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Extraction and Characterization of Native Starch From Black and Red Rice

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Abstract

The objective of the study is to extract and characterize red rice starch, a grain commonly consumed in the Northeast region of Brazil and to compare it with the starch obtained from black rice, widely spread in eastern countries. Starch was extracted by immersion of rice in sodium metabisulfite (0.2%), followed by milling, filtration, decantation and drying at 50 ℃. The obtained starches were characterized by the following parameters: physicochemical, physical, rheological, textural, structural, thermal and morphological. The highest starch extraction yield was found in red rice grains (67.84%), where it obtained higher water absorption and lower apparent viscosity, firmness and gum. Both starch gels indicate the behavior of non-Newtonian fluid in the specific case of pseudoplastic crystallinity type A and with polyhedral geometry. For the thermal analysis of red rice starch, it was observed higher gelatinization temperature and lower gelatinization enthalpy. Black and red rice grains can be considered an alternative source of starch, especially red rice, which has a lower cost due to its production in the country.

Keywords: *Oryza sativa*, Native starch, Technological properties

1. Introduction

The naturally grown pigmented rice cultivars are associated with many health benefits and are found to be present in the diverse shades of colors mainly red, purple, and black. These are highly valued and greater interest has been recently shown in them due to the presence of different polyphenols that are having multiple biological activities (Bhat and Riar, 2019). Rice is a widely consumed food because of its beneficial properties for health, constituting a source of energy due to the high concentration of starch, proteins, minerals and B vitamins (Ziegler *et al.*, 2016). Husked rice has a variety of natural colors, such as brown, red and black, depending on the content of anthocyanins in the episperm. Red rice is rich in nutritional and biofunctional components, such as proanthocyanidins, γ-aminobutyric acid, γ-oryzanol, dietary fibers, vitamins and minerals, compared to the common whole rice (Ding *et al.*, 2018). Black rice is a source of phytochemicals, with functional properties and high levels of antioxidants, which serve as protection for endothelial cells, acting in the prevention of cardiovascular diseases (Finocchiaro *et al.*, 2010). Black rice also contains a higher lipid content than non-pigmented and red rice, which makes it more palatable and attractive (Choi *et al.*, 2019).

One of the major constituents of rice is the starch, a polysaccharide with important properties such as biodegradability, biocompatibility and non-toxicity (Afolabi *et al.*, 2012). The interest in rice starch has increased due to its specific characteristics: white color, lack of odor, easy digestion and small particle size. These characteristics allow multiple applications for the starch, in both food and non-food industries (Ashogbon and Akintayo, 2012). Among its functions in the food industry, starch can facilitate processing, impart texture, serve as thickener, provide suspended solids or undergo modifications in its structure to perform specific functions (Silva *et al.*, 2013). The objective of the study is to extract and characterize the red rice starch, a grain commonly consumed in the Northeast region of Brazil and compare it with the starch obtained from black rice that is widespread in the Eastern countries.

2. Material and Methods

Black and red rice (Oryza sativa L.) grains, Type 1, Class medium, Subgroup whole, were purchased from the local market of the city of Campina Grande – PB, Brazil.

2.1 Extraction of Native Starches

Black and red rice starches were extracted according to Adebowale *et al.* (2005). Rice grains were immersed in sodium metabisulfite (0.2%), at 1:2 ratio (m/v) for 48 h, washed in running water for 3 min, crushed in a blender with distilled water at 1:2 rate (m/v) for 5 min, filtered in organza fabric, and the residue was crushed again three times, to increase process yield. Following filtration, the starch suspension was decanted for 24 h at 7 ℃. After the first decantation, the supernatant was discarded and 300 mL of distilled water were mixed with the precipitated starch to purify the sample. After more 24 h of decantation, the supernatant was discarded again and the same volume of water (300 mL) was mixed with the precipitate. This supernatant disposal and water addition process was performed 5 times. The decanted starch was dried in a forced air circulation oven at 50 ℃ until reaching a safe percentage of moisture, which does not allow the development of microorganisms.

2.1.1 Extraction Yield

Starch extraction yield was calculated according to Equation 1.

$$Y = \frac{ms}{mi} . 100 \tag{1}$$

Where, Y- yield, (%), ms- mass of starch obtained after drying (g), and mi- initial mass of crushed rice (g).

2.2 Starch Characterization

2.2.1 Physicochemical Characterization

Physicochemical analyses were carried out in triplicate, using the starch retained on the 80-mesh sieve (because it was visually more similar to the existing commercial starches): pH, titratable acidity, moisture content, ashes, starch and lipids were determined according to AACC (2000). Water activity at 25 ℃ was determined in an Aqualab 3TE water activity meter (Decagon).

2.2.2 Water and Oil Absorption Capacity

The method of Beuchat (1977) was used to determine the water and oil absorption capacity of the starches. Approximately 10 mL of distilled water or soybean oil were added to 1 g of starch in centrifuge tubes. The suspension was homogenized for 30 s, left at rest for 30 min and centrifuged for 15 min at 1000 rpm. The mass of water or oil absorbed (Equation 2) was expressed in g 100 g^{-1} (dry basis).

$$CA = (\text{tube mass after centrifugation}) - (\text{tube mass + dry sample}). 100 \tag{2}$$

Where, CA- capacity to absorb water or oil (g 100g^{-1}).

2.2.3 Syneresis

The syneresis (Equation 3) of rice starch gels was determined according to Farnsworth *et al.* (2006). After three days stored at 6 ℃, the gels were centrifuged at 3000 rpm for 10 min, where the ratio: starch / water (1:10) and temperature (80 ℃) were standardized in the preparation of suspensions.

$$Syneresis(\%) = \frac{mf}{mi}.100 \quad (3)$$

Where, mf- mass of water separated from the gel after centrifugation (g), and mi- initial mass of the gel (g).

2.2.4 Rheological Behavior

The apparent viscosity of the rice starch gels was determined in triplicate using the Brookfield DV-II+Pro viscosimeter at rotation speeds from 50 to 200 rpm. Starch-water (10%) suspensions were heated up to the point of gelatinization (80 ℃/30 min), and viscosity readings were taken at gelatinization temperature and room temperature (25 ℃). The results were analyzed in Origin 8.0 software.

2.2.5 Texture

The texture profile was tested using the TA-XT PLUS texture analyzer (Scarsdale, NY; Stable Micro Systems, UK). Instrument settings were compression mode, trigger type, auto5 g; pretest speed, 2.0 mm/s; posttest speed, 1.0 mm/s; test speed, 1.0 mm/s; compression height, height of samples; strain, 40%; interval between two compressions, 5 s; compression times, 2 s, aluminum cylinder with a diameter of 36 mm in diameter. Each sample should be measured twice and the final result was the average. The firmness, cohesiveness, guminess and adhesiveness values were calculated from the force-for-time plots obtained (Liu *et al.*, 2015).

2.2.6 X-ray Diffractograms

The X-ray diffractograms of the starches were obtained in diffractometer (Shimadzu, XRD – 7000) using copper Kα radiation (1.5418 Å), 40 kV, 30 mA, step of 0.05 and scanning rate of 0.5 °min^{-1} at room temperature. The scanning range of the diffraction was adjusted to angles from 5° to 65° (2θ). The diffractograms were used to identify the phases composing a crystalline material. The diffractogram was read from the graph of the record of counts per second versus diffraction angle 2θ, with the degree of crystallinity obtained according to Equation 4.

$$\%Crystallinity = \frac{Total\ Area - Amorphous\ Fraction\ Area}{Total\ Area}.100\% \quad (4)$$

2.2.7 FTIR

The FT-IR spectra of the starches were obtained using Spectrum 400 from Perkin Elmer in the region of 4000-600 cm^{-1}, using a resolution of $4cm^{-1}$ and 32scans. Gaussian function was used to deconvolute the FTIR peaks.

2.2.8 Differential Scanning Calorimetry (DSC)

The thermal properties of black and red rice starch were determined using a differential scanning calorimeter (2920 Modulated DSC, TA Instruments, New Castle, Del., U.S.A.) equipped with a refrigerated cooling system. Starch (12 mg, dry basis) was charged into a high-volume pot and distilled water (28 mg) was added. The samples were sealed and allowed to equilibrate overnight at room temperature before the DSC analysis. The sample pots were heated at a rate of 10 ℃ / min from 20 ℃, up to 200 ℃. An empty pan was used as a reference. The temperatures onset (To), peak temperature (Tp) and transition enthalpy (ΔH) were measured from the thermograms.

2.2.9 Scanning Electron Microscopy

Starch morphology was analyzed by the technique of Scanning Electron Microscopy (SEM). The samples were prepared by placing the solids on an aluminum sample holder, covered with carbon double-sided tapes, metallized with gold, in order to provide a reflecting surface for the electron beams, and then observed in the VEGA3 Tescan scanning electron microscope under vacuum of $1.5x10^{-3}$ Pa, with magnification of 2000x.

2.3 Data Statistical Analysis

All experiments were executed in triplicate, and the results were submitted to analysis of variance (ANOVA) at 5% of probability. The significant qualitative responses were submitted to the Tukey test adopting the same level of 5% of significance. All statistical analyses were developed using Statistica software version 7.0 (Stasoft, 2007).

3. Results and Discussion

3.1 Extraction Yield

Starch extraction yield was equal to 50.03 and 67.84% for black rice and red rice, respectively. Ashogbon and Akintayo (2012) studied starches of different rice cultivars and obtained extraction yields from 45.7 to 65%.

3.2 Physicochemical Analyses and Functional Properties

The results obtained for physicochemical and physical parameters of black and red rice starches are presented in Table 1.

Table 1. Physicochemical and physical parameters of black and red rice starches

Parameters	Black rice starch	Red rice starch
pH	5.26 ±0.01[a]	5.25 ±0.01[a]
Acidity (ml NaOH 1N100 g^{-1})	3.10 ±0.01[a]	3.11 ±0.03[a]
Moisture content (%)	7.83 ±0.05[b]	12.80 ±0.10[a]
Ash (%)	0.85 ±0.05[a]	0.52 ±0.02[b]
Starch (%)	91.06 ±1.12[a]	86.54 ±0.38[b]
Lipids (%)	0.26 ±0.02[a]	0.14 ±0.02[b]
Water activity (A_w)	0.345 ±0.006[a]	0.328 ±0.007[b]
Water absorption (g 100 g^{-1})	99.53 ±1.00[b]	111.6 ±1.82[a]
Oil absorption (g 100 g^{-1})	86.90 ±1.37[a]	78.90 ±0.30[b]
Syneresis (%)	13.47 ±0.05[b]	16.93 ±0.11[a]

Note: Means followed by the same lowercase letters in the rows do not differ statistically by Tukey test at 0.05 probability level.

The values of pH and acidity obtained for red and black rice starches did not differ statistically ($p>0.05$). Alencar *et al.* (2017) obtained for the black rice grain the value of 6.67 for pH, which is higher than the value found in the present study. Ashogbon and Akitayo (2012) observed in the paste of rice starch pH values from 5.3 to 6.9, which are close the ones found here. According to the current legislation for rice starches (Brazil, 1978), the maximum value allowed is 14% for moisture content. The starches of the present study are within the standard required by legislation for moisture content. Such higher acidity may be related to the types of rice used, the conditions of cultivation and processing.

Ash and starch contents differed statistically ($p>0.05$) between samples, with highest values in black rice starch. Ash contents were lower than 1%, indicating pure starches. Arns *et al.* (2015) obtained ash content of 0.20% in the starch of rice (cv. IRGA-424). Santos *et al.* (2019) found 1.97% ash for the black rice grain. An *et al.* (2016) obtained 69.68% starch for black rice grain and Almeida *et al.*, 2019 obtained 81.98% for native red rice starch. The varying content of starch in different rice starch samples could be attributed to variations in environmental conditions during grain development (Asaoka *et al.*, 1985) and cultural practice (Kim and Wiesenborn, 1995). Multiplying the value of the extraction yield by the starch content obtained in Table 1, red rice stands out with 56.25% of pure starch extraction in relation to 41.61% of black rice.

The lipid contents in the starches differed statistically ($p>0.05$). Low lipid contents were also found by Przetaczek-Roznowska (2017) in starches isolated from pumpkin, which ranged from 0.04 to 0.05%, and by Arns *et al.* (2015) in rice starches, which varied from 0.03 to 0.07%.

The water activity values obtained for red and black rice starches were not favorable for the development of bacteria (0.90), yeasts (0.80), fungi (0.60), halophile bacteria (0.65) and osmophilic yeasts (0.62), according to Fonseca and Cantarelli (1984).

The water absorption capacity indicates the volume of water absorbed by the starch granules of a certain sample subjected to a thermal treatment. Higher capacity to absorb water was observed in red rice starch. Similar results were reported by Almeida *et al.* (2013), who studied ying native and modified starch of taro. In relation to the oil absorption capacity, the black rice starch had higher capacity to absorb oil. Falade and Christopher (2015) obtained values from 112.55 to 150.19 g $100g^{-1}$ in starches of six varieties of Nigerian rice.

Higher value of syneresis was found in red rice starch, thus revealing that it releases greater volume of water in its handling.

3.3 Rheological Behavior

The rheological behavior of the starch gels at temperatures of 25 and 70 ℃ can be observed in Figure 1.

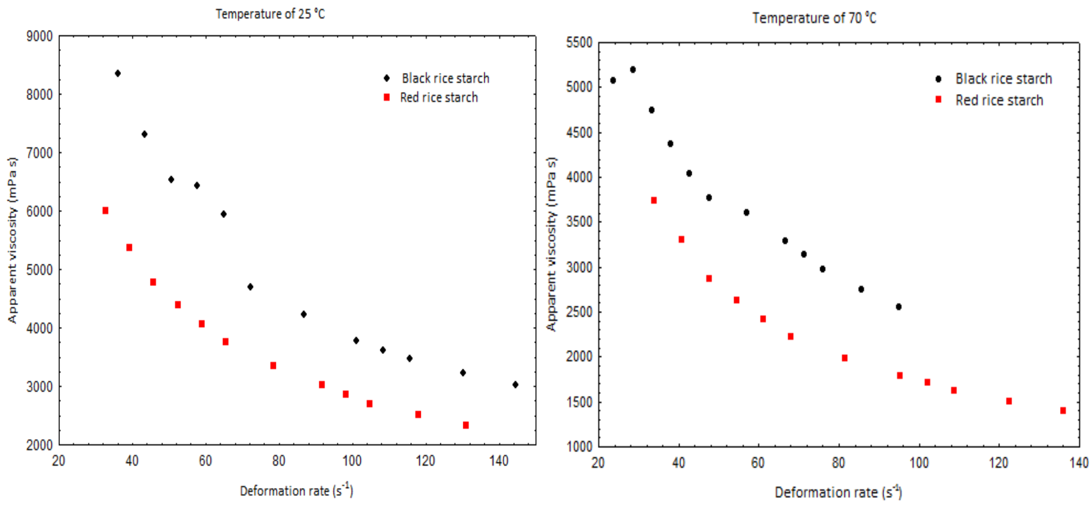

Figure 1. Rheological behavior of gels of black and red rice starches

Apparent viscosity decreased with the increase in deformation rate, indicating a behavior of non-Newtonian fluid with pseudoplastic characteristics. Such pseudoplastic behavior can be explained by the progressive orientation of the soluble starch molecules in the direction of the flow, as well as the disruption of the intramolecular and intermolecular bonding system in the starch network, which increases the sensitivity to the shearing force (Sun and Yoo, 2011). The same profile was found by Ma *et al.* (2019) in their studies with cornstarch added at different pectin concentrations

3.4 Texture

Table 2 presents the texture parameters of the starch gels. Firmness corresponds to the force applied to cause deformation in the sample and is correlated with the force to bite and compress the food in the mouth (Kalviainen *et al.*, 2000).

Table 2. Texture parameters of black and red rice starches

Starch source	Firmness (N)	Cohesiveness (dimensionless)	Elasticity (dimensionless)	Guminess (N)
Black rice	2.98 ±0.04[a]	0.47 ±0.05[b]	1.00 ±0.02[a]	1.41 ±0.02[a]
Red rice	2.16 ±0.10[b]	0.55 ±0.02[a]	1.00 ±0.08[a]	0.96 ±0.05[b]

Note: Means followed by the same lowercase letters in the columns do not differ statistically by Tukey test at 0.05 probability level.

The black rice starch showed greater firmness than the red rice starch. Przetaczek-Roznowska (2017) obtained higher firmness values of 11.80 and 22.98 N for corn and potato, respectively.

It is possible to correlate firmness with the rheological behavior found for the starches, so a greater firmness is directly related to higher viscosity, as observed in Figure 1, which shows that black rice starch showed higher viscosity than red rice starch at the evaluated temperatures.

In the present study, cohesiveness was higher in the red rice starch. Przetaczek-Roznowska (2017) found a lower value (0.2) in corn starch. Elasticity did not differ significantly between the two starches. This property measures the capacity of a product to return to its original form after being subjected to a compression force (Guimarães *et al.*, 2014). Gumminess is the energy required to disintegrate a semi-solid food (Silva *et al.*, 2013), and black rice starch showed higher gumminess than the red rice starch.

3.5 X-ray Diffractograms

The X-ray diffractograms of black and red rice starches Figure 2.

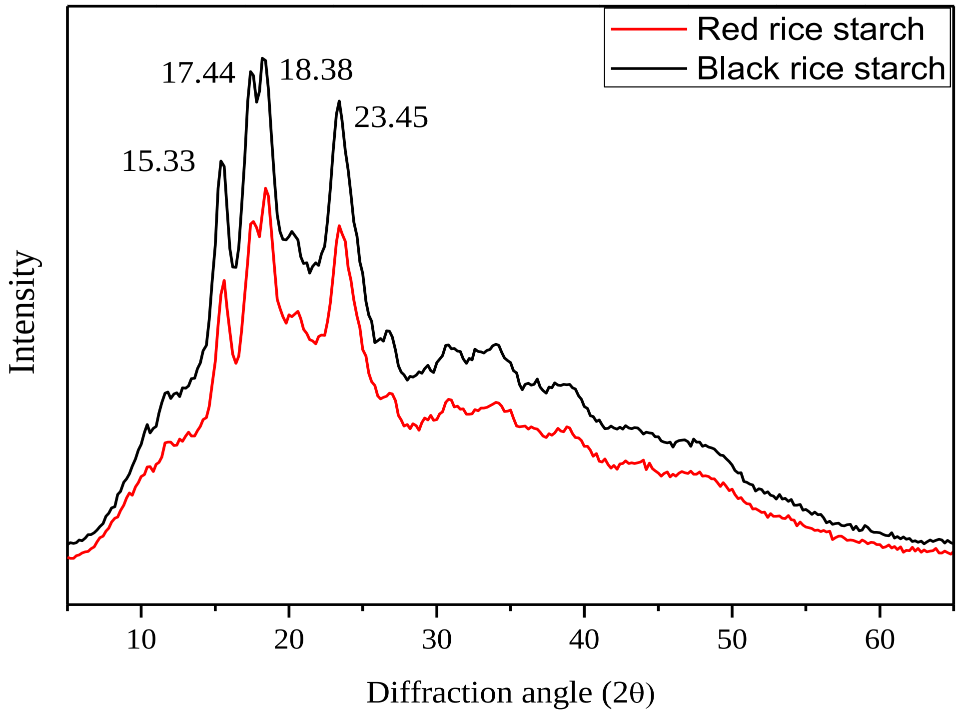

Figure 2. X-ray diffractograms of black and red rice starches

They presented intensity peaks at 2θ diffraction angles with values of 15.33°, 17.44°, 18.48° and 23.45° for black and red rice starch, being classified as type A crystallinity (Mir *et al.*, 2017). Minor differences in intensity are observed in the diffractogram between black and red rice starch. Ziegler *et al.* (2017) also found for the crystallinity of the brown, black and red type A rice starch structure, with peaks at (15°, 17°, 18°, 20° and 23°) according to the classification of Klein *et al.* (2013). The crystallinity degree obtained for black rice starch was 22.14% and for red rice starch was 20.88%, which is in agreement with the literature, which says that the degree of crystallinity (Xc) for native regular starches should be between 20% and 45% (Van Soest & Vliegenthart, 1997). According to the studies conducted by Ziegler *et al.* (2017) who analyzed starch isolated from brown, black and red pericarp whole grains of rice after storage at different temperatures (16, 24, 32 and 40 ℃), where X-ray patterns showed a type A crystal structure and on the first day of storage, the grains showed 25.2%, 26.3% and 27.6% relative crystallinity for brown, black and red pericarp grains, respectively. This result is very similar to those shown by Paraginski *et al.* (2014), with relative crystallinity of 24.9%, 23.7% and 26% for starch obtained from brown, red and yellow pericarp rice grains, respectively. Acute peaks are associated with the crystalline region, whereas diffuse peaks are associated with the amorphous region of the samples (Dharmaraj *et al.*, 2011).

3.6 FTIR

The infrared absorption spectra (FTIR) of the black and red rice starches are presented in Figure 3.

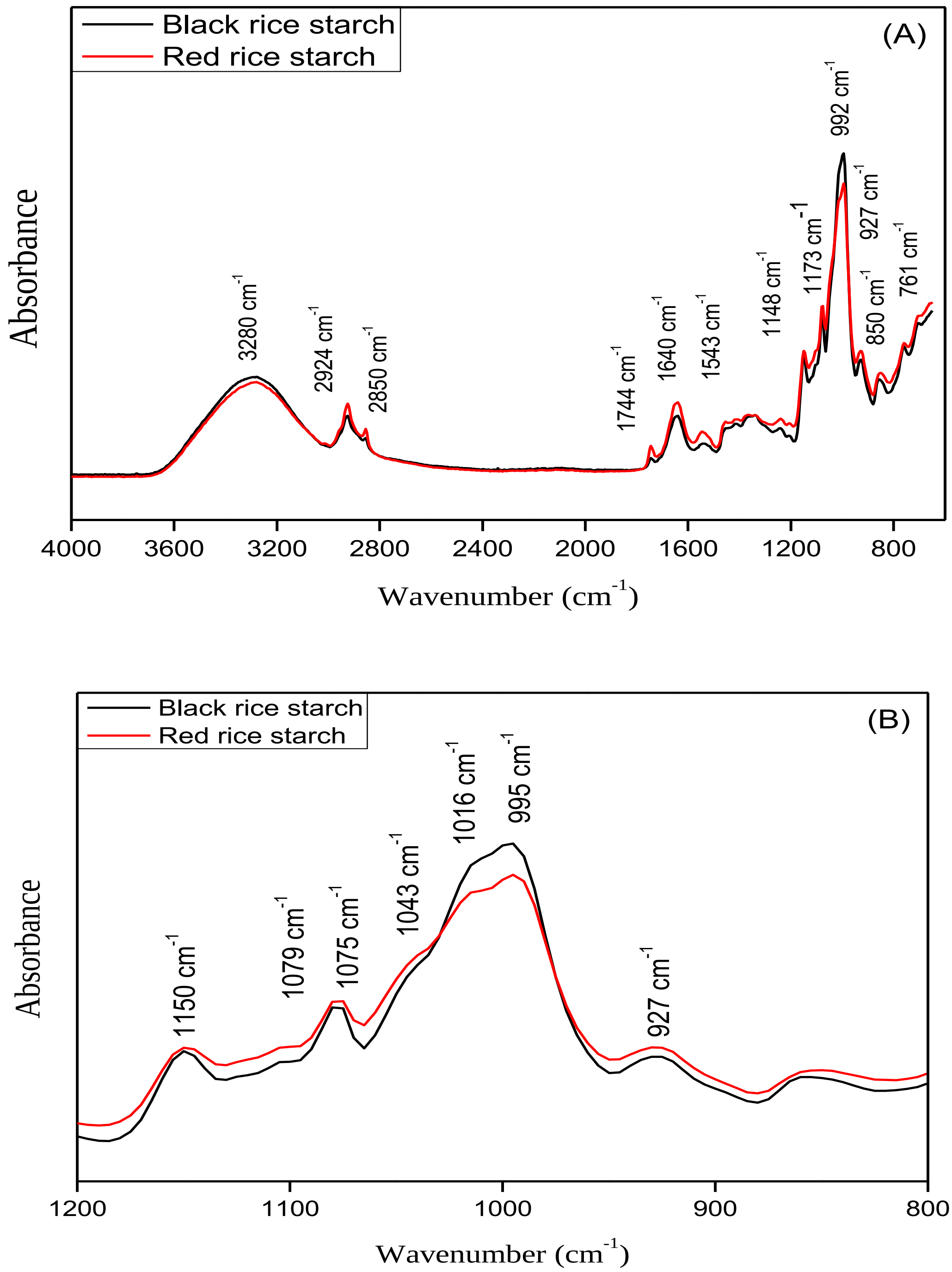

Figure 3. FTIR spectrograms of black and red starch samples (A) full wavelength analyzed (B) close up of 1200-800cm^{-1} region

The literature includes evidence of peaks detected in the maximum intensity spectral bands between 854 cm^{-1} and 1100.50 cm^{-1}, similar to those found in the starches of this study

(Valencia *et al.*, 2015; Ascheri *et al.*, 2014). The spectrum of native starch has bands in the region of 2900-3000 cm^{-1} (corresponding to the C-H stretch), at 1163, 1150, 1124 and 1103 cm^{-1}, which correspond to the C-O and C-C stretches with some contribution of the C-OH stretch. The bands at 1077, 1067, 1047, 1022, 994 and 928 cm^{-1} are attributed to the C-OH and CH_2 deformations (Zeng *et al.*, 2011).

The bands referring to the molecular deformation existing in the starch molecules at 3400 and 1650 cm^{-1} are attributed to the stretching and the angular deformation of -OH bonds, relative to the peak of moisture content. It is possible to observe the band close to 2930 cm^{-1}, attributed to the axial deformation of C-H bonds (lipids). The peaks in the region 1600 to 1200 cm^{-1} are related to C-H bending from CH_2 and the with hydroxyl bending of alcohols functional groups (Ferraz *et al.*, 2019). Bands in 1000 to 1200cm^{-1} region are considered characteristic starch bands and are attributed to the C-OH stretching and bending, the C-O-C from the glycoside link, and the CO and CC stretching (Pozo *et al.*, 2018). The vibrations of amylose and amylopectin, which are the main components of starch, were observed in the regions below 1000 cm^{-1} (Zeng *et al.*, 2011). Minor differences in intensity are observed between black and red rice starch.

3.7 Differential Scanning Calorimetry

The results of DSC analysis are presented in Table 3, for the parameters: enthalpy variation (ΔH) and temperatures of the thermal events.

Table 3. Values obtained in the DSC analysis for black and red rice starches.

Parameters	Black rice starch	Red rice starch
T_0 (℃) (gelatinization temperature)	57.45	70.46
T_p (℃)	63.42	75.45
$T_p - T_0$ (℃)	5.97	4.99
ΔH ($J\ g^{-1}$) (gelatinization enthalpy)	3.51	2.85

It can be observed that the gelatinization temperature (T_0) and peak temperature (T_p) of red rice starch was higher than that of black rice starch, demonstrating that the latter requires less energy for the swelling of particles through water absorption and increase of temperature (gelatinization). These values are similar to those found by Almeida *et al.* (2019) for native red rice starch. Bhat and Riar, (2019) found Tp values ranging from 62.53 to 67.84 ℃ for starches obtained from different varieties of pigmented rice. Weber *et al.*, (2009) observed for corn starch gelatinization temperatures between 64 and 105 ℃. Arns *et al.* (2015) found for rice starch higher values of gelatinization enthalpy, varying from 6.47 to 9.13 J g^{-1}. Fabian *et al.* (2011) found for rice bran starch a gelatinization temperature of 72.6℃ and a gelatinization enthalpy around 9.5 J g-1; It is noteworthy that ΔH of black and red rice starches are smaller, so it can be stated that the granules are poorly resistant to breakage with prolonged heating. It can be observed that the gelatinization temperature was higher in black rice starch, which indicates a greater loss of molecular organization or breakdown of hydrogen bonds in the granule (Alvani *et al.*, 2011).

3.8 Scanning Electron Microscopy

The micrographs of the starches are presented in Figure 4, which shows high similarity between black and red rice starches, both with disordered polyhedral structure, smooth surface and no cracks.

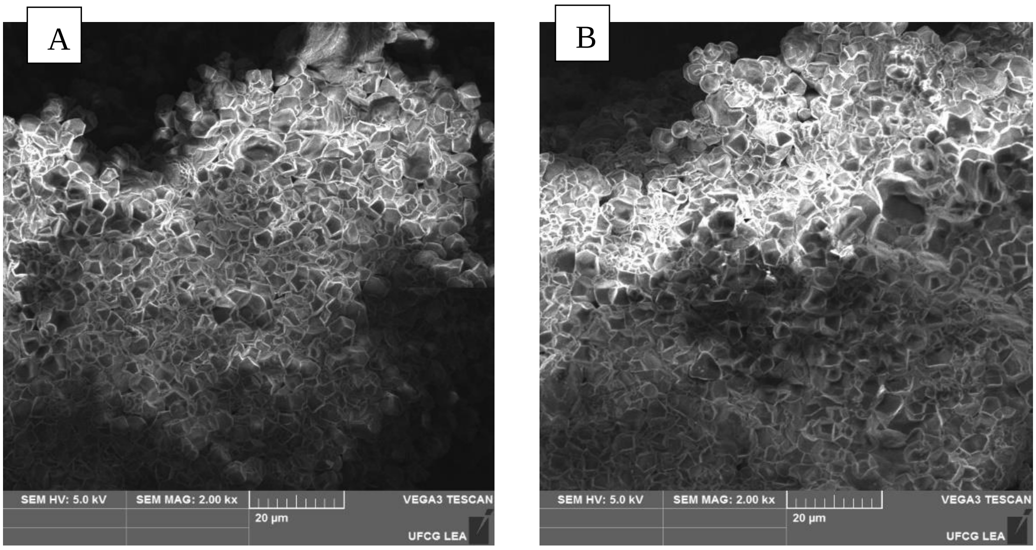

Figure 4. Micrographs of black rice starch (A) and red rice starch (B) magnified 2000 times.

The average sizes of starch granules were 3.38 μm for black rice and 3.55 μm for red rice, which are similar to the average size of rice starch granules (3 to 8 μm) reported by Souza *et al.* (2016).

Smooth starch surfaces with no cracks suggest purity in its extraction, but the morphological characteristics may vary according to the plant cultivar, growth stage, environmental conditions and methods of extraction and purification (Oyeyinka and Oyeyinka, 2018).

The red rice starch granules presented an irregular shape with edges and a polyhedral or polygonal geometry, where all surfaces are multi-faceted as reported by (Ramos *et al.*, 2019; Almeida *et al.*, 2019). Ziegler *et al.* (2017) found the same geometry for the starch granules obtained from brown, black and red rice and did not observe significant differences due to the grain pericarp coloration. Small differences in the appearance of the surfaces of the starch granules may be due to and the milling process of the rice during starch extraction.

4. Conclusion

Red rice presented higher extraction yield, while black rice showed higher purity. Both starch gels showed non-Newtonian pseudoplastic fluid behavior. The gelatinization temperature was higher in red rice starch, but the highest gelatinization enthalpy was found in black rice starch, which may indicate that black rice starch requires greater resistance to breakdown of its crystal structure. Both starches are classified as type a crystallinity and with polyhedral geometry. According to the quality presented by starches, they are suitable for use in various areas of food and non food industry, and black and red rice grains can be considered an alternative source for starch, especially the red rice that has lower cost due to its production in the country.

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