearate, oleate, linoleate and α-linolenate were analyzed. Epagri black rice showed larger levels of total fatty acids than the commercial black rice varieties, while no significant differences were detected among the fatty acids identified in red rice varieties.

Important differences were detected in bioactive compounds and antioxidant properties between commercial and Epagri varieties (Table 2). The contents of total phenolics and total flavonoids were significantly higher in Epagri rice genotypes than in commercial varieties for both pigmented rice. The total anthocyanin and total proanthocyanidin contents in black rice and red rice, respectively, also were greater in Epagri varieties. In addition, the antioxidant properties of black and red rice were higher in Epagri rice varieties than commercial varieties followed by both the methods analyzed ORAC and DPPH. However, when comparing both pigmented rice, black rice exhibited greater chemical composition, phytochemical profile, antioxidant properties and fatty acid composition than red rice.

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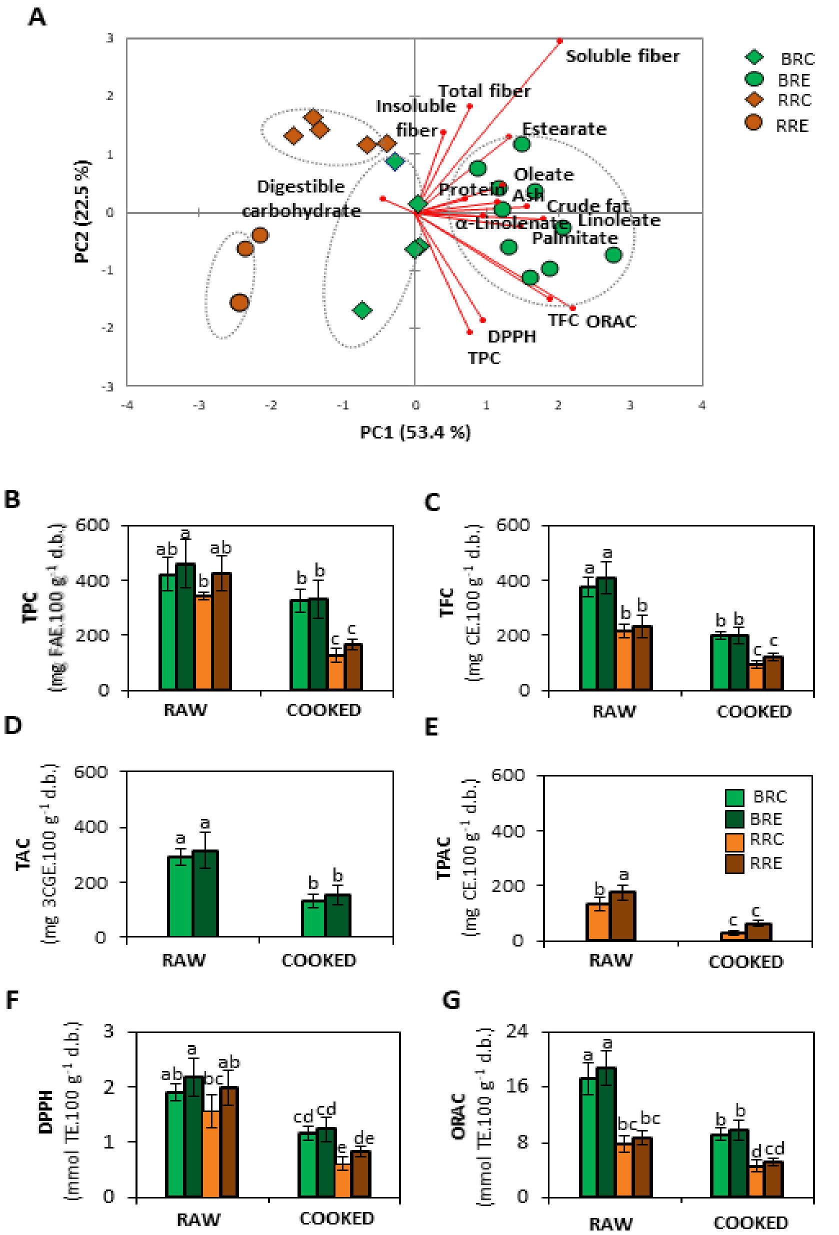
Table 2 - Proximal composition, fatty acids, bioactive compounds and antioxidant properties of the raw black and red rice.

	Commercial black rice	*Epagri´s black rice*	*Commercial red rice*	*Epagri´s red rice*
Chemical composition				
Protein (g/100g d.b.)	8.84±0.41^{b}	9.93±0.77^{a}	9.00±0.48^{ab}	8.75±0.64^{b}
Crude fat (g/100g d.b.)	2.68±0.16^{b}	3.62±0.32^{a}	2.78±0.18^{b}	2.45±0.19^{b}
Ash (g/100g d.b.)	1.68±0.13^{b}	1.80±0.08^{a}	1.58±0.08^{b}	1.37±0.09^{c}
Digestible carbohydrate (g/100g d.b.)	82.28±0.37^{a}	81.54±2.52^{a}	84.36±3.63^{a}	84.55±1.17^{a}
Soluble fiber (g/100g d.b.)	0.80±0.48^{a}	0.82±0.20^{a}	0.70±0.20^{ab}	0.22±0.05^{c}
Insoluble fiber (g/100g d.b.)	3.72±0.32^{c}	4.37±0.24^{b}	4.82±0.34^{a}	3.65±0,12^{c}
Total dietary fiber (g/100g d.b.)	4.52±0.41^{b}	5.19±0.35^{a}	5.52±0.25^{a}	3.85±0,12^{c}
Fatty acids				
Palmitate (16:0) (g/100g d.b.)	0.46±0.03^{b}	0.70±0.06^{a}	0.51±0.10^{b}	0.48±0.01^{b}
Estearate (18:0) (g/100g d.b.)	0.05±0.00^{b}	0.07±0.01^{a}	0.06±0.00^{ab}	0.04±0.00^{b}
Oleate (18:1) (g/100g d.b.)	1.02±0.06^{b}	1.32±0.17^{a}	1.18±0.03^{ab}	0.97±0.08^{b}
Linoleate (18:2) (g/100g d.b.)	1.10±0.06^{b}	1.47±0.09^{a}	0.99±0.05^{bc}	0.91±0.10^{c}
α-Linolenate (18:3) (g/100g d.b.)	0.04±0.00^{b}	0.05±0.00^{a}	0.04±0.00^{ab}	0.04±0.00^{b}
Bioactive compounds				
[1]TPC (mg FAE/100g d.b.)	419.60±20.40^{b}	459.50±16.40^{a}	342.42±14.59^{c}	423.70±18.20^{b}
[2]TFC (mg CE/100g d.b.)	375.70±15.80^{b}	407.40±16.10^{a}	216.50±16.40^{d}	233.90±18.40^{c}
[3]TAC (mg C3GE/100g d.b.)	290.90±19.20^{b}	340.30±17.5^{a}	-	-
[4]TPAC (mg CE/100g d.b.)	-	-	133.70±10.60^{b}	174.70±12.80^{a}
Antioxidant Properties				
[5]ORAC (mM TE/100g d.b.)	17.24±1.10^{b}	20.80±1.20^{a}	7.80±1.17^{d}	10.65±1.06^{c}
[6]DPPH (mM TE/100g d.b.)	1.90±0.14^{b}	2.18±0.14^{a}	1.56±0.14^{c}	1.97±0.12^{b}

[1]TPC, total phenolic content; [2]TFC, total flavonoid content; [3]TAC, total anthocyanin content; [4]TPAC, total proanthocyanidin content; [5]ORAC, oxygen radical absorbance capacity; [6]DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity. Values are means of at least three replicates ± standard errors of values. Different letters in the line indicate statistically significant differences (p<0.05). FAE, feluric acid equivalent; CE, catequin equivalent; TE, trolox equivalent; C3GE, cyanidin-3-*O*-glucoside equivalent; d.b., dry basis. Source: Authors (2022).

A non-supervised principal component analysis (PCA-biplot) was plotted from proximal composition, fatty acids, bioactive compounds and antioxidant properties of raw black and red rice to observe the main differences between the commercial and Epagri rice varieties (Figure 1A). PCA-biplot displayed notable separation not only between black and red rice, but also between commercial and Epagri varieties. Epagri black rice showed remarkable difference in the fatty acid composition such as stearate, oleate, linoleate and palmitate and α-linolenate, and chemical components, such as protein and fiber levels. In addition, it is important to note that black rice samples showed great contents of bioactive compounds and antioxidant capacity and these characteristics are even most prominent in Epagri varieties.

Figure 1 - Non-supervised principal component analysis (PCA-biplot) obtained from proximal composition, fatty acids, bioactive compounds and antioxidant properties (A). Impact of cooking processing on the TPC (B), TFC (C), TAC (D), TPAC (E), ORAC (F) and DPPH (G) of black and red rice.



BRC, raw commercial black rice; BRE, raw Epagri black rice; RRC, raw commercial red rice; RRE, raw Epagri red rice; TPC, total phenolic content; TFC, total flavonoid content; TAC, total anthocyanin content; TPAC, total proanthocyanidin content; ORAC, oxygen radical absorbance capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity. Source: Authors (2022).

3.2 Impact of cooking processing in phytochemical profile and antioxidant activity of BR and RR

The effect of cooking processing on bioactive compounds and antioxidant capacity of commercial and Epagri pigmented rice is observed in Figure 1. The cooking processing did not affect the total phenolics of commercial black rice, but caused a reduction by 28% in cooked Epagri black rice. Both samples of red rice, commercial and Epagri, were significantly affected by cooking, reducing the contents of total phenolic by 62 and 60%, respectively (Figure 1B). Total flavonoids were also negatively influenced by cooking, decreasing by 46 and 50% in the commercial and Epagri black rice genotypes. Similar behavior was observed by the red rice varieties, 55 and 48% of reduction in the commercial and Epagri genotypes, respectively (Figure 1C). Moreover, cooked samples of black rice retained higher contents of total phenolics and flavonoids when compared to cooked red rice.

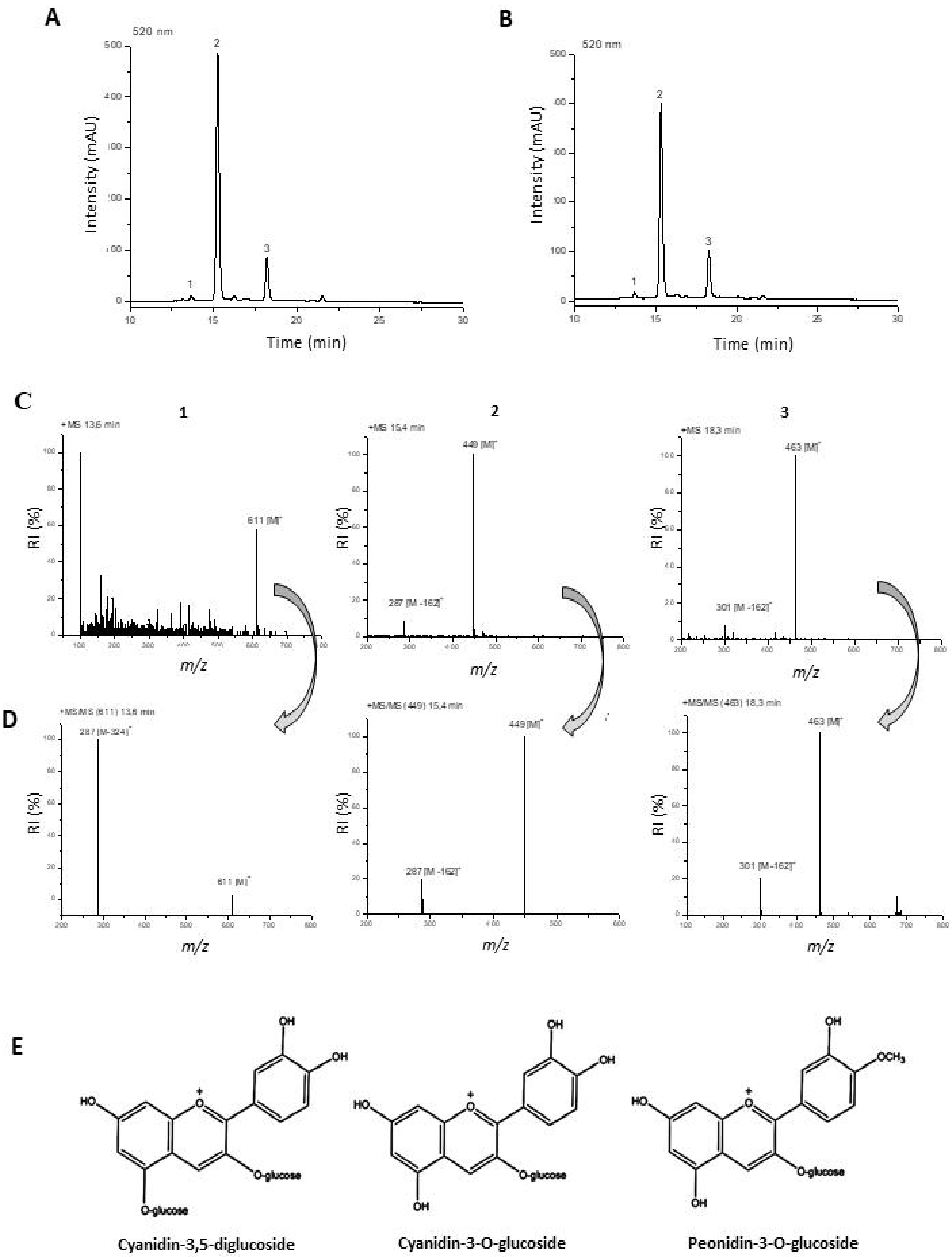
The levels of total proanthocyanidins and anthocyanins were analyzed in red and black rice, respectively, since they were the major type of flavonoids found in these varieties of pigmented rice. Proanthocyanidins content were highly impacted by cooking, showing a reduction of 79 and 64% in commercial and Epagri red rice varieties, respectively (Figure 1D). While, anthocyanins level where less affected, decreasing by 56% in commercial black rice and 51% in Epagri black rice (Figure 1C)

In addition, anthocyanins of raw and cooked black rice were identified by HPLC-DAD-MS/MS (Figure 2A and 2B, respectively), where is possible to observe a reduction in the peak area after rice cooking. The anthocyanins identified in raw and cooked samples were characterized by peak 1, 2 and 3, which correspond to cyanidin-3,5-diglucoside, cyanidin-3-O-glucoside and peonidin-3-O-glucoside, respectively (Figure 2C, 2D and 2E). Aalim et al. (2021) described that cyanidin-3-O-glucoside was the main phenolic compound, composing the majority of total anthocyanins in black rice. The fragmentation profile of peak 1, 2 and 3 of raw and cooked black rice did not show differences, evidencing that he raw and cooked samples showed the same anthocyanins composition, but with reduction in their levels after cooking.

Regarding the antioxidant properties, the cooking process affected similarly the commercial and Epagri rice varieties. Black and red rice showed a reduction of 47 and 41%, respectively, after cooking processing by ORAC analysis. When antioxidant properties were determined by DPPH analysis, commercial and Epagri black rice decreased by 39 and 44%, respectively, after cooking. While, in red rice varieties the antioxidant properties were most influenced by cooking, showing a reduction of 61 and 58% (Figure 1F and 1G). In addition, not only raw black rice but also the cooked samples showed greater antioxidant capacity than the red rice varieties.

It is important to highlight that part of the phenolics that were previously soluble by methanolic extract before processing are no longer easily solubilized due to several reaction which can undergo during cooking such as polymerization, interaction with other components of the grain and formation of the Maillard reaction product (Aalim et al., 2021; Massaretto et al., 2011), which can negatively impact the process of extracting bioactive compounds.

Figure 2 - Anthocyanins identification in raw black rice (A) and cooked black rice SCS120 Ônix variety (B). MS fragmentation profile of peak 1, 2 and 3 obtained in chromatograms A and B (C). MS/MS fragmentation profile of major peaks obtained in chromatogram C (D). Chemical structure of cyanidin-3,5-diglucoside, cyanidin-3-O-glucoside and peonidin-3-O-glucoside which were identified as peak 1, 2 and 3, respectively (E).



Source: Authors (2022).

3.3 Sensory evaluation of Epagri black and red rice

BR and RR were evaluated regarding to sensorial overall acceptability of their raw material and cooked rice by 87 tasters. Raw materials of BR and RR were analyzed by overall appearance as their color, shape and size. Whereas, the cooked

BR and RR were evaluated as overall acceptability considering their taste, flavor and texture (Table 3). In addition, they were questioned about purchase intention of the cooked products. The raw BR and RR presented 69% (6.21±2.11) and 71% (6.40±1.86) of acceptability by the tasters, respectively. Cooking induced a higher acceptability as both, BR and RR achieved 78% (6.99±2.02) and 81% (7.33±1.74) of consumer acceptance, respectively, which were significantly different when comparing with those no processed.

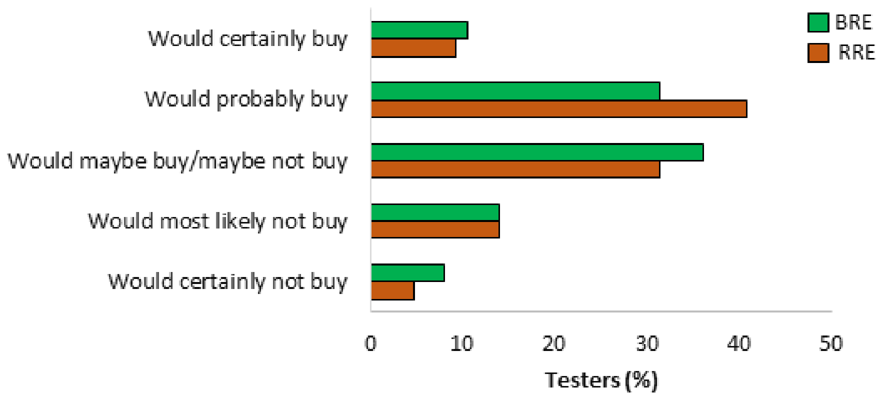
Table 3 - Sensory properties of the raw and cooked black rice (*Oryza sativa* var. SCS120 Ônix) and red rice (*Oryza sativa* var. SCS119 Rubi).

	Raw rice		***Cooked rice***	
	Black rice	**Red rice**	**Black rice**	**Red rice**
Size	7.30±1.04^{a}	6.25±1.68^{b}	-	-
Shape	7.41±1.39^{a}	6.62±1.87^{b}	-	-
Texture	-	-	6.71±1.45^{a}	6.68±1.37^{a}
Flavor	-	-	7.12±1.45^{a}	6.39±1.57^{b}
Taste	-	-	7.32±1.81^{a}	6.58±1.87^{b}
Color	7.52±1.34^{a}	7.43±1.44^{a}	6.34±1.25^{b}	6.28±1.39^{b}
Overall acceptability			7.33±1.74^{a}	6.40±1.86^{b}

Values are means of 87 untrained assessors' ± standard errors of the values. Different letters in the line indicate statistically significant differences (p<0.05). Source: Authors (2022).

Regarding the great sensorial acceptability, purchase intention test of the cooked pigmented rice either demonstrated a relevant interest in acquisition of the new varieties of rice (Figure 3). Around 50 and 42% of panelists informed that they "would probably and certainly buy" the cooked BR and RR, respectively if they were found on the marketplace, emphasizing the robust acceptability of cooked rice. In addition, between 31 and 36% of them reported that "would maybe and maybe not buy" the cooked rice. Lastly, just 19 and 22% of consumers assumed that "would certainly and most likely not buy" the cooked BR and RR, respectively.

Figure 3 - Purchase intention of black and red rice.



BRE, Epagri black rice; RRE, Epagri red rice. Source: Authors (2022).

The tasters were questioned about their motivation purchase the BR and RR. They answered that the main reasons that could make them to buy these pigmented varieties were the taste, color and the health benefits related to them. According with this, the great sensorial response obtained can be related with the presence of anthocyanins (Figure 1D) and proanthocyanidins (Figure 1E) in BR and RR, respectively. Reminding that these bioactive compounds are responsible for the natural color of the bran layers of the rice and also for the beneficial effects on human health as the reduction of the risks of developing some cancers, diabetes, obesity and cardiovascular diseases (Tiozon et al., 2021; Aalim et al., 2021; Samyor et al., 2017).

4. Conclusion

Epagri black rice showed higher levels of protein, crude fat, ash, insoluble fiber, total fiber and fatty acids than the commercial black rice, while red rice samples presented greater amounts of ash, soluble, insoluble and total fiber in commercial rice varieties than Epagri rice varieties. The contents of phenolics, flavonoids, anthocyanins, proanthocyanidins, and antioxidant properties were significantly higher in Epagri rice genotypes than in commercial varieties for both pigmented rice. However, when comparing both pigmented rice, black rice exhibited greater chemical composition, fatty acid composition, phytochemical profile and antioxidant properties than red rice. The cooking processing did not affect the total phenolics of commercial black rice and caused a reduction by 28% in cooked Epagri black rice, while in red rice, commercial and Epagri, reduced around 60%. Flavonoids showed a reduction of 50% in the commercial and Epagri genotypes after cooking. However, cooked samples of black rice retained higher contents of total phenolics and flavonoids when compared to cooked red rice. Proanthocyanidins were reduced by 79 and 64% in commercial and Epagri red rice varieties, respectively, while anthocyanins decreased by 56 and 51% in commercial black rice and Epagri black rice, respectively. Regarding the sensory analysis, raw black and red rice showed 69 and 71% of acceptability by the tasters, respectively. Cooking induced a higher acceptability of black and red rice by 78 and 81% of consumer acceptance, respectively. This work contributed with the diffusion of knowledge about the special types of rice cultivated in Brazil, highlighting the impact of cooking on the phytochemical and antioxidant properties of black and red rice. Results obtained in this work give support to future research as the development of studies based on other types of processing of pigmented rice in the food industry, such as extruded products (snacks), bakery, pasta, or its potential use as ingredient in plant-based foods. The high nutritional properties of pigmented rice stimulates the use of flour from by-products generated from the processing of rice to develop available foods with added nutritional value as other options to consumers.

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